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Cellulose bait improves the effectiveness of *Metarhizium anisopliae* as a microbial control of termites (Isoptera: Rhinotermitidae)

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Abstract

The efficacy of a new virulent *Metarhizium anisopliae* strain (SRRC 2558) obtained from *Coptotermes formosanus* in China was evaluated against the eastern subterranean termite, *Reticulitermes flavipes* and the Formosan subterranean termite, *C. formosanus*, in the laboratory. This new strain was compared with three other virulent *M. anisopliae* strains and was found to be highly infectious against termites. It caused 100% mortality to groups of 100 *R. flavipes* workers in containers with treated vermiculite/sand medium at a concentration of $\geqslant 3 \quad 10^6$ conidia/cm^3. However, in two-container choice devices, *R. flavipes* avoided containers treated with *M. anisopliae* at the concentration of $\geqslant 1.5 \quad 10^7$ conidia/cm^3. Cellulose powder bait containing *M. anisopliae* was tested against *R. flavipes* and *C. formosanus*. At effective concentration, the bait did not cause apparent repellence to *R. flavipes* and *C. formosanus*. Successful control of *R. flavipes* and *C. formosanus* groups (1000/group) was achieved at $1.5 \quad 10^8$ and $3 \quad 10^8$ conidia/g, respectively, in choice tests in which termites were provided with treated cellulose bait and untreated wood.

Keywords: Entomopathogenic fungus; *Reticulitermes flavipes*; *Coptotermes formosanus*; *Metarhizium anisopliae*; Termites; Biological control

1. Introduction

The study of pathogens for termite control started as early as 1965 (Smythe and Coppel, 1965; Yendol and Paschke, 1965). Most of these studies focused on *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Culliney and Grace, 2000; Grace, 1997; Rath, 2000). Both of these fungal species are widely distributed in soil and have very broad host ranges. They are generally proved to be effective against termites in laboratory studies but had little success in field trials (Hänel and Watson, 1983; Lai, 1977; Milner and Staples, 1996). The main reason for the failure of pathogens under field conditions against termites was the social behavior of these insects, i.e., the avoidance and sealing off the diseased termites and grooming among nest mates (Rath, 2000). The high carbon dioxide and naphthalene contents and secretions of termites may also inhibit the growth of pathogens in termite nests or galleries, and therefore, render termite control with pathogens unsuccessful under field conditions (Li et al., 1979; Rosengaus et al., 2000; Wright et al., 2000). Because of these reasons, biological control of termites using these pathogens has been generally regarded as unfeasible.

As a result of the environmental concerns and phasing out of some potent chemical termiticides in the market, there has been renewed interest in using pathogens for controlling termites in recent years (Almeida et al., 1997; Connick et al., 2001; Delate et al., 1995; Jones et al., 1996; Milner and Staples, 1996; Milner et al., 1998; Neves and Alves, 1999, 2000a,b; Osbrink et al., 2001; Ramakrishnan et al., 1999; Sajap et al., 1997; Zoberi and Grace, 1995). *M. anisopliae* is especially recommended for practical control of termites as a bioinsecticide because it: (1) will not infect humans or higher animals; (2)

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is virulent to all species of termites tested; (3) has robust conidia that are easy to formulate and store; and (4) has conidia that can survive >18 months in termite nests (Milner and Staples, 1996). One commercial product based on *M. anisopliae* was registered for termite control in the U.S. in 1994 but was later withdrawn from the market due to lack of virulence and low field efficacy. A U.S. patent on control of termites with *M. anisopliae* was also granted in 1997 (Milner et al., 1997).

Virulence and repellency are two factors that must be addressed to improve the efficacy of *M. anisopliae*. A high level of virulence is essential to control termites with minimum cost and the highest efficacy. Low repellency of the fungal conidia or a non-repellent fungal conidial formulation is necessary to induce a continuous infection to termite colonies. However, it is often true that highly virulent fungal strains are also highly repellent (Staples and Milner, 2000). To overcome this obstacle, a non-repellent fungal conidial formula is needed in order to kill termite colonies.

We initiated an exploration for more virulent *M. anisopliae* strains and developing non-repellent bait formulations in 1999. The study was focused on *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* (Shiraki) because they both are serious pests in the U.S. (Snyder, 1954; Su and Scheffrahn, 1988). In the preliminary studies, strain SRRC 2558 was compared to ESC1 (active ingredient in BioBlast), FI-610 [a virulent strain from Australia (Milner, 2003)], and a new strain (SRRC 2502, Fungal Collection, USDA ARS, Southern Regional Research Center, New Orleans, LA) isolated from *R. flavipes*. The strain SRRC 2558 was obtained from the Formosan subterranean termite, *C. formosanus*, from China. It was the most virulent against *R. flavipes* when compared with other three virulent strains in preliminary studies (Wang and Powell, unpublished data). In this study, we report our results on evaluation of the efficacy of *M. anisopliae* strain SRRC 2558 and a non-repellent formulation for control of *R. flavipes* and *C. formosanus* colonies under laboratory conditions.

2. Materials and methods

2.1. Preparation of conidial suspension

Cultures of *M. anisopliae* strain SRRC 2558 were grown on potato dextrose agar (Difco, Becton–Dickson, Sparks, MD) at 27 or 30 °C in the dark for 10–19 days. Conidial suspensions were made by lightly scraping the fungal culture surface with a sterile "L" shaped stick (cell spreader) into a 60-ml plastic container. The conidial clumps were suspended in distilled water with 0.01% Tween 80 (ICI Americas, Norwich, NY). The suspensions were vortexed for 5 min on a Vortex-Genie 2 laboratory mixer (Daigger, Vernon Hills, IL) to dissociate conidial clumps. Then, the suspensions were filtered through one layer of cloth (36 × 36 threads/cm^2, threads of 0.3–0.4 mm thick) to remove conidial clumps and mycelial debris. Concentration of the suspensions was determined using a Neubauer hemocytometer under phase-contrast microscopy at 400 × magnification. Conidial suspensions were diluted to a range of 5 × 10^5–1 × 10^8 conidia/ml, depending on the experiment. Conidial suspensions were stored at 5 °C and were used on the same day of or the day after preparation. The pure fungal culture was deposited in the Fungal Collection, USDA ARS, Southern Regional Research Center, New Orleans, Louisiana as strain No. SRRC 2558.

2.2. Collection and preparation of termites

Reticulitermes flavipes workers and soldiers were collected from cardboard bait buried near trees in the field in Stoneville, Mississippi. *C. formosanus* workers and soldiers were collected by the same method in NewOrleans, Louisiana. The termite colonies were kept in large plastic containers (33.5 × 26.5 × 10.0 cm) in the laboratory at room temperature (25–28 °C) for 0–3 months. The containers were filled with pine wood stakes (*Pinus* sp.) and corrugated cardboard as termite food. One day prior to testing, termite workers older than third instar were counted and transferred to 9.0 × 1.5 cm plastic petri dishes filled with a wet filter paper. Dead and weak termites (identified by their slow movement) were replaced with healthy termites immediately before the test.

2.3. Experiment 1. Virulence of *M. anisopliae* strain SRRC 2558 against *R. flavipes* in petri dishes

The test was conducted in 9.0 × 1.5 cm plastic petri dishes with one 8.2 cm diameter filter paper (grade 1 Whatman, Clifton, NJ). The area of the bottom half of the dish is 58.1 cm^2. One milliliter of *M. anisopliae* conidial suspensions was applied to the filter paper by a pipet at the concentrations of 0, 4 × 10^6, 1 × 10^7, and 4 × 10^7 conidia/ml. The conidial suspensions were applied in 10–15 drops evenly distributed on the filter paper. Thirty termite workers were added to each petri dish. Each concentration was applied to four replicates, with each replicate representing a different colony of termites. *M. anisopliae* conidia were harvested from a 10-day-old fungal culture at 30 °C. Live termites were counted at 14 and 28 days.

2.4. Experiment 2. Virulence of *M. anisopliae* strain SRRC 2558 against *R. flavipes* in containers with treated vermiculite/sand medium

This test was performed to examine the virulence of *M. anisopliae* against *R. flavipes* in a condition similar to soil treatment in the field. The test was conducted in

round plastic containers of 5.1 cm diameter by 3.5 cm high. A southern yellow pine (*Pinus* sp.) block (1.9 1.9 1.9 cm) was put in each container. Then the container was filled with 30 cm^3 *M. anisopliae* treated medium. The medium was composed of vermiculite:sand:conidial suspension at 16:14:9 by volume. The concentrations of the *M. anisopliae* conidial suspensions were 0, 5 10^5, 1 10^6, 5 10^6, 1 10^7, and 5 10^7 conidia/ml equivalent to 0, 1.5 10^5, 3 10^5, 1.5 10^6, 3 10^6, and 1.5 10^7 conidia/cm^3 in the medium. These concentrations were chosen based on preliminary tests that showed the effective rates fell into this range. The medium was mixed with the *M. anisopliae* suspension thoroughly using a sterile plastic stick. One hundred *R. flavipes* workers were added to each container. Each treatment was replicated three times, with each replicate representing a different colony. *M. anisopliae* conidia were harvested from a 14-day-old culture at 30 °C. Termite mortality was recorded at 21 days.

2.5. Experiment 3. Repellency of M. anisopliae strain SRRC 2558 to R. flavipes in two-container choice device

The test device consists of two plastic containers of similar size (5.1 cm diameter 3.5 cm high), which were connected by a transparent flexible PVC tube (15 cm long, 1.3 cm outer diameter, 0.9 cm inner diameter). One container was filled with 30 cm^3 vermiculite/sand moistened with 9 ml of 0.01% Tween 80, and the other container was filled with 30 cm^3 medium mixed with 9 ml of the *M. anisopliae* suspension. Both containers in the control were filled with 0.01% Tween 80 moistened medium. The medium was prepared as in Experiment 2. Concentrations of *M. anisopliae* were 2 10^6, 1 10^7, 5 10^7, and 1 10^8 conidia/ml equivalent to 6 10^5, 3 10^6, 1.5 10^7, and 3 10^7 conidia/cm^3 in medium. Two hundred *R. flavipes* workers were added to the container filled with untreated medium at the test date. Each concentration was replicated three times with each representing a different colony. *M. anisopliae* conidia were harvested from 14-day-old culture at 30 °C. Location of the termites was checked every day until 61 days. Termite mortality was recorded at 61 days.

2.6. Experiment 4. Concentration response of R. flavipes to M. anisopliae strain SRRC 2558 treated cellulose bait

The test was conducted in round plastic containers (5.1 3.5 cm). The medium was moistened with distilled water. The food was a pine block (1.9 1.9 1.9 cm) at one side and 1 g (dry weight) cellulose bait at the opposite side of the bottom of the container. The bait was made by mixing cellulose powder (Sigma Chemical, St. Louis, MO) with *M. anisopliae* conidial suspension at 1:3 w/w. The final conidial concentrations in the bait were 3 10^6, 1.5 10^7, 3 10^7, and 1.5 10^8 conidia/g (dry weight). The cellulose in the control was mixed with 0.01% Tween 80. Sixty *R. flavipes* workers were added to each container. Each treatment was replicated three times, each replicate represented a different colony. *M. anisopliae* conidia were harvested from a 14-day-old culture at 30 °C. Termite mortality was recorded at 28 days.

2.7. Experiment 5. Concentration response of C. formosanus to M. anisopliae strain SRRC 2558 treated cellulose bait

Similar to Experiment 4, this experiment examines the response of *C. formosanus* to the bait treatment. The conidial concentrations in the bait were 1.5 10^7 and 3 10^7 conidia/g (dry weight). Eighty *C. formosanus* termites (72 workers and 8 soldiers) were added to each container. Each treatment was replicated three times using termites from the same colony. *M. anisopliae* conidia were harvested from a 10-day-old culture at 30 °C. Termite mortality was recorded at 21 days.

2.8. Experiment 6. Control of R. flavipes large groups with M. anisopliae strain SRRC 2558 treated cellulose bait

This experiment used much larger groups of termites than Experiment 4 to examine the efficacy of the cellulose bait formulation against large groups of termites. Large plastic containers of 15.5 cm diameter 4 cm high were filled with 2 cm high vermiculite/sand medium as described in Experiment 3. The medium was moistened with distilled water. A piece of aspen wood (2.5 1.7 0.5 cm) was put at the bottom near the side of the container. Cellulose bait was added on the top of the vermiculite/sand at the opposite side of the container on the test day (Fig. 1A). The cellulose bait was made by mixing 2.5 g cellulose and 7.5 ml *M. anisopliae* conidial suspension. The conidial concentrations in the bait were 3 10^7 and 1.5 10^8 conidia/g (dry weight). One thousand termites (estimated by weight) were put in each container 1 day before treatment. Each treatment was applied to four replicates, each representing a different colony. The soldier proportions of the four colonies were 0.8, 1.5, 0.5, and 2.5%. They were natural soldier ratios of the colonies kept in the laboratory. *M. anisopliae* conidia were harvested from a 19-day-old culture at 30 °C. Termite mortality was observed every 1–3 days until 60 days.

2.9. Experiment 7. Control of C. formosanus large groups with M. anisopliae strain SRRC 2558 treated cellulose bait

Preliminary tests showed that *M. anisopliae* SRRC 2558 was also very virulent to *C. formosanus*. Similar to Experiment 6, this experiment was intended to examine the efficacy of *M. anisopliae* against large groups of *C.*

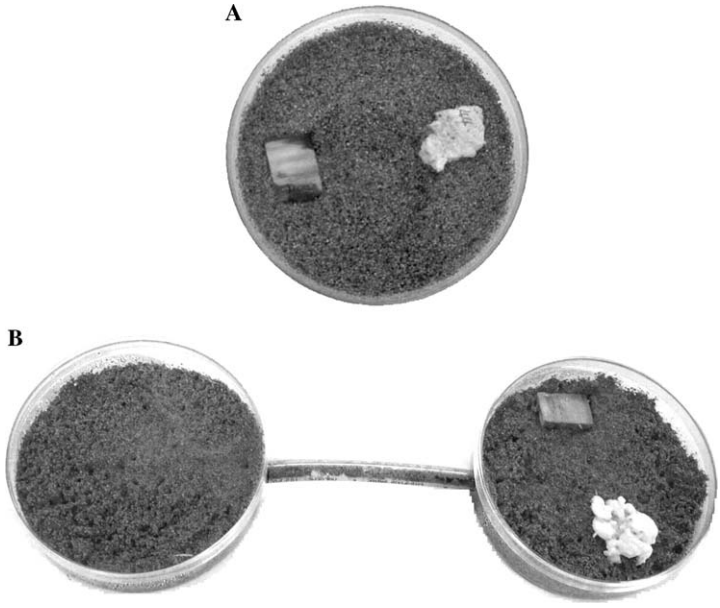

Fig. 1. Device for testing *Metarhizium anisopliae* bait against large groups of *Reticulitermes flavipes* (A) and *Coptotermes formosanus* (B).

formosanus. The test device was two large containers of 15.5 cm diameter by 4 cm high that were connected by a transparent flexible plastic tube (15 cm long, 1.3 cm outside diameter, and 0.9 cm inside diameter) (Fig. 1B). The containers were filled with 2 cm vermiculite/sand medium as described in Experiment 2. Cellulose bait and a piece of aspen wood (2.5 1.7 0.5 cm) were put in one of the containers. The water content in the cellulose bait was 73%. The conidia concentrations in the bait were 3 10^7, 1.5 10^8, or 3 10^8 conidia/g (dry weight). One thousand termites (estimated by weight) were put in each device (evenly distributed to the two containers) 2 days before treatment. Each treatment was applied to four replicates, each representing a different colony. The soldier proportions of the four colonies were 15, 9, 3, and 2%. *M. anisopliae* conidia were harvested from 13-day-old culture at 27 °C. Termite mortality was observed every 1–3 days until 32 days.

All experimental units were maintained in an incubator under dark at 25 °C and 82% RH.

2.10. Statistical analysis

Mortality data were transformed (arcsine of the square root) and analyzed using analysis of variance (ANOVA) for comparison among treatment concentrations. PROC MIXED in the SAS software was used (SAS Institute, 2000). Means were compared by the least significant difference (LSD) at $\alpha = 0.05$ after ANOVA (SAS Institute, 2000). Corrected mortality from fungal treatments was calculated using the formula by Abbott (1925). One-tailed tests were used for comparisons among different concentrations since higher concentrations are thought to cause higher mortality than lower concentrations.

3. Results

3.1. Experiment 1

Under direct contact with *M. anisopliae* strain SRRC 2558 in petri dishes, *R. flavipes* exhibited significantly mortality when compared with the control at the concentration of 4 10^7 conidia/ml ($P < 0.10$, LSD) at 14 days ($F = 11.2$; $df = 3, 9$; $P < 0.01$) and at 28 days ($F = 22.3$; $df = 3, 9$; $P < 0.01$) after treatment (Fig. 2). The corrected mortality at the concentration of 4 10^7 conidia/ml at 14 days was $58.2 \pm 16.2\%$ (mean $\pm$ SE). The concentration 1 10^7 conidia/ml only caused significant mortality at 28 days ($P = 0.06$, LSD). No significant mortality was detected at the treatment concentration of 4 10^6 conidia/ml ($P > 0.10$, LSD).

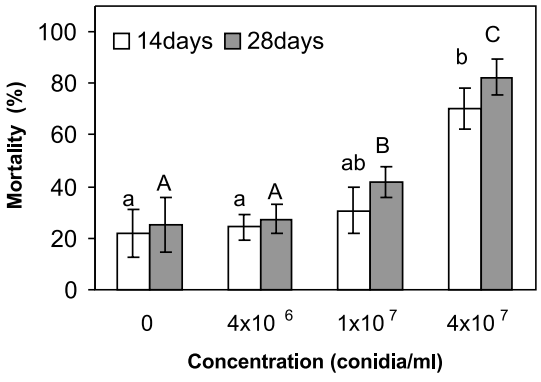

Fig. 2. Concentration mortality response of *Metarhizium anisopliae* strain SRRC 2558 against *Reticulitermes flavipes* in petri dishes. Bars with different same-case letters are significantly different ($P < 0.10$, LSD). Lower case letters indicate comparisons at 14 days. Upper case letters indicate comparisons at 28 days. Means are four colonies with 30 workers in each colony.

3.2. Experiment 2

Reticulitermes flavipes exposed to *M. anisopliae* strain SRRC 2558 treated vermiculite/sand medium suffered significant mortality at the concentration of $\geqslant 3 \quad 10^5$ conidia/cm^3 ($P < 0.10$, LSD) ($F = 19.5$; $df = 5, 10$; $P < 0.01$). One hundred percent mortality occurred at the concentrations of $3 \quad 10^6$ and $1.5 \quad 10^7$ conidia/cm^3 (Fig. 3).

3.3. Experiment 3

When *R. flavipes* were released into the untreated containers in the two-container choice devices, they immediately began to burrow into the medium. In the control, *R. flavipes* entered and occupied the second container (treated container) at 1–2 days (Table 1). The treatments $1.5 \quad 10^7$ and $3 \quad 10^7$ conidia/cm^3 caused apparent repellency to termites. The termites occupied the treated container as early as 4 days at the highest concentration ($3 \quad 10^7$ conidia/cm^3). The response of the three *R. flavipes* colonies toward the *M. anisopliae* treatment varied greatly in terms of duration of avoidance. Colony 3 avoided the treated container throughout the test period at the concentrations of $1.5 \quad 10^7$ and $3 \quad 10^7$ conidia/cm^3. No apparent repellency was found with the $3 \quad 10^6$ and $6 \quad 10^5$ conidia/cm^3 treatments. Although high mortality occurred in some colonies at the concentration of $\geqslant 3 \quad 10^6$ conidia/cm^3, there were no significant differences in termite mortality between the treatments and the control because of the high variance ($F = 0.8$; $df = 4, 10$; $P = 0.52$).

3.4. Experiments 4 and 5

When *R. flavipes* and *C. formosanus* were provided with a choice of treated cellulose bait and untreated wood, they did not avoid contacting and feeding on the cellulose bait in all of the treatments. At 4 days, dead termite bodies were seen on top of the media and at the bottom of the containers in the treatment $\geqslant 1.5 \quad 10^7$ conidia/g. *R. flavipes* suffered significant mortality at the concentration of $\geqslant 1.5 \quad 10^7$ conidia/g ($P < 0.10$, LSD) ($F = 6.6$; $df = 4, 8$; $P = 0.01$) (Fig. 4). One hundred percent *R. flavipes* mortality was achieved at the concentration of $\geqslant 3 \quad 10^7$ conidia/g. Similar to *R. flavipes*, *C. formosanus* suffered significant mortality at $1.5 \quad 10^7$ and $3 \quad 10^7$ conidia/g ($F = 23.3$; $df = 2, 9$; $P < 0.01$) (Fig. 5). The treatments caused 83.6 ± 13.8 and $99.2 \pm 0.7\%$ control mortality, respectively.

3.5. Experiments 6 and 7

Large groups of *R. flavipes* and *C. formosanus* exposed to cellulose bait containing *M. anisopliae* did not

Table 1
Repellency of *Metarhizium anisopliae* strain SRRC 2558 against *Reticulitermes flavipes* in two-container choice devices

Treatment concentration (conidia/cm^3)	Colony	Termite response to treated media		
		First day of entrance	First day of occupancy	Mortality at 61 days (%)
$3 \quad 10^7$	1	9	22	43
	2	1	4	100
	3	29	NA[a]	47
$1.5 \quad 10^7$	1	12	15	48
	2	15	19	21
	3	12	NA	99
$3 \quad 10^6$	1	4	8	98
	2	1	1	46
	3	2	5	28
$6 \quad 10^5$	1	1	2	35
	2	1	1	35
	3	1	1	30
Control	1	2	2	23
	2	2	2	42
	3	1	1	19

[a] Termites never occupied the treated container.

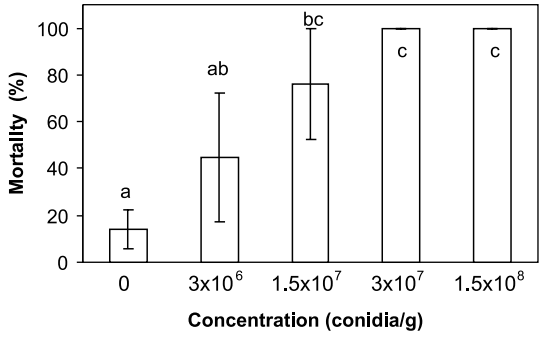

Fig. 4. Effect of *Metarhizium anisopliae* strain SRRC 2558 against *Reticulitermes flavipes* in containers with untreated wood and treated cellulose bait. Bars with different letters are significantly different ($P < 0.10$, LSD). Means are three colonies with 60 workers in each colony.

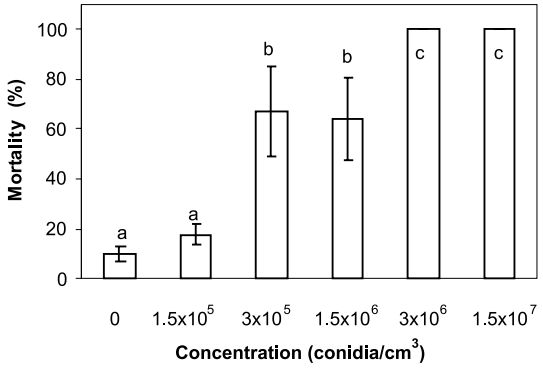

Fig. 3. Concentration mortality response of *Metarhizium anisopliae* strain SRRC 2558 against *Reticulitermes flavipes* in containers with treated vermiculite/sand medium. Bars with different letters are significantly different ($P < 0.10$, LSD). Means are three colonies with 100 workers in each colony.

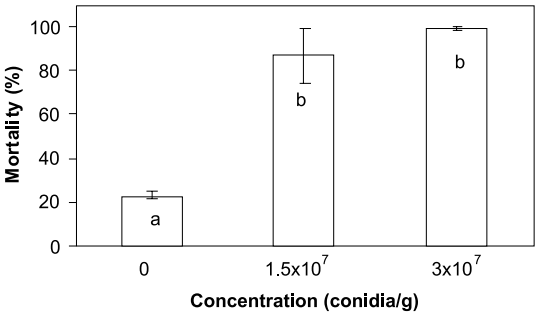

Fig. 5. Effect of *Metarhizium anisopliae* strain SRRC 2558 against *Coptotermes formosanus* in containers with untreated wood and treated cellulose bait. Bars with different letters are significantly different ($P < 0.10$, LSD). Means are three replicates with 80 workers and soldiers in each replicate.

exhibit apparent avoidance to the bait. Dead termites began to appear within 5 days after the treatment. They were either on top of the medium or buried in the medium. All of the four *R. flavipes* groups were killed at 1.5 10^8 conidia/g at 60 days after treatments. Their survival time ranged from 28 to 60 days with a mean survival time of 39.5 days. The lower concentration (3 10^7 conidia/g) only had $10.3 \pm 5.6\%$ control mortality (corrected as Abbott, 1925) ($P = 0.08$, LSD) ($F = 477.8$; $df = 2, 6$; $P < 0.01$). The mortality in the control was $32.1 \pm 0.4\%$. All of the four *C. formosanus* groups were killed at 3 10^8 conidia/g at 32 days after exposure. Their survival time ranged from 11 to 32 days with a mean survival time of 17.3 days. Significant *C. formosanus* mortality occurred at 1.5 10^8 conidia/g ($P = 0.004$, LSD) ($F = 16.6$; $df = 3, 11$; $P < 0.01$). No significant *C. formosanus* mortality occurred at 3 10^7 conidia/g ($P = 0.78$, LSD). The control group had an average mortality of $27.7 \pm 7.6\%$.

4. Discussion

Our study indicated that the efficacy of *M. anisopliae* against termites varied greatly under various test conditions. *M. anisopliae* was less effective against termites when they were provided with a choice of untreated medium (as in field conditions) by avoiding contact with the treated medium. Experimentation in non-choice containers with treated media indicated that *M. anisopliae* caused 100% mortality at the concentration of $\geqslant 3$ 10^6 conidia/cm^3, whereas experimentation in two-container choice tests caused high mortality to only one colony at 1.5 10^7 and 1.5 10^7 conidia/cm^3, which were clearly repellent to termites. From this study, we may speculate that treating soil with *M. anisopliae* will not likely eliminate termite colonies in the field through direct contact and spreading of *M. anisopliae* by termites in soil.

The use of palatable baits greatly increased the effectiveness of *M. anisopliae* against *R. flavipes*. In containers provided with both untreated wood and treated cellulose baits, termites readily foraged upon treated baits. Cellulose bait treatment caused 100% mortality to *R. flavipes* and *C. formosanus* groups at the concentrations of 1.5 10^8 and 3 10^8 conidia/g, respectively, without causing repellency to termites.

Cellulose bait containing virulent *M. anisopliae* conidia could eliminate termite groups under laboratory conditions. It took 32–60 days to kill the *R. flavipes* and *C. formosanus* groups (1000 per group), which is comparable to chitin synthesis inhibitor treatments. Rojas and Morales-Ramos (2001) showed that an average of 9 weeks was needed to kill the *C. formosanus* groups by cellulose bait containing chitin synthesis inhibitors (2500 per group).

To confirm that the termite mortality was indeed caused by the conidia, we tested the viability of conidia preparation. A sample stored at −15 °C for 2 weeks was inoculated and cultured in potato dextrose broth for 23 h at 30 °C. Sporulation rate was 16%. Because fresh conidia (<1-day-old) were used, the sporulation rates of the preparations should be higher than 16%. More data on the viability of fresh conidia would be helpful in explaining the virulence of the fungus strain.

The two experiments with cellulose bait demonstrated that larger groups of termites tend to be more resistant to fungal treatment, and therefore, are more likely to survive the treatment. This phenomenon was also found in a study by Rosengaus et al. (1998) on resistance of a dampwood termite, *Zootermopsis angusticollis* (Hagen), to fungal infections. The reason is that termite social interactions can effectively minimize deleterious stresses such as starvation, poisoning, and disease (Boucias et al., 1996; DeSouza et al., 2001). This has practical implications in the field of termite control. A *R. flavipes* or *C. formosanus* colony may have hundreds of thousands of termites and a large foraging area. It may take a long treatment period and many treatment sites to eliminate field colonies using *M. anisopliae*.

Most field studies failed to eliminate termite colonies by using fungal pathogens. These failed experiences had prevented the fungus from becoming a stand-alone termite treatment measure. Developing a palatable formulation with appropriate concentration is the key to improve its efficacy. With the study of improved bait formula and virulent strains, we hope to achieve better control of termite colonies and enable pathogens to become a useful element in the Integrated Pest Management system.

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References

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

Almeida, J.E.M., Alves, S.B., Pereira, R.M., 1997. Selection of *Beauveria* spp. isolates for control of the termite *Heterotermes tenuis* (Hagen 1858). J. Appl. Entomol. 121, 539–543.

Boucias, D.G., Stokes, C., Storey, G., Pendland, J.C., 1996. The effects of imidacloprid on the termite *Reticulitermes flavipes* and its interaction with the mycopathogen *Beauveria bassiana*. Pflanzenschutz-Nachrichten 49, 103–144.

Connick, W.J., Osbrink, W.L.A., Wright, M.S., Williams, K.S., Daigle, D.J., Boykin, D.L., Lax, A.R., 2001. Increased mortality of *Coptotermes formosanus* (Isoptera, Rhinotermitidae) exposed to eicosanoid biosynthesis inhibitors and *Serratia marcescens* (Eubacteriales: Enterobacteriaceae). Environ. Entomol. 30, 449–455.

Culliney, T.W., Grace, J.K., 2000. Prospects for the biological control of subterranean termites (Isoptera: Rhinotermitidae), with special reference to *Coptotermes formosanus*. Bull. Entomol. Res. 90, 9–21.

Delate, K.M., Grace, J.K., Tome, C.H.M., 1995. Potential use of pathogenic fungi in baits to control the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Appl. Entomol. 119, 429–433.

DeSouza, O., Miramontes, O., Santos, C.A., Bernardo, D.L., 2001. Social facilitation affecting tolerance to poisoning in termites. Insect. Soc. 48, 21–24.

Grace, J.K., 1997. Biological control strategies for suppression of termites. J. Agric. Entomol. 14, 281–289.

Hänel, H., Watson, J.A.L., 1983. Preliminary field tests on the use of *Metarhizium anisopliae* for the control of *Nasutitermes exitiosus* (Hill) (Isoptera: Termitidae). Bull. Entomol. Res. 73, 305–313.

Jones, W.E., Grace, J.K., Tamashiro, M., 1996. Virulence of seven isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Environ. Entomol. 25, 481–487.

Lai, P.Y., 1977. Biology and ecology of the Formosan subterranean termite, *Coptotermes formosanus*, and its susceptibility to the entomogenous fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. Ph.D. dissertation. University of Hawaii, Honolulu, HI.

Li, D., Zheng, J.-K., Zhao, Y., Huang, M.-Z., 1979. Pathogenicity of microbes on *Coptotermes formosanus* in laboratory studies. Entomol. Knowledge 16, 162–165 (in Chinese).

Milner, R.J., 2003. Application of biological control agents in mound building termites—experiences with *Metarhizium* in Australia. Sociobiology 41, 419–428.

Milner, R.J., Staples, J.A., 1996. Biological control of termites: results and experiences within a CSIRO project in Australia. Biocontrol Sci. Technol. 6, 3–9.

Milner, R.J., Staples, J.A., Lenz, J.A., Lutton, M., McRae, G.G., Watson, J.A.L., 1997. Insect pest control. US Patent #5,595,746.

Milner, R.J., Staples, J.A., Lutton, G.G., 1998. The selection of an isolate of the hyphomycete fungus, *Metarhizium anisopliae*, for the control of termites in Australia. Biol. Control 11, 240–247.

Neves, P.J., Alves, S.B., 1999. Associated control of *Cornitermes cumulans* (Kollar, 1832) (Isoptera: Termitidae) with *Metarhizium anisopliae*, *Beauveria bassiana* and imidacloprid. Sci. Agric. 56, 305–311.

Neves, P.J., Alves, S.B., 2000a. Selection of *Beauveria bassiana* (Bals.) Buill. and *Metarhizium anisopliae* (Metsch.) Sorok. strains for control of *Cornitermes cumulans* (Kollar). Brazilian Arch. Biol. Technol. 43, 373–378.

Neves, P.J., Alves, S.B., 2000b. Grooming capacity inhibition in *Cornitermes cumulans* (Kollar) (Isoptera: Termitidae) inoculated with entomopathogenic fungi and treated with imidacloprid. Ann. Soc. Entomol. Brazil 29, 537–545.

Osbrink, W.L.A., Williams, K.S., Connick Jr, W.J., Wright, M.S., Lax, A.R., 2001. Virulence of bacteria associated with the Formosan subterranean termite (Isoptera: Rhinotermitidae) in New Orleans, LA. Environ. Entomol. 30, 443–448.

Ramakrishnan, R., Suiter, D.R., Nakatsu, C.H., Humber, R.A., Bennett, G.W., 1999. Imidacloprid-enhanced *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) susceptibility to the entomopathogen *Metarhizium anisopliae*. J. Econ. Entomol. 92, 1125–1132.

Rath, A.C., 2000. The use of entomopathogenic fungi for control of termites. Biocontrol Sci. Technol. 10, 563–581.

Rojas, M.G., Morales-Ramos, J.A., 2001. Bait matrix for delivery of chitin synthesis inhibitors to the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94, 506–510.

Rosengaus, R., Maxmen, A., Coates, L., Traniello, J., 1998. Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). Behav. Ecol. Sociobiol. 44, 125–134.

Rosengaus, R.B., Lefebvre, M.L., Traniello, J.F.A., 2000. Inhibition of fungal conidial germination by *Nasutitermes*: evidence for a possible antiseptic role of soldier defensive secretions. J. Chem. Ecol. 26, 21–39.

Sajap, A.S., Atim, A.B., Husin, H., 1997. Isolation of *Conidiobolus coronatus* (Zygomycetes: Entomophthorales) from soil and its effect on *Coptotermes curvignathus* (Isoptera: Rhinotermithidae). Sociobiology 30, 257–262.

SAS Institute, 2000. SAS OnlineDoc, Version 8. SAS Institute, Cary, NC.

Smythe, R.V., Coppel, H.C., 1965. The susceptibility of *Reticulitermes flavipes* (Kollar) and other termite species to an experimental preparation of *Bacillus thuringiensis* Berliner. J. Invertebr. Pathol. 7, 423–426.

Snyder, T.E., 1954. Order Isoptera—The termites of the United States and Canada. National Pest Control Association, New York. 64 pp.

Staples, J.A., Milner, R.J., 2000. A laboratory evaluation of the repellency of *Metarhizium anisopliae* conidia to *Coptotermes lacteus* (Isoptera, Rhinotermitidae). Sociobiology 36, 133–148.

Su, N.-Y., Scheffrahn, R.H., 1988. Foraging population and territory of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment. Sociobiology 14, 353–359.

Wright, M.S., Lax, A.R., Henderson, G., Chen, J., 2000. Growth response of *Metarhizium anisopliae* to two Formosan subterranean termite nest volatiles, naphthalene and fenchone. Mycologia 92, 42–45.

Yendol, W.G., Paschke, J.D., 1965. Pathology of an entomophthora infection in the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). J. Invertebr. Pathol. 7, 414–422.

Zoberi, M.H., Grace, J.F., 1995. *Metarhizium anisopliae*, a fungal pathogen of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Mycologia 87, 354–359.