

Virulence of Seven Isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

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Environ. Entomol. 25(2): 481-487 (1996)

ABSTRACT Three strains of *Metarhizium anisopliae* (Metschnikoff) Sorokin and 4 strains of *Beauveria bassiana* (Balsamo) Vuillemin were evaluated for potential use as remedial control agents against *Coptotermes formosanus* Shiraki. The 7 isolates were screened for relative pathogenicity, and the median lethal time eliciting 50% mortality (LT₅₀) was calculated. In general, the *M. anisopliae* strains were more virulent, with lower LT₅₀ values, than were the *B. bassiana* strains. However, *B. bassiana* strain 787 also showed a high level of virulence. The LT₅₀ values ranged from 0.13 to 4.5 d. Determination of the median lethal concentration required to achieve 50% mortality (LC₅₀) with the remaining 6 isolates showed that *B. bassiana* strain 787 had the lowest LC₅₀ value but was closely followed by all 3 *M. anisopliae* strains. The LC₅₀ values for 4 of the isolates ranged from 33 to 40 conidia per termite. The isolates were tested for transmissibility and survivorship under simulated nest conditions. Termite workers dusted with dry conidia (6×10^5 to 2.4×10^7 conidia per termite) were capable of transmitting the pathogen to other colony members. The isolates differed in their inoculum potential, rate of mortality, and response by other workers to fungus-killed cadavers. However, all isolates showed the ability to grow, sporulate, and produce mycosis under these artificial conditions, indicating the potential for causing an epizootic in treated nests. Based on these results, *B. bassiana* strain 787 was thought to possess the highest potential as a remedial control agent for *C. formosanus* because of the moderately low LT₅₀ value of 2.9 d and the low LC₅₀ value of 33 conidia per termite in combination with its transmissibility and performance in nesting material.

KEY WORDS *Coptotermes formosanus*, *Metarhizium*, *Beauveria*, microbial control, entomopathogenic fungi, termite control

Coptotermes formosanus SHIRAKI is the most important economic pest in the Hawaiian Islands (Tamashiro et al. 1987) and a serious pest of structures worldwide in locations between 35° north and south latitude (Su and Tamashiro 1987). Although remedial control of the Formosan subterranean termite has traditionally relied upon the application of large quantities of soil insecticides beneath and around structures (Su and Scheffrahn 1990, Grace et al. 1993), alternatives such as physical barriers (Su et al. 1991b, Tamashiro et al. 1991), in situ wood treatments (Grace and Yamamoto 1992), and baits (Su et al. 1987) are of great interest.

Candidate baits for use against the Formosan subterranean termite include organic (Su et al. 1991a) and inorganic (Grace and Abdallay 1990, Grace 1991) insecticides, insect growth regulators (Haverty et al. 1989; Su 1994), and the fungal pathogens *Beauveria bassiana* (Balsamo) Vuille-

min and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Lai 1982, Hanel and Watson 1983). Bait efficacy depends upon successful delivery of a slow-acting control agent throughout the colony. Insect pathogens are attractive candidate baits because of their self-replicating nature and safety to nontarget animals. The mode of action of an entomopathogen is especially desirable because it is possible for a small amount of inoculum to be spread throughout a nest before detection, resulting in an epizootic. Social interactions (grooming and food sharing) are expected to disperse the inoculum (Kramm et al. 1982), and the confined area and the high relative humidity inside a subterranean colony are conducive to the growth and survival of pathogenic agents such as fungi (Ignoffo 1992).

However, typical termite behavior of isolating and avoiding abnormal colony members has been cited as a major impediment to the transfer of entomopathogens within the colony (Logan et al. 1990). Indeed, Kramm et al. (1982) and Zoberi and Grace (1990) found that when fungus-killed

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Table 1. Description and source of fungal isolates

Species	ARSEF no.	Source insect of isolate	Collection site (yr)
<i>Beauveria bassiana</i> (Balsamo) Vuillemin	787	Unknown sp. (Coleoptera: Tenebrionidae)	Barbalha, Ceara, Brazil (1982)
	1683	<i>Anomala cuprea</i> Hope (Coleoptera: Scarabaeidae)	Nagano, Japan (1980)
	3040	<i>Hypothenemus obscurus</i> (F.) (Coleoptera: Scolytidae)	Kona, Hawaii, USA (1990)
	3041	<i>Reticulitermes flavipes</i> (Kollar) (Isoptera: Rhinotermitidae)	Ontario, Canada (1988)
<i>Metarhizium anisopliae</i> (Metschnikoff) Sorokin	346	<i>Aphodius tasmaniae</i> Hope (Coleoptera: Scarabaeidae)	Australia (?)
	472	? <i>Dasygnathus</i> (Coleoptera: Scarabaeidae)	New South Wales, Australia (1980)
	2162	Chafer larva (Coleoptera: Scarabaeidae)	Papua, New Guinea (1986)

cadavers were introduced to laboratory groups of active *Reticulitermes flavipes* (Kollar) workers, limited conidial transfer because of termite avoidance and burial of the bodies in the substrate resulted in low mortality. Pereira and Stimac (1992) noted similar behavior by ants. However, vectoring of the pathogen was successful in similar laboratory tests using living fungal-infected *R. flavipes* workers (Kramm et al. 1982, Grace and Zoberi 1992).

Our study was initiated to evaluate the potential of 4 strains of *B. bassiana* and 3 strains of *M. anisopliae* as bait toxicants for remedial control of *C. formosanus*. Relative pathogenicity and virulence of all isolates was determined, along with their transmissibility and survivorship under simulated nesting conditions.

Materials and Methods

Fungal Isolates. Seven fungal isolates were selected based on their association with soil-borne insects, or geographic origin (Table 1). Two *B. bassiana* strains and 3 *M. anisopliae* strains were obtained from the USDA-ARS Collection of Entomopathogenic Fungi (ARSEF), USDA-ARS Plant Protection Unit, U.S. Plant Soil and Nutritional Laboratory, Ithaca, NY. Two other *B. bassiana* strains were also included in this study, 1 isolated locally in Hawaii and the other isolated in Canada from *R. flavipes*. Both of these isolates were sent to USDA-ARS to be cataloged as strain numbers 3040 and 3041, respectively. An additional strain of *Metarhizium* was isolated from *C. formosanus* in Hawaii but required confirmation of its identification before testing. This isolate was also sent to USDA-ARS, where it was cataloged as strain number 3045.

Preparation of Plate Cultures and Conidial Suspensions. Cultures of all 7 isolates were maintained on Sabouraud dextrose agar (Difco, Detroit, MI) supplemented with 0.5% yeast extract (Difco, Detroit, MI) (SDAY) at pH 6.8 and grown at 25°C in the dark. All isolates were periodically reisolated from termite cadavers to prevent any loss of virulence associated with extensive subculturing. To

determine pathogenicity of the fungi to *C. formosanus* workers, each isolate was cultured on SDAY for 14 d. Cultures (2 wk old) were chosen as a standard to ensure that all isolates had sufficient time to mature and sporulate.

Conidial suspensions used to determine the median lethal concentration for each isolate were prepared from 14-d-old SDAY plate cultures by lightly scraping the fungal surface with a sterile surgical blade. The loosened conidial clumps and mycelium were harvested and suspended in a solution of 3.0-mM KH_2PO_4 with 0.01% Tween 80. The suspension was first vortexed for 15 min to dissociate conidial clumps then filtered through sterile nylon organdy cloth (4 threads per millimeter) to remove the mycelial debris and any large clumps of conidia. Conidial concentrations in the filtrate were determined using a Neubauer hemocytometer under phase-contrast microscopy. Conidial suspensions were serially diluted to achieve a range of 1×10^4 – 1×10^7 conidia per millimeter. Each termite worker received 4.0 μl of an aliquot ranging in concentration from 1×10^2 to 2×10^5 conidia per termite.

Source of Termites. *C. formosanus* workers (pseudergates, or undifferentiated individuals older than 3rd instar) were collected from 3 active field colonies immediately before use in laboratory assays, using the trapping technique described by Tamashiro et al. (1973). Two of the colonies were located on the Manoa campus of the University of Hawaii. The 3rd colony was collected from a residential backyard in Kaneohe, HI.

Relative Pathogenicity Bioassay. Three replicates of 30 termite workers each were placed on standard plate cultures of each fungal isolate for a 10-min exposure period. A separate culture plate was used for each replicate. After exposure, each group of 30 termites was carefully removed from the culture plate and placed in a plastic petri dish (5.5 cm diameter) lined with a moistened Whatman No. 2 filter paper disk. Termites were incubated at 25°C in the dark. Mortality was recorded on 1, 2, 3, 4, and 8 d after exposure.

Dosage Mortality Assessment. Whatman No. 2 filter paper disks (6 mm diameter) were placed in the bottom of each well of several 96-well flat bottom polystyrene microtiter plates (Cat. No. 001-012-9050, Dynatech, Chantilly, VA). A 4- μ l aliquot of the appropriate suspension concentration (or control suspension solution) was applied by micropipette to the dorsal surface of each termite at the base of the head. Conidial suspensions were prepared as previously stated. The experimental design was blocked by termite colony, with 3 colonies \times 3 replicates of 8 termite workers for each conidial concentration per fungal isolate. Treated termites were held in the dark at 25°C. Resulting mortality was recorded daily for 8 d.

Survival of Fungi on Termite Nest Material. *C. formosanus* workers fill voids with nest material or carton, consisting of masticated wood fibers, soil particles, saliva, and fecal material. Carton material was collected from termite traps to determine if it could support fungal pathogen growth. Carton was finely ground by mortar and pestle then sterilized by autoclaving. Termite cadavers produced by each of the fungal strains were placed individually in 5.5-cm glass petri dishes (3 replicates per strain) containing 10 g each of moistened sterile crushed carton and incubated at 25°C in the dark for 14 d. At the end of that period, the dishes were examined for fungal growth and conidial production. A virulence bioassay was performed by placing 30 termites in each dish for 3 h. They were then removed to a clean petri dish lined with moistened filter paper as for the pathogenicity bioassay and held at 25°C in the dark. Mortality was assessed after 8 d.

Transmission of Conidia by Termite Workers. The conidial transmission assay was similar to that used by Grace (1991) to evaluate the toxicity of borate powders. Test vessels consisted of 45-ml polystyrene vials partially filled with 10 g of sieved and autoclaved clay soil moistened with 2–3 ml distilled water. A short length of wood tongue depressor (1.5 by 2.5 cm; Puritan No. 25-705, hardwood, Guilford, ME) was included as a food source for the termites. Conidia from standard plate cultures of each *M. anisopliae* strain were aspirated, using a vacuum pump, into sterile 15-ml polystyrene test tubes (Falcon No. 2057, Becton Dickinson, Lincoln Park, NJ). Conidia were harvested from *B. bassiana* cultures by scraping the surface of the plates with a sterile scalpel blade, oven-dried for 30 min at 30°C to minimize clumping, then placed into similar 15-ml test tubes. The different harvesting methods were required because of the difference in sporulation characteristics of the 2 fungal species; *M. anisopliae* conidia grow in thick dry clumps that detach readily upon maturation, *B. bassiana* conidiphores are clustered in tight hydrophilic packets requiring either mechanical disturbance or slight desiccation to dissociate them. Inoculation occurred by placing 10 *C. formosanus* workers in a

test tube containing conidia, gently rolling the tube on its side to coat the termites with the conidial dust, then allowing the workers to walk out of the tube into a collection dish. The inoculated workers were then allowed to walk into a 2nd collection dish before they were added to the assay vials. This procedure was performed to minimize transfer of loose conidia.

Each assay vial contained a total of 100 termite workers, of which either 0 (controls), 10, 20, or 100% were inoculated with conidia. Five replicates of each inoculation population density were used for each fungal isolate. The uninoculated individuals in each group were added to the vials 4 h before adding the inoculated workers. This was to allow the termites to acclimate and begin normal tunneling behavior in the soil provided. Worker mortality was recorded after 16 d incubation at 25°C in the dark.

The average amounts of conidia per termite were determined by vortexing 5 groups of 10 inoculated workers for each isolate in a sterile test tube containing 1 ml of 3.0-mM KH_2PO_4 plus 0.01% Tween 80. Aliquots were serially diluted as necessary, and conidia were counted using a Neubauer hemacytometer. Mean conidia concentrations represent the averages of 5 counts per tube of 5 replicates per strain (25 counts per treatment).

Statistical Analysis. All proportional mortality data taken were transformed by the arcsine of the square root and analyzed using analysis of variance (ANOVA), and means were separated by Ryan-Einot-Gabriel-Welsch multiple F test at ≤ 0.05 (SAS Institute 1987). Dosage–mortality data were probit-transformed to determine the median lethal concentrations (LC_{50}) by day for each isolate, and the median lethal time (LT_{50}) for all effective dosages using probit analysis (SAS Institute 1987). No significant differences were found between the 3 termite colonies used to test for fungal pathogenicity and dosage mortality. Therefore, data for all colonies were pooled.

Results and Discussion

The initial screening for pathogenicity of all 7 isolates indicated that all 3 *M. anisopliae* strains and 3 of the 4 *B. bassiana* strains caused high termite mortality (83–100%) by day 8 (Table 2). Exposure to *B. bassiana* strain 1683 elicited significantly lower mortality ($12.4 \pm 7.9\%$) along with an extremely long estimated median lethal time ($\text{LT}_{50} = 20.8$ d), eliminating this strain from further consideration as a control agent. Calculations of LT_{50} s for the 6 remaining isolates reflect that, in general, the *M. anisopliae* strains elicited quicker mortality (0.13–2.0 d) than did the *B. bassiana* strains (2.1–4.5 d). These results are supported by previous work by Lai et al. (1982), who found a similar pattern of activity with several isolates of these 2 fungal pathogens. Regression equations generated from probit transformed mortality data (Table 2)

Table 2. Mean percentage mortality after 8 d and median lethal time (LT₅₀) calculated from workers exposed for 10 min to 14-d-old cultures

Fungal species	ARSEF strain no.	% Dead at day 8 (± SD)	LT ₅₀	Limits (d)	Regression equation ^a
<i>B. bassiana</i>	787	99.5 ± 1.3ab	2.9	— ^b	$m = 2.96 + 6.44t$
	1683	12.4 ± 7.9c	20.8	13.6–53.1	$m = 3.09 + 2.35t$
	3040	83.3 ± 23.3b	4.5	1.6–1 × 10 ¹⁷	$m = 2.58 + 3.96t$
	3041	94.4 ± 13.6ab	2.1	— ^c	$m = 1.08 + 3.37t$
<i>M. anisopliae</i>	346	100 ± 0a	<0.13	— ^d	—
	472	100 ± 0a	1.64	0.9–2.4	$m = 1.28 + 5.90t$
	2162	100 ± 0a	2.0	1.8–2.3	$m = 1.45 + 4.65t$

Within a column, means followed by the same letter are not significantly different ($P = 0.05$).

^a m , Probit-transformed mortality; t , time in days.

^b Significantly heterogeneous ($\chi^2 = 161.6$, $P < 0.0001$).

^c Significantly heterogeneous ($\chi^2 = 85.9$, $P < 0.0001$).

^d Mortality reached 100% in <3 h.

indicate that mortality by *B. bassiana* strain 787 increases the most with time. This observation, in conjunction with the high resulting mortality, marks this strain as a strong candidate for producing an epizootic.

Metarhizium anisopliae strain 346 caused 100% worker mortality in only 3 h following exposure to sporulating plate cultures, suggesting the presence of a potent exotoxin produced by the fungus. Both fungal species are reported to produce exoproteases with insecticidal activity—the cyclic peptide beauverin in *B. bassiana* (Hamill et al. 1969) and the destruxin family of cyclic peptides in *M. anisopliae* (Pais et al. 1981). However, chemotoxicity may be a mortality factor only when conidial concentrations are high. Termite workers in our 1st experiment were so heavily coated with conidia from *M. anisopliae* strain 346 that they could no longer walk freely.

Topical application of conidia suspensions was performed to assess the concentrations required to cause infection and mortality more accurately. Results of the probit-transformed mortality data obtained from applying a known amount of conidia per termite were used to determine the LC₅₀ for each isolate (Table 3). Values for LC₅₀ reflect the relative virulence of each isolate. *B. bassiana* strain 787 had the lowest LC₅₀, indicating that it is the most virulent at low conidial concentrations. This value is closely followed by all *M. anisopliae* strains, although strain 346 produced highly vari-

able results across the range of doses tested. The remaining 2 strains of *B. bassiana*, strains 3040 and 3041, required ≈4.5 times more conidia to produce the same level of pathogenesis. However, subsequent topical application results using 3041 by Wells et al. (1995) indicated that this strain is capable of producing higher mortality.

The differences observed in fungus-induced mortality with 3 equivalent conidial doses (Table 4) illustrate further the general trends for the isolates. At the lowest concentration, only 2 isolates elicited greater than 80% mortality—*B. bassiana* strain 787 (81.9%) and *M. anisopliae* strain 2162 (91.7%). The levels of mortality for remaining isolates ranged from 44.4 to 72.2% and were not statistically different. As expected, there was an increase in mortality with increasing conidial concentration. However, 2 *B. bassiana* strains (3040 and 3041) showed the strongest positive correlation between mortality and dose. The remaining isolates demonstrated high levels of mortality with the midrange conidial concentrations.

Figure 1 depicts the relationship between conidial concentration and time required to achieve 50% mortality. In this graph, a steeper slope reflects a stronger correlation between dose and time; that is, the higher the concentration, the less time required to reach the LT₅₀. Conversely, the isolates showing flatter lines are more time-dependent, requiring more time to become lethal. For a fungal pathogen to be successful in producing an epizo-

Table 3. Median lethal concentration (LC₅₀) of conidia calculated from termites 8 d after topical application of fungal suspensions

Fungal species	ARSEF strain no.	LC ₅₀ ^a	Limits (conidia)	Regression equation ^b
<i>B. bassiana</i>	787	33	11 – 81	$m = 2.98 + 1.33c$
	3040	146	6 – 5 × 10 ³	$m = 3.31 + 0.78c$
	3041	157	46 – 472	$m = 2.53 + 0.91c$
<i>M. anisopliae</i>	346	39	— ^c	$m = 3.18 + 1.14c$
	472	40	1.4– 1.8	$m = 2.90 + 1.31c$
	2162	36	24 – 51	$m = 2.75 + 1.45c$

^a Concentrations are in number of conidia per termite.

^b m , Probit transformed mortality; c , Concentration.

^c Significantly heterogeneous ($\chi^2 = 122.7$, $df = 5$, $P < 0.001$).

Table 4. Mean percentage mortality of termite workers 8 d after inoculation with 3 equivalent conidial concentrations for each isolate

Fungal species	ARSEF strain no.	Mean % mortality \pm SD ^a		
		2×10^2	1×10^3	2×10^5
<i>B. bassiana</i>	787	81.9 \pm 12.7ab	95.8 \pm 7.4a	100 \pm 0a
	3040	44.4 \pm 25.1c	70.8 \pm 15.3c	97.9 \pm 4.8a
	3041	57.6 \pm 22.3bc	81.9 \pm 18.9b	99.3 \pm 2.9a
<i>M. anisopliae</i>	346	52.8 \pm 25.6c	93.3 \pm 13.8ab	98.6 \pm 4.0a
	472	72.2 \pm 22.3bc	97.2 \pm 9.1a	100 \pm 0a
	2162	91.7 \pm 10.8a	100 \pm 0a	100 \pm 0a

Within a column, means followed by the same letter are not significantly different ($P = 0.05$).

^a Concentrations are in number of conidia per termite.

otic, it should cause mortality after a reasonable lag period to allow the maximum number of contacts with the infected carrier before morbidity, and it should remain effective at decreasing doses (Carruthers and Soper 1987). In other words, a worker that has picked up conidia must be able to travel through the colony, where it encounters other individuals before morbidity. It must then engage in grooming behavior with these other individuals, who then become infected. From this point, a slow geometric progression of infectious contact would occur with the concentration of inoculum decreasing. From these criteria, the leading epizootic candidates are *B. bassiana* strain 787 and all 3 *M. anisopliae* strains. However, results of *M. anisopliae* strains 346 and 2162 at increasing doses showed significantly heterogeneous data with chi-square values at 434 and 125, respectively. This may indicate some type of instability in the conidial population or an erratic response to the exotoxins produced by these strains. This makes both of the strains unreliable for epizootic purposes.

Our pathogenicity tests were conducted under completely artificial conditions. Therefore, to gain more insight into pathogenic potentials in the field, a final test was conducted to see if the isolates could use the carton nesting material as a growth medium and retain their virulence. Both *B. bassi-*

ana and *M. anisopliae* commonly infect soil-dwelling insects (Storey and McCoy 1992), although at the time of this study, only *B. bassiana* had been isolated and positively identified from subterranean termites (Zoberi and Grace 1990). All 6 isolates tested grew and proliferated in the crushed carton and produced significantly greater mortality in termite workers than did the unexposed controls ($10 \pm 0.01\%$). However, the percentage mortality caused by each of the isolates was low, ranging from 30 to 57%. Mortalities among treatments were not significantly different from each other ($P = 0.05$).

Although isolates grown on termite carton material did not differ in the amount of mortality produced, differences in fungal growth patterns and termite responses to fungal-killed cadavers were apparent. *B. bassiana* strains 787 and 3040 and *M. anisopliae* strain 472 produced a great deal of mycelial growth throughout the carton, whereas the growth of *B. bassiana* strain 3041 and *M. anisopliae* strains 346 and 2162 was restricted to the area immediately surrounding the cadavers. Termite workers showed no adverse behavioral response to cadavers killed by *B. bassiana* strains 787 and 3040, leaving them in the open, while all other cadavers were buried in the substrate. Isolation of infective cadavers and unhealthy colony members is an effective defense mechanism in termites (Kramm et al. 1982, Zoberi and Grace 1990). Absence of such a behavioral response to the 2 *B. bassiana* strains should encourage the spread of the pathogen throughout the termite gallery system, leading to epizootis.

Regardless of the low level of mortality, the results were encouraging enough to proceed with testing for epizootic activity under different population densities. Termite workers coated with conidial dusts carried from 6×10^5 to 2.4×10^7 conidia per termite, demonstrating a high inoculum potential (Table 5). At the population exposure level of 1 in 10, >80% mortality resulted from *B. bassiana* strains 787 and 3041 and *M. anisopliae* strain 346. The remaining isolates achieved <50% control. When the ratio was increased to 2 in 10, the same 3 isolates stood out with $\geq 98\%$ mortality, and the remaining isolates were still significantly lower. Similar mortalities have been reported for

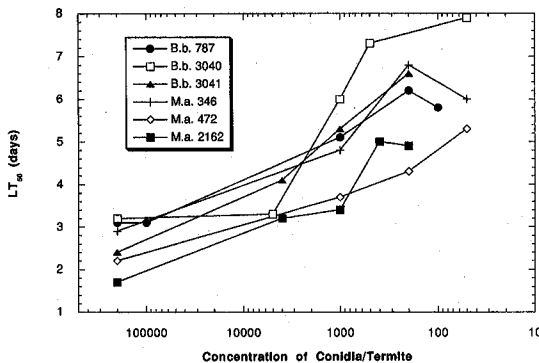


Fig. 1. Median lethal time required to achieve 50% mortality (LT_{50}) of *C. formosanus* workers following topical application of different concentrations of conidia from isolates of *B. bassiana* (B.b.) and *M. anisopliae* (M.a.).

Table 5. Mean percentage mortality of *C. formosanus* workers 16 d after inoculation of a portion of the test population with conidial dust

Fungal species	ARSEF strain no.	Conidia per termite	% Mortality \pm SD for each density ^a		
			1:10	2:10	10:10
<i>B. bassiana</i>	787	2.4×10^7	94 \pm 7a	98 \pm 4a	100 \pm 0a
	3040	8.0×10^6	40 \pm 9b	49 \pm 12b	100 \pm 0a
	3041	7.2×10^6	84 \pm 18a	100 \pm 0a	100 \pm 0a
<i>M. anisopliae</i>	346	1.4×10^6	89 \pm 24a	100 \pm 0a	100 \pm 0a
	472	8.3×10^5	43 \pm 18b	61 \pm 14b	100 \pm 0a
	2162	6.0×10^5	38 \pm 13b	65 \pm 25b	100 \pm 0a

Within a column, means followed by the same letter are not significantly different ($P = 0.05$). Mean \pm SD% mortality in uninoculated controls was 10 ± 4 for *B. bassiana* and 6 ± 1 for *M. anisopliae*.

^a Ratios indicate the number of inoculated individuals per total number in each test population.

R. flavipes workers treated with zinc borate dust where 10% of the population were dusted (Grace and Abdallay 1990). However, the fungal-induced mortality in this study greatly exceeded the mortality reported for *C. formosanus* workers dusted with borate powders (Grace 1991). This indicates that conidia may be transmitted more readily than insecticidal dusts among individuals of this species.

For a potential termite bait, high activity at a low concentration, low variability in termite responses, and delayed toxicity are desirable characteristics. Based on these criteria, *B. bassiana* strain 787 showed the most promise and *B. bassiana* strain 1683 and *M. anisopliae* strain 346 the least among the isolates evaluated. The ability to proliferate and maintain virulence when grown in the the habitat of the host is also highly desirable (Ignoffo 1992). Although Fernandes (1991) was able to eradicate colonies of *Cornitermes cumulans* (Kollar) by inoculating termite mounds with large quantities of *B. bassiana* and *M. anisopliae* conidia, delivery of a sufficient quantity of inoculum to the large and diffuse gallery systems associated with rhinotermitid colonies could be extremely difficult (Grace 1992). However, our study demonstrates that there are differences within strains of the same fungal species and between species in relative virulence, time required for mortality, and induction of defensive avoidance behavior in *C. formosanus* workers. Preferential isolates can be identified by a combination of bioassays and field testing. Our current results suggest that *B. bassiana* strain 787 may be a particularly promising candidate for use in *C. formosanus* baiting systems. Research is continuing with this and other fungal isolates for Formosan subterranean termite control.

Acknowledgments

We are grateful to R. Yamamoto, C. Arakaki, and E. Recel (University of Hawaii) for their hard work and technical assistance; and to L. LeBeck, M. Cornelius, C. Clement (University of Hawaii), and N. Reimer (Hawaii State Department of Agriculture) for reviewing the manuscript. This study was supported by USDA-ARS Specific Cooperative Agreements 58-6615-9-012 and 58-

6615-4-037. This is Journal Series No. 4113 of the Hawaii Institute of Tropical Agriculture and Human Resources.

References Cited

- Bao, L. -L., and W. G. Yendol. 1971. Infection of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) with the fungus *Beauveria bassiana* (Balsamo) Vuill. Entomophaga 16: 343-352.
- Carruthers, R. I., and R. S. Soper. 1987. Fungal diseases, pp. 357-416. In J. R. Fuxa and Y. Tanada [eds.], Epizootiology of insect diseases, Wiley, New York.
- Fernandes, P. M. 1991. Controle microbiano de *Cornitermes cumulans* (Kollar, 1832) utilizando *Beauveria bassiana* (Bals.) Vuill. e *Metarhizium anisopliae* (Metsch.) Sorok. Ph.D. dissertation, ESALQ—University de Sao Paulo, Piracicaba, Brazil.
- Grace, J. K. 1991. Response of eastern and Formosan subterranean termites (Isoptera: Rhinotermitidae) to borate dust and soil treatments. J. Econ. Entomol. 84: 1753-1757.
1992. Termite distribution, colony size, and potential for damage, pp. 67-76. In W. H. Robinson [ed.], Proceedings of the National Conference on Urban Entomology, College Park, MD.
- Grace, J. K., and A. Abdallay. 1990. Termiticidal activity of boron dusts (Isoptera: Rhinotermitidae). J. Appl. Entomol. 109: 283-288.
- Grace, J. K., and R. T. Yamamoto. 1992. Termiticidal effects of a glycol borate wood surface treatment. Forest Prod. J. 42(11/12): 46-48.
- Grace, J. K., J. R. Yates, M. Tamashiro, and R. T. Yamamoto. 1993. Persistence of organochlorine insecticides for Formosan subterranean termite (Isoptera: Rhinotermitidae) control in Hawaii J. Econ. Entomol. 86: 761-766.
- Grace, J. K., and M. H. Zoberi. 1992. Experimental evidence for transmission of *Beauveria bassiana* by *Reticulitermes flavipes* workers (Isoptera: Rhinotermitidae). Sociobiology 20: 23-28.
- Hamill, R. L., C. E. Higgins, H. E. Boaz, and M. Gorman. 1969. The structure of beauvericin, a new depsipeptide antibiotic toxic to *Artemia salina*. Tetrahedron Lett. 49: 4255-5258.
- Hanel, H., and J.A.L. Watson. 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.
- Haverty, M. I., N.-Y. Su, M. Tamashiro, and R. T. Yamamoto. 1989. Concentration-dependent pro-soldier induction and feeding deterrence: potential of

- two insect growth regulators for remedial control of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 82: 1370-1374.
- Ignoffo, C. M. 1992.** Environmental factors affecting persistence of entomopathogens. *Fla. Entomol.* 75: 516-525.
- Kramm, K. R., D. F. West, and P. G. Rockenbach. 1982.** Termite pathogens: transfer of the entomopathogen *Metarhizium anisopliae* between *Reticulitermes* sp. termites. *J. Invertebr. Pathol.* 40: 1-6.
- Lai, P. Y., M. Tamashiro, and J. K. Fujii. 1982.** Pathogenicity of six strains of entomogenous fungi to *Coptotermes formosanus*. *J. Invertebr. Pathol.* 39: 1-5.
- Logan, J.W.M., R. H. Cowie, and T. G. Wood. 1990.** Termite (Isoptera) control in agriculture and forestry by non-chemical methods: a review. *Bull. Entomol. Res.* 80: 309-330.
- Pais, M., B. C. Das, and P. Ferron. 1981.** Depsipeptides from *Metarhizium anisopliae*. *Phytochemistry (Oxf.)* 20: 715-723.
- Pereira, R. M., and J. L. Stimac. 1992.** Transmission of *Beauveria bassiana* within nests of *Solenopsis invicta* (Hymenoptera: Formicidae) in the laboratory. *Environ. Entomol.* 21: 1427-1432.
- SAS Institute. 1987.** SAS/STAT guide for personal computers, version 6 ed. SAS Institute, Cary, NC.
- Storey, G. K., and C. W. McCoy. 1992.** Potential for biological control of soil insects using microbial pesticides in the Caribbean. *Fla. Entomol.* 75: 533-539.
- Su, N.-Y. 1994.** Field evaluation of hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 87: 389-397.
- Su, N.-Y., and R. H. Scheffrahn. 1990.** Comparison of eleven soil termiticides against the Formosan subterranean termite and eastern subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 83: 1918-1924.
- Su, N.-Y., and M. Tamashiro. 1987.** An overview of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the world, pp. 3-14. *In* M. Tamashiro and N.-Y. Su [eds.], *Biology and control of the Formosan subterranean termite*. Hawaii Institute of Tropical Agriculture and Human Resources Research and Extension Series 083. University of Hawaii, Honolulu.
- Su, N.-Y., M. Tamashiro, and M. I. Haverty. 1987.** Characterization of slow-acting insecticides for the remedial control of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 80: 1-4.
- Su, N.-Y., P. M. Ban, and R. H. Scheffrahn. 1991a.** Population suppression of field colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae) by dihaloalkyl arylsulfone (A-9248) baits. *J. Econ. Entomol.* 84: 1525-1531.
- Su, N.-Y., R. H. Scheffrahn, and P. M. Ban. 1991b.** Uniform size particle barrier: a physical exclusion device against subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 84: 912-916.
- Tamashiro, M., J. K. Fujii, and P. Y. Lai. 1973.** A simple method to observe, trap and prepare large numbers of subterranean termites for laboratory and field experiments. *Environ. Entomol.* 2: 721-722.
- Tamashiro, M., J. R. Yates, and R. H. Ebesu. 1987.** The Formosan subterranean termite in Hawaii: problems and control, pp. 15-22. *In* M. Tamashiro and N.-Y. Su [eds.], *Biology and control of the Formosan subterranean termite*. Hawaii Institute of Tropical Agriculture and Human Resources Research and Extension Series 083. University of Hawaii, Honolulu.
- Tamashiro, M., J. R. Yates, R. T. Yamamoto, and R. H. Ebesu. 1991.** Tunneling behavior of the Formosan subterranean termite and basalt barriers. *Sociobiology* 19: 163-170.
- Wells, J. D., J. R. Fuxa, and G. Henderson. 1995.** Virulence of four fungal pathogens to *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *J. Entomol. Sci.* 30: 208-215.
- Zoberi, M. H., and J. K. Grace. 1990.** Isolation of the pathogen *Beauveria bassiana* from *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiology* 16: 289-296.

Received for publication 9 November 1994; accepted 2 November 1995.