Virulence of Four Fungal Pathogens to *Coptotermes formosanus* (Isoptera: Rhinotermitidae)[1]

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ABSTRACT Four species and 10 isolates of entomogenous fungi were tested for virulence against the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Five isolates of *Beauveria bassiana* (Balsamo) Vuillemin and three of *Metarhizium anisopliae* (Metschnikoff) Sorokin were virulent to *C. formosanus.* The isolates of these two fungal species were chosen because they originated from different phylogenetic groups of host insects. The *B. bassiana* isolates originated from hosts in the Orthoptera, Lepidoptera, Homoptera, Dermaptera, and Isoptera; the *M. anisopliae* isolates came from hosts in the Coleoptera, Isoptera, and Homoptera. The *B. bassiana* isolate from an isopteran was most virulent to *C. formosanus,* when LD_{50} and mean time until death were both considered. There was no other pattern in virulence of isolates relative to the phylogeny of their original insect hosts. All five *B. bassiana* isolates and one of the three *M. anisopliae* isolates produced full growth of external mycelium and conidia on the dead termites. Termites exposed to fungal doses killing > 67% of the insects died more quickly than those exposed to doses killing < 67%. *Metarhizium flavoviride* Gams and Rozsypal was pathogenic to *C. formosanus* but had low virulence (1% infection in bioassays). Termites that walked across a hyphal mat of *Conidiobolus coronatus* (Constantin) Batko suffered 100% mortality within 2 d, but this fungus did not produce conidia in culture on media.

KEY WORDS Formosan subterranean termite, *Coptotermes formosanus, Beauveria bassiana, Metarhizium anisopliae, Metarhizium flavoviride, Conidiobolus coronatus,* fungi, entomogenous, virulence.

Subterranean termites (Rhinotermitidae) are promising candidates for control with entomogenous fungi. Their social behavior, preference for humid conditions, and contact with soil are factors that favor fungal pathogens. Termites are among our most costly insect pests (Su 1994). Entomogenous fungi are safe to humans and other non-target organisms (Laird et al. 1990), which is important in the urban setting in which termites often are pests. There have been several laboratory tests of fungal pathogenicity and virulence to termites (e.g., Boa and Yendol 1971, Hänel 1982, Kramm and West 1982, Lai et al. 1982), and there has been limited success in initiation of epizootics in field populations (Hänel and Watson 1983).

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The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, has successfully invaded new regions and produced major structural damage around the world (Su and Tamashiro 1987). Within the last few decades, well-established populations have been found scattered across the southeastern USA (Su and Tamashiro 1987), and a colony was recently discovered in southern California (Atkinson et al. 1993). Pathogens such as fungi might be useful against *C. formosanus* because they have the potential to spread within termite colonies. If *C. formosanus* has invaded a structure with a moisture source, then it is unlikely to be eliminated with a soil-insecticide barrier treatment; costly fumigation also is necessary. However, a fungus introduced to eliminate termites inside the structure, coupled with a soil insecticide barrier to terminate further invasion and contact with the unusually extensive colonies of *C. formosanus* in soil (King and Spink 1969), has the potential to be an effective control measure. Lai et al. (1982) measured the virulence of *Beauveria* spp. and *Metarhizium anisopliae* (Metschnikoff) Sorokin against *C. formosanus* in Hawaii, but they tested only one isolate of *B. bassiana* (Balsamo) Vuillemin and were unable to detect significant differences among fungal isolates.

Our purpose was to determine the virulence of four fungal pathogens to *C. formosanus* and to determine whether *B. bassiana* and *M. anisopliae* isolates from phylogenetically different host insects differed in their virulence toward *C. formosanus*. The long-term goal of the research was to select fungal isolates for possible further development as microbial insecticides.

Materials and Methods

Four species and 10 isolates of entomopathogenic fungi were used in the experiment (Table 1). Nine of the isolates were obtained from the USDA-ARS Collection of Entomopathogenic Fungi (Ithaca, NY). One isolate, C 56096, was from the American Type Culture Collection (Rockville, MD). The termites were collected near Lake Charles, LA, and kept in a container with moist vermiculite and cardboard for approximately 11 months prior to testing.

Our techniques were adapted from those of Lai et al. (1982). The fungi were grown on Sabouraud dextrose agar + yeast at 26.7°C, and sterile conditions were maintained throughout the preparation of suspensions. Conidia were harvested by flooding the plate with distilled H_2O and then scraping the colony with forceps. A suspension was formed by stirring the conidia for 20 min in 250 ml 0.005% Triton X-100 and distilled H_2O, which was then filtered through cheese cloth. The concentration of conidia in each suspension was determined with a counting chamber (Petroff-Hausser, Hausser Scientific Partnership, Horsham, PA). All suspensions were stored at -6° until use. Viability of conidia at the time of treatment was measured by mixing a drop of suspension with a drop of Sabouraud broth on a microscope slide and incubating under high humidity for 24 h at room temperature. All suspensions displayed > 98% germination of conidia.

For the bioassay, termites were anesthetized with CO_2, and 1 µl of suspension was applied to the ventral surface of each with a microdispenser (Drummond Scientific Co., Broomall, PA). The bioassay of each fungal isolate included six fungal doses plus a control. Three replicates of 10 insects each

Table 1. Origins of the fungal isolates.

Fungal species	Isolate number*	Original host species	Host order/ family	Geographical origin
Metarhizium flavoviride	F 3606	*Zonocerus variegatus*	Orthoptera/ Pyrgomorphidae	Unknown
Metarhizium anisopliae	F 724	*Cerotoma arcuata*	Coleoptera/ Chrysomelidae	Brazil
"				
"	F 3045	*Coptotermes formosanus*	Isoptera/ Rhinotermitidae	Hawaii
"				
"				
"	C 56096	Unknown	Homoptera/ Cicadidae	Unknown
"				
"				
Beauveria bassiana	F 356	Unknown	Orthoptera/ Acrididae	New South Wales, Australia
"				
"	F 533	*Ostrinia nubilalis*	Lepidoptera/ Pyralidae	P. R. China
"				
"				
"	F 714	*Nilaparvata lugens*	Homoptera/ Delphacidae	Hupei, P. R. China
"				
"				
"	F 796	Unknown	Dermaptera	Colombia
"				
"	F 3041	*Reticulitermes flavipes*	Isoptera/ Rhinotermitidae	Ontario, Canada
"				
"				
Conidiobolus coronatus	F 511	*Nilaparvata lugens*	Homoptera/ Delphacidae	Philippines

* F = USDA/ARS Collection of Entomopathogenic Fungi; C = American Type Culture Collection.

were treated with each dose, and 0.005% Triton X-100 served as the control. After inoculation, each replicate of 10 termites was placed in a 60 × 15 mm Petri dish with a 0.5 cm^2 piece of paper towel. The dishes were held within 2-liter plastic containers lined with wet paper towels at 24.5 - 26.5°C. The insects were examined daily; dead individuals were removed and incubated under the same conditions for 2 d to allow for external fungal growth. After 8 d, the number of live termites was noted (a few were missing and presumed cannibalized). There was no mortality of control insects.

Mortality due to the pathogen was calculated as the number of termite cadavers that grew the appropriate fungal species (some cadavers grew saprophytes, particularly *Aspergillus* spp.) divided by the number of individuals that could be accounted for (i.e., 30 minus those missing). Probit analysis was performed with the POLO-PC program (Russell et al. 1977). Mean numbers of days from inoculation until death were analyzed by the General Linear Models Procedure with Tukey's Studentized Range (HSD) test for comparison of means (SAS Institute 1987).

Results and Discussion

Both *B. bassiana* and *M. anisopliae* were virulent to *C. formosanus* (Table 2). The rank of LD_{50}'s (median lethal doses) from these two fungi were interspersed, indicating that fungal strain rather than species had more effect on virulence.

Among the *B. bassiana* isolates, F 3041 was more virulent than F 356, F 533, or F 796, based on nonoverlap of 90% confidence limits of the LD_{50}'s (Table 2). The F 3041 isolate also killed *C. formosanus* significantly ($P < 0.05$) more quickly than the other *B. bassiana* isolates, with the exception of F 714 (Table 3). Thus, the isolate from a termite was the most virulent for *C. formosanus*. No broader phylogenetic pattern was observed in the rank of LD_{50}'s. For example F 533 was isolated from a host relatively distantly related to *C. formosanus,* yet its LD_{50} was similar to those (F 356 and F 796) isolated from insects in orders thought to be phylogenetically close to Isoptera (Hennig 1981, Kristensen 1981).

Among the isolates of *M. anisopliae*, the LD_{50} for C 56096 was 4.8 - 7.9 times those for the other two isolates (Table 2). However, C 56096 and F 724 killed termites more quickly ($P < 0.05$) than F 3045 (Table 3). Thus, F 724, originally isolated from a chrysomelid, was the most virulent of the three isolates if both LD_{50} and time until death are considered. Isolate F 3045 was the only fungus in the experiment isolated from *C. formosanus*. Therefore, there was no phylogenetic pattern of original host to *M. anisopliae* virulence in *C. formosanus,* perhaps because only three isolates were tested.

For purposes of microbial control of *C. formosanus,* capability of a fungus to be transmitted among members of a termite colony is an important parameter, particularly if applied in baits. Termite-to-termite transmission is important because, unlike chemicals, a fungal pathogen can reproduce and magnify the effects of the initial inoculum throughout its host population, provided that it can transfer from host to host. Obviously, one fungal characteristic important to transmission will be the production of conidia, the transmissive stage of these

Table 2. Log-dose-probit parameters and growth characteristics of fungal isolates evaluated against *Coptotermes formosanus*.

Fungal species	Isolate*	Growth of conidia**	Probit Statistics			
			n	slope ± SE	LD_{50} (90% CL)+	Chi‡
Metarhizium flavoviride	F 3606		205	only two termites in the entire bioassay (dose range 0.2-4.7) killed by *M. flavoviride*		
M. anisopliae	F 724	+	205	0.76 ± 0.198	0.5 (–)	18.43 a
"	F 3045	++	204	0.62 ± 0.192	0.9 (–)	38.15 a
"	C 56096	+	206	0.92 ± 0.232	4.2 (2.14 - 17.61)	1.31
Beauveria bassiana	F 3041	++	206	1.15 ± 0.205	0.4 (0.21 - 0.88)	7.83 a
"	F 714	++	205	1.00 ± 0.199	1.6 (–)	17.75 a
"	F 356	++	203	1.45 ± 0.258	1.9 (0.91 - 23.11)	13.54 a
"	F 533	++	196	0.47 ± 0.199	4.8 (2.05 - 25.48)	3.51
"	F 796	++	204	0.64 ± 0.212	7.1 (2.53 - 168.58)	1.40

* F = USDA/ARS Collection of Entomopathogenic Fungi; C = American Type Culture Collection.

** + patchy growth with mycelium and conidia covering < 25% of cadaver surface in at least 1/2 of the cases; ++ = full growth with mycelium and conidia covering all or almost all of the cadaver surface in most cases.

+ conidia/insect, X 10^4. POLO-PC calculates confidence limits only when g < 0.5.

‡ a = data heterogenus.

Table 3. Number of days after treatment until death caused by each fungal isolate.

Fungal species	Isolate Number*	Mean days (n, SE) until death at doses causing mortality of**: < 33.3%	33.3- 66.7%	> 66.7%	Mean days (n, SE) until death at all doses**
Metarhizium flavoviride	F 3606	7.0 (2, 1.00)	–	–	7.0 (2, 1.00)
M. anisopliae	F 724	–	5.3 B, ab (53, 0.27)	3.7 A, a (51, 0.12)	4.5 (104, 0.16) a
"	F 3045	6.5 B, b (21, 0.38)	6.0 AB, bc (26, 0.39)	5.2 A, b (28, 0.31)	5.9 (75, 0.21) cd
"	C 56096	4.4 A, a (16, 0.41)	4.0 A, a (21, 0.21)	–	4.2 (37, 0.21) a
Beauveria bassiana	F 3041	5.4 B, ab (10, 0.64)	5.8 B, bc (51, 0.27)	3.3 A, a (24, 0.09)	5.0 (85, 0.21) ab
"	F 714	5.7 A, ab (16, 0.46)	4.9 A, ab (30, 0.34)	5.5 A, b (51, 0.24)	5.4 (97, 0.18) bc
"	F 356	6.3 A, ab (9, 0.41)	6.1 A, bc (32, 0.32)	–	6.1 (41, 0.27) cd
"	F 533	6.4 A, b (9, 0.67)	6.0 A, bc (68, 0.20)	–	6.0 (77, 0.19) cd
"	F 796	5.9 A, ab (19, 0.41)	6.8 A, c (22, 0.44)	–	6.4 (41, 0.30) d
Means for all Isolates		5.8 B (100, 0.19)	5.6 B (303, 0.11)	4.5 A (154, 0.13)	

* F = USDA/ARS Collection of Entomopathogenic Fungi; C = American Type Culture Collection.

** Means followed by the same capital letter are not significantly different from other means horizontally, and means followed by the same lower-case letter are not significantly different from other means vertically ($P < 0.05$, General Linear Models Procedure, Tukey's Studentized Range [HSD] test [SAS Institute 1987]). Isolate F3606 was not included in the anlaysis due to the low number of observations.

deuteromycetes. All of the isolates of *B. bassiana* and *M. anisopliae* produced conidia on the cadavers within 2 d after death of the insect. All of *B. bassiana* isolates grew conidia profusely over most or all of the body surface of each termite in almost all cases, as did *M. anisopliae* isolate F 3045 (Table 2). However, the other two isolates of *M. anisopliae* usually grew only in patches covering less than 25% of the cadaver's surface.

Time until death could be another factor affecting fungal capability for transmission and, therefore, efficacy in microbial control. In previous research, a fungal disease spread more efficiently among *Reticulitermes* sp. from living termites exposed to the fungus than from termites killed by the fungus, due to termite behavior (Kramm et al. 1982). Thus, a fungus that kills slowly might prove more effective than one producing rapid mortality, though this has not been tested experimentally. For all isolates combined in the current research, doses killing > 66.7% of the termites caused significantly quicker mortality than doses killing < 66.7%. Three of the isolates (F 724, F 3045, and F 3041) also killed termites significantly more rapidly at the doses causing high rates of mortality (Table 3).

Results of our bioassays were highly variable. The POLO-PC program (Russell et al. 1977) gave 95% confidence limits of LD_{50}'s only for the three isolates with non-heterogenous data; thus, 90% confidence limits are presented in Table 2. It would be difficult to develop more exacting procedures than the ones used in the current research (individual, topical application; daily examination). Variability in response is not unusual in bioassays with entomopathogenic fungi (e.g., Lai et al. 1982, Pell et al. 1993, James and Lighthart 1994, Puterka et al. 1994).

Two other fungal species were bioassayed. *Metarhizium flavoviride* Gams and Rozsypal infected only two insects and, therefore, had low virulence for *C. formosanus*. *Conidiobolus coronatus* (Costantin) Batko was lethal when termites were forced to walk across a hypahal mat in Petri dishes (100% mortality in < 2 d, n = 40). Such rapid mortality, along with a lack of external fungal growth on the cadavers, indicated that death was due to some cause other than parasitic development of the fungus. This fungal species would not produce conidia under our culture conditions (Sabouraud dextrose agar + yeast, 26.7°C).

Thus, our results indicate that *B. bassiana* isolate F 3041 and *M. anisopliae* isolate 3045 are the best candidates for control of *C. formosanus* among the 10 isolates tested. These two isolates, the only ones that originally came from termites, were virulent and produced good conidial growth on the cadavers. Further research is necessary to determine whether and to what degree they would spread within a termite colony if foraging workers were to become infected.

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References Cited

Atkinson, T. H., M. C. Rust and J. L. Smith. 1993. The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), established in California. Pan-Pac. Entomol. 69: 111-113.

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.

Hänel, H. 1982. Selection of a fungus species suitable for the biological control of the termite *Nasutitermes exitiosus* (Hill). Z. Angew. Entomol. 94: 237-245.

Hänel, 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.

Hennig, W. 1981. Insect phylogeny. Wiley & Sons, Chichester.

James, R. R. and B. Lighthart. 1994. Susceptibility of the convergent lady beetle (Coleoptera: Coccinellidae) to four entomogenous fungi. Environ. Entomol. 23: 190-192.

King, E. G. and W. T. Spink. 1969. Foraging galleries of the Formosan subterranean termite, *Coptotermes formosanus*, in Louisiana. Ann. Entomol. Soc. Am. 62: 536-542.

Kramm, K. R. and D. F. West. 1982. Termite pathogens: effect of ingested *Metarhizium, Beauveria,* and *Gliocladium* conidia on worker termites (*Reticulitermes* sp.). J. Invertebr. Pathol. 40: 7-11.

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.

Kristensen, N. P. 1981. Phylogeny of insect orders. Annu. Rev. Entomol. 26: 135-157.

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.

Laird, M., L. A. Lacy and E. W. Davidson [eds.]. 1990. Safety of microbial insecticides. CRC Press, Boca Raton, FL.

Pell, J. K., N. Wilding, A. L. Player and S. J. Clark. 1993. Selection of an isolate of *Zoophthora radicans* (Zygomycetes: Entomopthorales) for biocontrol of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae). J. Invertebr. Pathol. 61: 75-80.

Puterka, G. J., R. A. Humber and T. J. Poprawski. 1994. Virulence of fungal pathogens (Imperfect Fungi: Hyphomycetes) to pear psylla (Homoptera: Psyllidae). Environ. Entomol. 23: 514-520.

Russell, R. M., J. L. Robertson and N. E. Savin. 1977. POLO: a new computer program for probit analysis. Bull. Entomol. Soc. Am. 23: 209-213.

SAS Institute. 1987. SAS/STAT guide for personal computers, version 6 ed. SAS Institute, Cary, NC.

Su, N. - Y. 1994. Field evaluation of a hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 87: 389-397.

Su, N. -Y. and M. Tamashiro. 1987. An overview of the Formosan subterranean termite (Isoptera: Rhinotermitidiae) 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, Honolulu.