# Virulence and *in vitro* Characteristics of Pathogenic Fungi Isolated from Soil by Baiting with *Coptotermes formosanus* (Isoptera: Rhinotermitidae)[1]

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**Abstract** *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) was used as "bait" to isolate pathogenic fungi from soil. Ninety soil samples were collected from woodlands and pastures in the vicinities of Baton Rouge, New Orleans, and Lake Charles, LA, from which six *Metarhizium anisopliae* (Metsch.) Sorokin and nine *Beauveria bassiana* (Balsamo) Vuillemin isolates were obtained. Numbers of fungal isolates from the three sampling locations did not differ, but more isolates were found in woodlands than in pastures. Median lethal doses ($LD_{50}$s) of these fungal species to *C. formosanus* were interspersed, indicating that fungal isolates rather than species had the greatest effect on virulence. Among nine Louisiana and two USDA isolates of *B. bassiana*, $LD_{50}$s ranged from $4.95 \times 10^3$ to $4.96 \times 10^5$ conidia/termite, a difference of 100X. $LD_{50}$s of six Louisiana and four USDA isolates of *M. anisopliae* ranged from $7.89 \times 10^3$ to $1.22 \times 10^5$ conidia/termite. Survival time also was used to compare virulence; *M. anisopliae* infections caused significantly shorter host survival times than *B. bassiana*. *In vitro* growth characteristics were significantly correlated with virulence against termites, suggesting that the characteristics of a fungus growing on agar might contribute to estimating the fungal virulence *in vivo*.

**Key Words** Formosan subterranean termite, *Coptotermes formosanus, Metarhizium anisopliae, Beauveria bassiana,* fungi, virulence, baiting technique

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), is a promising candidate for control with entomogenous fungi. Its social behavior, preference for humid conditions and contact with soil are factors that favor fungal pathogens (Milner 1997, Wells et al. 1995). A mature colony of *C. formosanus* can cause great damage in a short time. Unprotected homes built over strong colonies have been almost destroyed in 2 yrs (Tamashiro et al. 1987). The use of fungi as biological agents against termites is an attractive alternative to present technology. Entomogenous fungi are safe to humans and other non-target organisms (Laird et al. 1990), which is important in the urban setting where termites are often pests. Among the many entomogenous fungi, *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin are potentially the most useful in termite biocontrol. They are distributed worldwide in soil where termites are usually found (Ko 1982; Milner and Lutton 1976, Milner et al. 1998a, b). Many isolates are virulent against termites (Jones et al. 1996, Ko 1982, Lai et al. 1982, Milner et al.

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1998a, Wells et al. 1995), and they are relatively inexpensive to grow in quantity (Delate et al. 1995, Grace 1997, Jackson et al. 2000, Milner 1992).

A crucial first step for biological control of termites is the selection of a suitable isolate for further development. Because soil is an important reservoir of entomopathogens (Fuxa and Kunimi 1997), new isolates or species of entomopathogens often are found directly or indirectly through soil sampling (Ko 1982, Milner et al. 1998a, Sajap et al. 1997). Isolations of fungal entomopathogens from *C. formosanus* are relatively rare, despite frequent anecdotal observations of various fungal growths associated with weak termite colonies in the laboratory (Beard 1974). Frequently, the most effective isolate is obtained from an epizootic in the target pest under natural conditions (Milner 1992). In the case of termites, there are no confirmed reports of *M. anisopliae* or *B. bassiana* causing natural mortality in the field (Milner et al. 1998a), and thus previous studies have mostly involved isolates obtained from other, often unrelated, insects (Hanel 1981, Lai et al. 1982, Kramm and West 1982). Semi-selective media have been used to detect entomogenous genera, such as *Metarhizium, Beauveria,* and *Conidiobolus* (Milner et al. 1998a, Sajap et al. 1997). However, most successful microbial control agents have been obtained from host insects rather than by selective medium or from existing culture collections (Jackson et al. 2000).

The first objective in this paper was to isolate from soil fungi pathogenic to *C. formosanus* by using the termites in a "baiting" technique. The soil samples were collected from different locations and ecosystems in Louisiana. Fungal isolates (four *M. anisopliae* and two *B. bassiana*) also were obtained from the USDA-ARS Collection of Entomopathogenic Fungi (Ithaca, NY). A second objective was to compare the virulence and *in vitro* growth of these fungal isolates in *C. formosanus* and to select isolates for possible further development as microbial insecticides.

## Materials and Methods

**Sampling.** Ninety soil samples were taken from woodlands and pastures in the vicinities of Baton Rouge, New Orleans, and Lake Charles, LA, in 1999. At each location, three sites were selected in woodlands and pastures and; five samples were collected by core sampler (15 cm diam, 10 to 15 cm depth), approximately 3 m apart in a transect from each site. Each soil sample was stored in an individual plastic bag (Whirl-Pak sterile sample bag 15 × 22 cm); and if necessary, the samples were stored at 4 to 6°C up to 2 wks prior to processing.

**Soil pH and moisture.** Soil pH was measured in three replicate 10 g sub-samples from each soil sample (Corning pH meter, Corning Science Products, Coming, NY, USA). Another 50 g subsample of soil from each sample was saturated with distilled water. A soil moisture meter probe (Lincoln Irrigation Inc., Lincoln, NE) was then applied to the saturated soil, and its indicator needle was calibrated to the maximum reading (100% water content). Water content was then determined for the original sample and sterile water was added, if necessary, so that all soil samples were adjusted to 70% water content before fungal isolation.

**Fungal isolation and culture.** Formosan subterranean termites were used as a "bait" to isolate pathogenic fungi from soil. Termite workers were collected from a colony infesting trees in Lake Charles and reared in the laboratory. One hundred termite workers were released into a 100 g subsample from each soil collection in a Pyrex® storage dish (100 mm diam, 80 mm high) and covered with a lid. After 24 h incubation at 27°C, a piece of wet filter paper (100 mm diam) was placed on top of the

soil for 10 to 20 min. The termites trapped on the filter paper then were transferred into an empty Petri dish (100 × 15 mm) for surface decontamination of the termites, which is essential for removal of saprophytes on the body surface. All termites from each test jar were placed in 1% NaClO, plus 0.05% of Tween 100, for 1 min, and they then were briefly rinsed in 2 to 3 changes of sterile water. All washed termites were put on sterile filter paper to dry and transferred to four Petri dishes (100 × 15 mm), 25 termites/dish, with a wet piece of filter paper in each dish as the source of food and moisture. Petri dishes were incubated at 27°C without light for up to 12 days, and mortality was recorded daily. Dead termites were surface decontaminated as previously described, except that they also were immersed in 70% alcohol for 5 s before NaClO treatment. The termites then were placed individually on a general culture medium, Sabouraud dextrose agar + yeast (SDAY) (Goettel and Inglis 1997), and incubated up to 12 days at 27°C. After prolific conidiogenesis was observed, the fungus was transferred onto SDAY agar.

**Purification and identification.** Conidia were harvested by flooding the plate with 0.05% Triton X-100 in sterile distilled water. The concentration of conidia in the suspension was determined by hemocytometer (Petroff-Hausser, Hausser Scientific Partnership, Horsham, PA) and serially diluted to $10^4$ conidia/ml. One hundred µl of this suspension were then inoculated onto SDAY in a Petri dish (100 × 15 mm) and spread over the agar with a sterile spreader (30 mm wide) on an inoculating turntable (114 mm, VWRbrand®, VWR Corporation, OH). The dishes were incubated at 27°C for 30 h, and a single germinated conidium was excised from the agar by microscalpel and transferred to the center of a new SDAY Petri dish. This dish was sealed with Parafilm® and cultured at 27°C without light for 12 days. All fungal isolates from soil were subjected to this process in order to ensure single genotypes. After purification, all isolates were stored at –20°C in silica gel (Smith 1993). Each fungus was identified by its morphological characters (Humber 1997) and confirmed through the USDA-ARS Collection of Entomopathogenic Fungi (Ithaca, NY).

**USDA fungal collections.** Six fungal isolates were obtained from the USDA-ARS Collection of Entomopathogenic Fungi (Ithaca, NY), including four isolates of *M. anisopliae* (F3045 from *C. formosanus* in Hawaii; F724 from a chrysomelid beetle in Brazil, F683 from a scarab beetle in China; and F794 from leaf litter in Wisconsin) and two isolates of *B. bassiana* (F714 from a delphacid in China and F356 from an acridid in Australia). One isolate of *M. anisopliae,* Bio-blast® (biological termiticide), was obtained from the Ecoscience Corporation (Worcester, MA).

**Fungal *in vitro* growth characteristics.** A single germinated spore of each fungal isolate (30 h after inoculation) was obtained as described above and transferred to the center of a SDAY dish. This was done three times for each isolate. Each dish was sealed by parafilm and cultured at 27°C for 12 days without light. Based on their *in vitro* growth, the colonies for all fungal isolates were categorized into two groups according to standard characteristics (Hawksworth 1974, Klein 1996): one having colonies with compact growth, flat elevation, and >50% of the surface area sporulating after 12 days of growth; the other with filamentous growth, raised elevation, and <50% sporulation.

**Virulence bioassay.** Our techniques were adapted from those of Wells et al. (1995). The fungi were grown on SDAY at 27°C. Conidia were harvested under sterile conditions by flooding the plate with sterile distilled $H_2O$ and then scraping the colony with forceps. Conidia were stirred into suspension for 20 min in 200 mL 0.05% Triton X-100 and distilled $H_2O$, which was filtered through cheesecloth. The concentration of

conidia in each suspension was determined with a hemocytometer. All suspensions were stored at 6°C until used. Viability of conidia at the time of treatment was measured by mixing a drop of suspension into a drop of Sabouraud broth on a microscope slide and incubating under high humidity for 24 h at 27°C (Goettel and Inglis 1997). All suspensions displayed >98% germination of conidia.

For the bioassay, termites were anesthetized with a frozen refrigerant pack (20.32 × 17.78 × 3.81 mm), which was positioned under the Petri dish with the termites to be tested for 1 min, and 0.5 μl of conidia suspension was applied to the ventral surface of each termite with a micro-dispenser (Drummond Scientific Co., Broomall, PA). The bioassay of each fungal isolate included five fungal doses plus a control, with the range of doses determined in a preliminary test. Four replications of 10 insects each were treated with each dose, and 0.05% Triton X-100 in distilled water served as the control. After inoculation, each replicate of 10 termites was placed in a 100 × 15 mm Petri dish with a piece of wet filter paper (Whatman #1, diam 90 mm), on which two pieces of wet balsa wood (approximately 50 × 30 × 1.5 mm) were provided as food and shelter. The dishes were all sealed with parafilm and incubated at 27°C. The insects were examined daily; dead individuals were removed and incubated under the same conditions for 5 days to allow for external fungal growth. After 10 days, the number of live termites was recorded; a few were occasionally missing and presumed cannibalized. 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. The control mortalities were less than 7.5% for each fungal isolate and were corrected by POLO-PC program for $LD_{50}$ (Russell et al. 1977).

**Statistical analysis.** The $\chi^2$ statistic determined the differences in number of pathogenic fungi isolated among the different locations (cities) and the differences between two ecosystems at each location. Logistic regression analysis (SAS Institute 1996) was used to determine the effects of soil pH and water content on the number of detected fungal isolates. Probit analysis of fungal isolates was performed with the POLO-PC program (Russell et al. 1977). The data, which included censored as well as uncensored data at all doses for each fungal isolate, were subjected to survival analysis (Anonymous 1995, Kalbfleisch and Prentice 1980). The Kaplan-Meier product-limit estimator was used to test for significances among survival times, and pairwise comparisons were made with a log linear rank test (Kalbfleisch and Prentice 1980) in the STATISTICA program (Anonymous 1995). Differences in $LD_{50}$ between isolates in two categories of *in vitro* fungal characteristics on agar and between isolates from two soil ecosystems were analyzed by analysis of variance (ANOVA) (SAS Institute 1996).

## Results

A total of 15 isolates of *M. anisopliae* and *B. bassiana* were detected from 90 soil samples (Table 1). *Metarhizium anisopliae* was obtained from 6.7% of all samples, while 10% of samples contained *B. bassiana*. There were no significant differences ($\chi^2$ = 0.000; df = 2; $P$ = 1.000) among the numbers of fungal isolates from Baton Rouge, New Orleans, and Lake Charles, LA. However, significantly ($\chi^2$ = 3.920; df = 1; $P$ = 0.048) more isolates were found in woodlands than in pastures. *Metarhizium anisopliae* was not isolated from pasture soils.

Soil pH ranged from acid to neutral for all sampling sites in three locations, and

**Table 1. Entomopathogenic fungi isolated by baiting soil with *C. formosanus***

| Sampling locations in Louisiana | Sampling environments for each location | Sampling site in each environment* | Date of soil samples | Number of fungal isolates | Fungal species | Assigned isolate numbers |
|---|---|---|---|---|---|---|
| New Orleans | Woodland | 1 | 6/17/1999 | 1 | *Metarhizium anisopliae* | G-6170 |
| | | 2 | 8/2/1999 | 1 | *Beauveria bassiana* | W-8021 |
| | | 3 | 8/18/1999 | 1 | *Beauveria bassiana* | W-8181 |
| | pasture | 1 | 6/17/1999 | 0 | / | / |
| | | 2 | 8/2/1999 | 1 | *Beauveria bassiana* | W-8022 |
| | | 3 | 8/18/1999 | 1 | *Beauveria bassiana* | W-8182 |
| Baton Rouge | Woodland | 1 | 6/29/1999 | 2 | *Beauveria bassiana* | W-6291 |
| | | | | | *Metarhizium, anisopliae* | G-6292 |
| | | 2 | 7/2/1999 | 0 | / | / |
| | | 3 | 7/15/1999 | 2 | *Beauveria bassiana* | W-7151 |
| | | | | | *Metarhizium anisopliae* | G-7152 |
| | pasture | 1 | 6/29/1999 | 0 | / | / |
| | | 2 | 7/2/1999 | 0 | / | / |
| | | 3 | 7/15/1999 | 1 | *Beauveria bassiana* | W-7150 |

**Table 1. Continued.**

| Sampling locations in Louisiana | Sampling environments for each location | Sampling site in each environment* | Date of soil samples | Number of fungal isolates | Fungal species | Assigned isolate numbers |
|---|---|---|---|---|---|---|
| Lake Charles | Woodland | 1 | 6/18/1999 | 1 | Metarhizium anisopliae | G-6180 |
| | | 2 | 7/19/1999 | 2 | Beauveria bassiana | W-7191 |
| | | | | | Metarhizium anisopliae | G-7192 |
| | | 3 | 8/3/1999 | 1 | Metarhizium anisopliae | G-8032 |
| | pasture | 1 | 6/18/1999 | 0 | / | / |
| | | 2 | 7/19/1999 | 0 | / | / |
| | | 3 | 8/3/1999 | 1 | Beauveria bassiana | W-8031 |
| Total | | | | 15 | 2 | |

* Five soil samples in each sampling site.

there was no significant (Table 2) ($\chi^2$ = 0.205; df = 1; $P$ = 0.650) pH effect on the number of fungal isolates. Soil samples collected from different sites in different locations varied greatly in water content, ranging from 5 to 80%. There was no significant (Table 2) ($\chi^2$ = 0.642; df = 1; $P$ = 0.423) impact of soil water content on the number of detected fungal isolates.

Table 3 summarizes the $LD_{50}$ parameter from virulence bioassays, which indicated that both *M. anisopliae* and *B. bassiana* were virulent to *C. formosanus*. The ranks of $LD_{50}$s (median lethal doses) from these two fungi were interspersed, showing that fungal strains rather than species had greater effect on virulence. There was no difference in $LD_{50}$s between fungi isolated from woodland versus pasture ecosystems ($F$ = 0.020; df = 1, 13; $P$ = 0.888).

Survival times differed significantly ($\chi^2$ = 795.466; df = 21; $P$ < 0.0001) among overall fungal isolates. The differences in survival time between each pair of isolates of *B. bassiana* and *M. anisopliae* are presented in Table 4. *Metarhizium anisopliae* isolate G8032, which originated from our termite baiting of Louisiana soil, induced survival times significantly shorter than any other *M. anisopliae* or *B. bassiana* isolate (Table 4, Fig. 1). Interestingly, *M. anisopliae* isolates overall produced significantly shorter survival times (mean number of days of survival = 7.96 ± 0.033) than *B. bassiana* (8.37 ± 0.003) ($\chi^2$ = 18.714; df = 1; $P$ < 0.0001) (Fig. 1).

Two groups of fungal isolates could be categorized based on their colony characteristics (Hawksworth 1974, Klein 1996). Category 1 had a compact growth colony with flat elevation and >50% of the surface area of the fungal colony sporulated after 12 days of growth. Category 2 had filamentous growth with raised elevation and <50% sporulation after 12 days. The fungal isolates in category 1 had lower $LD_{50}$s in termites (mean $LD_{50}$ = 6.11 ± 0.443 × $10^4$ conidia/insect) than those in category 2 (mean $LD_{50}$ = 2.72 ± 1.746 × $10^5$ conidia/insect). ($F$ = 16.380; df =1, 20; $P$ < 0.001). The correlation between *in vitro* growth characteristics and $LD_{50}$ in termites was significant ($R$ = 0.67; $P$ = 0.0006).

## Discussion

Termites were very susceptible to isolates of *Metarhizium* and *Beauveria* in the current study, although virulence differed by as much as 16X among *M. anisopliae*

**Table 2. Physical characteristics of soil from which fungi were isolated**

| Sampling locations in Louisiana | Sampling environments for each location* | Soil pH (mean ± SE) | Soil water content (% ± SE) |
|---|---|---|---|
| New Orleans | Woodland | 6.12 ± 0.76 | 40.67 ± 14.62 |
| | pasture | 6.34 ± 0.47 | 15.33 ± 5.49 |
| Baton Rouge | Woodland | 5.78 ± 1.16 | 31.67 ± 17.69 |
| | pasture | 6.02 ± 0.76 | 34.67 ± 26.49 |
| Lake Charles | Woodland | 3.81 ± 0.51 | 8.00 ± 2.54 |
| | pasture | 5.77 ± 1.27 | 43.33 ± 32.16 |

* Total 15 soil samples in each location.

isolates and 100X among *B. bassiana* isolates. These results are similar to those of other studies of fungi in *C. formosanus* and other termite species (Almeida et al. 1997, Delate et al. 1995, Hanel 1981, Lai et al. 1982, Milner et al. 1998a, Wells et al. 1995). Similarly, a large number of isolates of *M. anisopliae* from termite colonies or from termite-derived material in Australia were virulent to termites (Milner et al. 1998a). However, termites are rarely infected naturally in the field (Milner 1997, Milner et al. 1998a, Roberts and Humber 1981), which suggests that their defensive behavior may play a major role in defending fungal attack.

Survival time could be an important factor affecting fungal capability for transmission in a termite colony and, therefore, efficacy in microbial control (Wells et al. 1995). Milner et al. (1998b) reported that the most effective fungal isolate of *M. anisopliae* against termites in laboratory and field trials was not the most virulent, causing the shortest survival time. Thus, long survival time may be an advantage in transmission, because the termites live longer, are more ambulatory, and have a greater tendency to spread spores (Kramm et al. 1982). The significant difference in survival time among different fungal isolates in the current research might be considered in selecting promising isolates for termite microbial control.

Fungal *in vitro* growth characteristics were correlated with $LD_{50}$s in termites. This suggests that a method may be developed to categorize isolates of *M. anisopliae* or *B. bassiana* by preliminary screening into different groups, based visually on their colony characteristics on a culture medium instead of virulence bioassays. In addition to their lower $LD_{50}$s, category 1 isolates (>50% sporulated colony area) produced more conidia (J. Sun, J. R. Fuxa, G. Henderson, unpubl. data) than category 2 isolates (<50% sporulated colony area), which should enhance their commercial production. Thus, these fungal characteristics may eventually prove useful in efficient screening of a large number of isolates against termites.

Using termites as a "bait" to detect pathogens in soil was an effective way to isolate fungi (Ko 1982) and is more efficient than isolation of pathogens from dead, field-collected termites. Separating bait termites from their nest significantly increased their chances to be infected, because termite nests are physically and chemically different from adjacent soils (Badawi et al. 1982). Termites modify soil by differential selection of soil particles, incorporation of saliva and excreta into the lining of galleries, and modification of organic matter during its passage in the termite gut (Lee and Wood 1971). Also, fecal pellets and fumigation of nest with volatiles significantly decrease germination rates of fungal spores and colony growth (Rosengaus et al. 1998, Wright et al. 2000). The baiting technique used in this study had the additional advantage of enabling detection and isolation of *C. formosanus* pathogens with little interference from saprophytic organisms. Baiting also may prove effective in fungal ecology. For example, we isolated more fungi from woodlands than from pastures (Table 1), although soil pH and water content did not affect the number of isolates (Table 2). For practical reasons, it may be advantageous to search for suitable strains of pathogens in the environment of the target pest (Milner et al. 1998b), where ecological factors have selected for fungal survival, reproduction, and transmission in populations of the target insect.

The occurrence of fungi pathogenic to *C. formosanus* in soil (Table 1) suggests that they might be important to natural mortality of termites founding new colonies. More than 16% of soil samples collected from Louisiana in the current research contained fungi pathogenic to Formosan subterranean termites, primarily *M. anisopliae* and *B. bassiana.* As many as 75% of soil samples collected from Kamuela in

**Table 3. Log-dose-profit parameters and colony growth characteristics on SDAY for the isolates of *M. anisopliae* and *B. bassiana* against *C. formosanus***

| Fungal species | Isolate number* | Slope ± SE (ld-p line) | $LD_{50}$(95%CL)** (conidia/termite) | $\chi^2$ Value*** | Geographical origin† | Original host species | Category of fungi‡ |
|---|---|---|---|---|---|---|---|
| *M. anisopliae* | F 3045 | 1.30 ± 0.12 | 0.08 (0.03-0.13) | 4.7 | Hawaii (Oahu) | *C. formosanus* | 1 |
| " | F 724 | 1.87 ± 0.19 | 0.56 (0.47-0.67) | 0.5 | Brazil | *Cerotoma arcuata* | 1 |
| " | F 683 | 1.46 ± 0.08 | 0.43 (0.28-0.63) | 11.8 a | P. R. China | Coleoptera: Scarabaeidae | 1 |
| " | F 794 | 1.18 ± 0.08 | 0.30 (0.18-0.48) | 10.6 a | Wisconsin | Leaf litter (suger maple) | 2 |
| *B. bassiana* | F 714 | 1.04 ± 0.92 | 0.05 (0.03-0.07) | 1.7 | P. R. China | *Nilaparvata lugens* | 1 |
| " | F 356 | 3.00 ± 0.18 | 0.80 (0.67-0.95) | 7.1 | Australia | Orthoptera: Acrididae | 1 |
| *M. anisopliae* | Bioblast | 1.30 ± 0.01 | 0.09 (0.06-0.14) | 3.2 | commercial | unknown | 1 |
| " | G7192 | 2.10 ± 0.19 | 0.68 (0.44-0.92) | 4.5 | Lake Charles (W) | *C. formosanus* | 1 |
| " | G7152 | 1.34 ± 0.11 | 0.70 (0.57-0.87) | 2.0 | Baton Rouge (W) | " | 1 |
| " | G6292 | 1.63 ± 0.13 | 0.73 (0.62-0.85) | 3.5 | Baton Rouge (W) | " | 1 |
| " | G6180 | 2.37 ± 0.23 | 1.08 (0.93-1.25) | 1.3 | Lake Charles (W) | " | 2 |
| " | G6170 | 1.27 ± 0.09 | 1.20 (0.96-1.49) | 0.3 | New Orleans (W) | " | 2 |
| " | G8032 | 2.40 ± 0.17 | 1.22 (1.07-1.38) | 2.8 | Lake Charles (W) | " | 2 |

**Table 3. Continued.**

| Fungal species | Isolate number* | Slope ± SE (ld-p line) | $LD_{50}$(95%CL)** (conidia/termite) | $\chi^2$ Value*** | Geographical origin† | Original host species | Category of fungi‡ |
|---|---|---|---|---|---|---|---|
| *B. bassiana* | W7150 | 2.06 ± 0.18 | 0.67 (0.44-0.95) | 6.2 | Baton Rouge (P) | " | 1 |
| " | W8022 | 1.65 ± 0.14 | 0.09 (0.51-1.42) | 7.5 | New Orleans (P) | " | 1 |
| " | W8182 | 1.31 ± 0.09 | 1.67 (1.33-2.07) | 1.7 | New Orleans (P) | " | 1 |
| " | W8181 | 3.88 ± 0.35 | 2.72 (2.52-2.90) | 3.5 | New Orleans (W) | " | 2 |
| " | W8021 | 1.53 ± 0.11 | 2.39 (2.04-2.81) | 2.4 | New Orleans (W) | " | 2 |
| " | W6291 | 1.33 ± 0.19 | 3.99 (3.22-5.29) | 2.6 | Baton Rouge (W) | " | 2 |
| " | W7191 | 1.15 ± 0.09 | 4.65 (3.25-7.03) | 3.1 | Lake Charles (W) | " | 2 |
| " | W7151 | 1.63 ± 0.13 | 4.69 (3.90-5.58) | 3.1 | Baton Rouge (W) | " | 2 |
| " | W8031 | 1.26 ± 0.12 | 4.96 (2.84-16.38) | 9.0 a | Lake Charles (W) | " | 2 |

* F = USDA/ARS Collection of Entomopathogenic Fungi; G and W = fungi from baiting isolation.

** conida × $10^5$/insect, POLO-PC calculates confidence limits only when g < 0.5.

*** a = data heterogeneous at $P < 0.05$, a large $\chi^2$ indicates a poor fit of the data by the probit analysis model.

† (P) = pasture, (W) = woodlands.

‡ 1 = Compact growth with flat elevation and sporulation >50% of total colony growth area on agar after 12 days at 27°C; 2 = Filamentous growth with raised elevation and sporulation <50% of total growth area on agar after 12 days at 27°C.

**Table 4. Kaplan-Meier product limit estimates and pairwise comparisons of survival time (days) for each fungal isolate by a log linear rank test***

| Fungal isolate | Fungal isolate | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G6170 | Biobl. | F683 | G7152 | F714 | W8021 | G6292 | W8182 | W7150 | W8022 | G7192 |
| G8032 | 8.866 | 13.090 | 12.156 | 14.574 | 10.287 | 8.945 | 12.231 | 10.866 | 14.369 | 10.166 | 10.376 |
| | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| G6170 | — | 5.975 | 4.468 | 7.241 | 2.115 | 0.670 | 4.525 | 2.744 | 7.317 | 2.383 | 2.565 |
| | | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P = 0.053$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| Bioblast | | — | −1.766 | 0.986 | −4.439 | −6.051 | −2.239 | −3.881 | 0.615 | −4.421 | −4.363 |
| | | | $P = 0.078$ | $P = 0.324$ | $P < 0.001$ | $P < 0.001$ | $P = 0.025$ | $P < 0.001$ | $P = 0.538$ | $P < 0.001$ | $P < 0.001$ |
| F683 | | | — | 2.791 | −2.675 | −4.220 | −3.117 | −2.076 | 2.593 | −2.504 | −2.423 |
| | | | | $P = 0.005$ | $P = 0.008$ | $P < 0.001$ | $P = 0.755$ | $P = 0.04$ | $P = 0.009$ | $P = 0.012$ | $P = 0.015$ |
| G7152 | | | | — | −5.387 | −7.020 | −3.085 | −4.921 | −0.122 | −5.309 | −5.159 |
| | | | | | $P < 0.001$ | $P < 0.001$ | $P = 0.002$ | $P < 0.001$ | $P = 0.903$ | $P < 0.001$ | $P < 0.001$ |
| F714 | | | | | — | −1.398 | 2.450 | 0.720 | 5.429 | 0.445 | 0.502 |
| | | | | | | $P = 0.162$ | $P = 0.014$ | $P = 0.472$ | $P < 0.001$ | $P = 0.656$ | $P = 0.616$ |
| W8021 | | | | | | — | 3.961 | 2.135 | 6.989 | 1.853 | 1.912 |
| | | | | | | | $P < 0.001$ | $P = 0.033$ | $P < 0.001$ | $P = 0.064$ | $P = 0.056$ |
| G6292 | | | | | | | — | −1.816 | 3.076 | −2.801 | −2.015 |
| | | | | | | | | $P = 0.069$ | $P = 0.002$ | $P = 0.038$ | $P = 0.044$ |
| W8182 | | | | | | | | — | 4.859 | −0.326 | −0.247 |
| | | | | | | | | | $P < 0.001$ | $P = 0.744$ | $P = 0.805$ |
| W7150 | | | | | | | | | — | −5.156 | −5.122 |
| | | | | | | | | | | $P < 0.001$ | $P < 0.001$ |
| W8022 | | | | | | | | | | — | 0.001 |
| | | | | | | | | | | | $P = 0.999$ |
| G7192 | | | | | | | | | | | — |

**Table 4. Continued.**

| Fungal isolate | Fungal isolate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | G6180 | W7151 | F724 | F356 | W8181 | F794 | F3045 | W7191 | W6291 | W8031 |
| G8032 | 14.070 | 10.066 | 15.333 | 12.404 | 10.809 | 11.951 | 9.715 | 14.884 | 14.600 | 15.828 |
| | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| G6170 | 6.637 | 2.677 | 8.349 | 5.100 | 2.930 | 4.445 | 2.317 | 8.202 | 7.924 | 9.291 |
| | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| Bioblast | 0.398 | −4.485 | 1.967 | −1.849 | −3.640 | −2.604 | −5.202 | 1.295 | 1.202 | 2.525 |
| | $P = 0.691$ | $P < 0.001$ | $P = 0.049$ | $P = 0.064$ | $P < 0.001$ | $P = 0.009$ | $P < 0.001$ | $P = 0.196$ | $P = 0.229$ | $P = 0.012$ |
| F683 | 2.201 | −2.399 | 3.865 | 0.195 | −1.803 | −0.543 | −2.975 | 3.406 | 3.269 | 4.619 |
| | $P = 0.03$ | $P = 0.016$ | $P = 0.000$ | $P = 0.845$ | $P = 0.071$ | $P = 0.587$ | $P = 0.003$ | $P < 0.001$ | $P = 0.001$ | $P < 0.001$ |
| G7152 | −0.630 | −5.197 | 1.072 | −2.591 | −4.619 | −3.458 | −5.897 | 0.633 | 0.439 | 1.852 |
| | $P = 0.529$ | $P < 0.001$ | $P = 0.284$ | $P = 0.009$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P = 0.527$ | $P = 0.661$ | 0.064 |
| F714 | 4.902 | 0.769 | 6.633 | 3.232 | 0.966 | 2.508 | 0.394 | 6.451 | 6.291 | 7.640 |
| | $P < 0.001$ | $P = 0.442$ | $P < 0.001$ | $P = 0.001$ | $P = 0.334$ | $P = 0.012$ | $P = 0.694$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| W8021 | 6.496 | 2.102 | 8.259 | 4.653 | 2.481 | 4.052 | 1.820 | 8.070 | 7.895 | 9.188 |
| | $P < 0.001$ | $P = 0.036$ | $P < 0.001$ | $P < 0.001$ | $P = 0.013$ | $P < 0.001$ | $P = 0.069$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| G6292 | 2.570 | −1.800 | 4.329 | 0.753 | −1.485 | −0.065 | −2.298 | 4.038 | 3.928 | 5.295 |
| | $P = 0.010$ | $P = 0.072$ | $P < 0.001$ | $P = 0.452$ | $P = 0.138$ | $P = 0.948$ | $P = 0.021$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| W8182 | 4.331 | −0.113 | 6.125 | 2.429 | 0.335 | 1.859 | −0.443 | 5.901 | 5.704 | 7.000 |
| | $P < 0.001$ | $P = 0.909$ | $P < 0.001$ | $P = 0.015$ | $P = 0.738$ | $P = 0.063$ | $P = 0.658$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| W7150 | −0.379 | −4.983 | 1.324 | −2.363 | −4.539 | −3.286 | −5.642 | 0.908 | 0.807 | 2.208 |
| | $P = 0.704$ | $P < 0.001$ | $P = 0.185$ | $P = 0.018$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P = 0.316$ | $P = 0.419$ | $P = 0.027$ |
| W8022 | 4.793 | 0.183 | 6.538 | 2.758 | 0.685 | 2.172 | −0.163 | 6.228 | 6.116 | 7.380 |
| | $P < 0.001$ | $P = 0.855$ | $P < 0.001$ | $P = 0.005$ | $P = 0.494$ | $P = 0.029$ | $P = 0.871$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |

**Table 4. Continued.**

| Fungal isolate | Fungal isolate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | G6180 | W7151 | F724 | F356 | W8181 | F794 | F3045 | W7191 | W6291 | W8031 |
| G7192 | 4.709 | 0.274 | 6.458 | 2.837 | 0.578 | 2.111 | −0.101 | 6.235 | 6.106 | 7.439 |
| | $P < 0.001$ | $P = 0.784$ | $P < 0.001$ | $P = 0.005$ | $P = 0.564$ | $P = 0.035$ | $P = 0.919$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| G6180 | — | −4.737 | 1.681 | −2.121 | −4.046 | −2.866 | −5.395 | 1.154 | 1.105 | 2.369 |
| | | $P < 0.001$ | $P = 0.093$ | $P = 0.034$ | $P < 0.001$ | $P = 0.004$ | $P < 0.001$ | $P = 0.249$ | $P = 0.310$ | $P = 0.018$ |
| G7151 | | — | 6.502 | 2.563 | 0.544 | 2.070 | −0.263 | 6.136 | 6.159 | 7.317 |
| | | | $P < 0.001$ | $P = 0.010$ | $P = 0.587$ | $P = 0.038$ | $P = 0.792$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| F724 | | | — | −3.845 | −5.799 | −4.687 | −7.229 | −0.539 | −0.680 | 0.701 |
| | | | | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P = 0.589$ | $P = 0.496$ | $P = 0.483$ |
| F356 | | | | — | −2.068 | −0.654 | −3.000 | 3.388 | 3.428 | 4.674 |
| | | | | | $P = 0.038$ | $P = 0.513$ | $P = 0.003$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| W8181 | | | | | — | 1.393 | −0.993 | 5.507 | 5.294 | 6.618 |
| | | | | | | $P = 0.164$ | $P = 0.321$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| F794 | | | | | | — | −2.519 | 4.342 | 4.176 | 5.476 |
| | | | | | | | $P = 0.012$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| F3045 | | | | | | | — | 6.893 | 6.818 | 8.003 |
| | | | | | | | | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| W7191 | | | | | | | | — | −0.073 | 1.342 |
| | | | | | | | | | $P = 0.942$ | $P = 0.179$ |
| W6291 | | | | | | | | | — | 1.299 |
| | | | | | | | | | | $P = 0.194$ |
| G8031 | | | | | | | | | | — |

* $\chi^2 = 795.5$, df = 21, $P < 0.0001$.

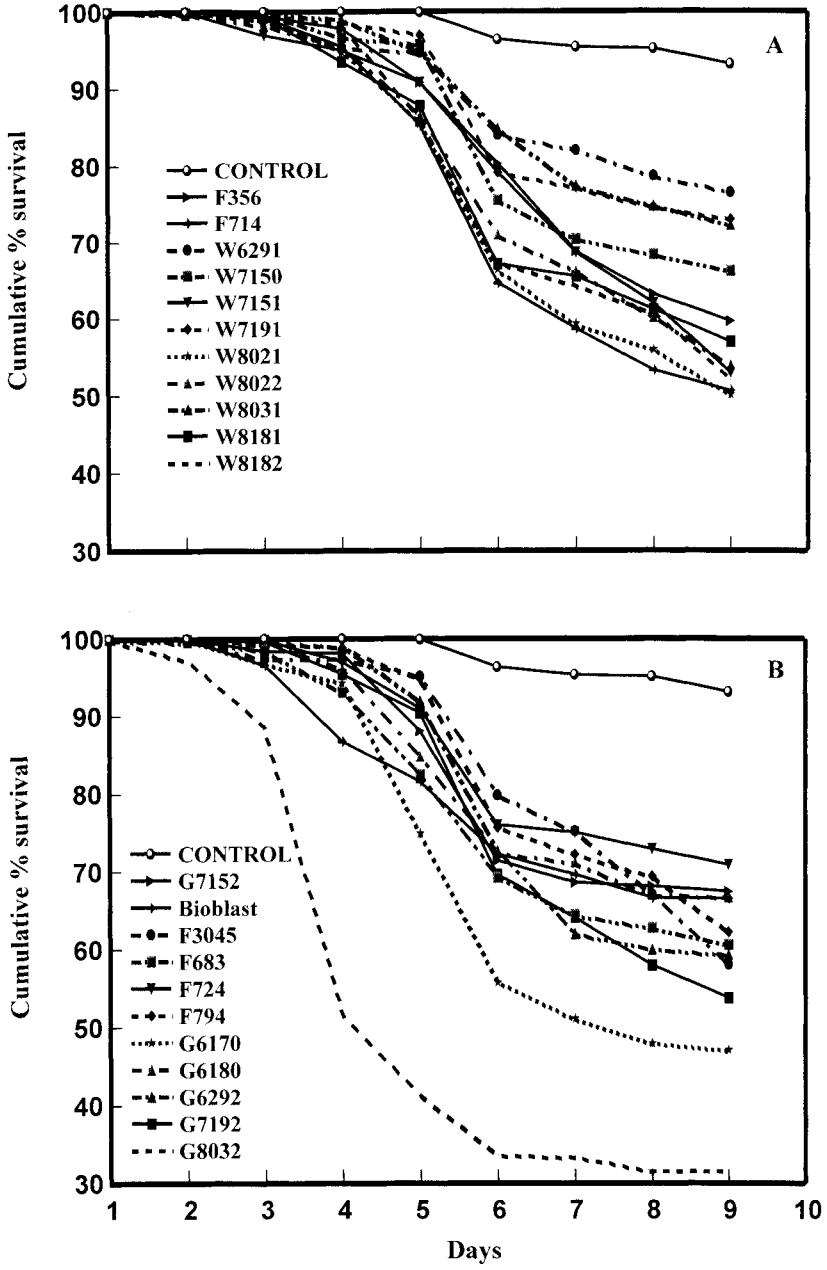


Fig. 1. Cumulative percentage survival of termites after inoculation with *B. bassiana* (A) or *M. anisopliae* (B).

Hawaii contained factors pernicious to termites, mainly entomogenous fungi (Ko 1982); *C. formosanus* is an important pest of wood structures in Hilo and Kona on the island of Hawaii, but it has never been observed in Kamuela (Tamashiro et al. 1980). The success rate of colony founding pairs in a new soil environment is generally very low (Fei 2000, McMahan 1962, Rosengaus and Traniello 1993), although *C. formosanus* appears to be relatively free of serious fungal diseases in its nests (Milner 1997, Milner et al. 1998a, Roberts and Humber 1981). A possible reason for this low success rate is the presence of *M. anisopliae* and *B. bassiana* (Ko 1982, Jackson et al. 2000), which are distributed in soils worldwide (McCoy et al. 1988).

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