

Potential use of pathogenic fungi in baits to control the Formosan subterranean termite (*Isopt.*, *Rhinotermitidae*)

K. M. Delate, J. K. Grace and C. H. M. Tome

Department of Entomology, University of Hawaii, Honolulu, Hawaii, USA

Abstract: A laboratory choice test was developed to assess the efficacy and acceptability of sporulating strains of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin to foraging Formosan subterranean termites, *Coptotermes formosanus* Shiraki. Filter paper disks were saturated with agar media and inoculated with either *B. bassiana* strain 787 or 3041, or *M. anisopliae* strain 346, 472 or 2162. Filter papers containing eight-day-old cultures were rolled into cylinders with the sporulating culture on the inside of the cylinder, and placed in one of two plastic jars, connected by a glass tube. Termites were placed in the other jar (refugia), and allowed to forage throughout both jars. This apparatus simulates the use of a fungal bait for subterranean termite control in the field. Termites did not avoid contact with the fungal baits, and fed upon the agar-treated filter papers. Exposure to *M. anisopliae* strains 346 and 2162 resulted in rapid termite mortality while exposure to the *B. bassiana* strains caused slower, but escalating, mortality. Efficacy of the fungi was not affected by the degree of sterility of the termite tunnelling medium. Living cultures of entomopathogenic fungi hold promise for further development as baits for subterranean termite control.

1 Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was first collected in Honolulu around 1907 (ZIMMERMAN, 1948), and was probably introduced to Hawaii prior to 1869, an area devoid of its natural enemies and competitors. Today, it is the most serious insect pest in Hawaii, costing residents approximately US \$100 million each year for control and repair of damages (YATES and TAMASHIRO, 1990). Although primarily known for damage to structures throughout the tropics and subtropics (SU and TAMASHIRO, 1987), *C. formosanus* also attacks field and tree crops, with estimates of the number of host plants in Hawaii ranging from 48 species (LAI et al., 1983) to our current list of 57 species.

Alternatives to conventional soil insecticides for remedial termite control include insecticidal baits (SU et al., 1987; GRACE and ABDALLAY, 1990; GRACE, 1992) and insect pathogens such as the entomogenous fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin (ZOBBERI and GRACE, 1990b; GRACE, 1993). Baits are particularly attractive control methods since they require only a small amount of insecticide and, ideally, only a portion of the colony is required to come into contact with the bait material to allow distribution by the foragers through the entire colony. Fungal pathogens exhibit characteristics similar to those of desirable bait toxicants, in that they are slow-acting, self-replicating agents which can be vectored throughout the colony (GRACE and ZOBBERI, 1992).

Insect biological control by pathogenic organisms can occur via classical methods (introduction and establishment), augmentation (inundative or inoculative

releases), manipulation of the environment, or conservation of existing microbial agents (LACEY and HARPER, 1986; FUXA, 1987; GRACE, 1994). Fungal pathogens offer great promise for biological control of soil-inhabiting insects (STOREY and MCCOY, 1992) because desiccation of fungal spores and destruction by UV radiation and temperature extremes are mitigated in the soil matrix.

Despite the limited reports of pathogens isolated from termites (LOGAN et al., 1990; ZOBBERI and GRACE, 1990a, b), several successful microbial control experiments with termites have been reported (TOUMANOFF and ROMBAUT, 1965; BAO and YENDOL, 1971; KRAMM et al., 1982; LAI et al., 1982; PRESTON et al., 1982; HANEL and WATSON, 1983; ZOBBERI and GRACE, 1990b; SUZUKI, 1991; FERNANDES, 1991; GRACE and ZOBBERI, 1992; GRACE, 1993). In Hawaii, LEONG (1966) obtained complete control of *C. formosanus* exposed to sporulating cultures of *M. anisopliae* and *B. bassiana* in laboratory studies. *Coptotermes formosanus* fills voids in its colony with nest material, or carton, consisting of masticated wood fibres, soil particles, salivary and faecal secretions (ZIMMERMAN, 1948), which offers an additional matrix for the proliferation of an introduced fungus into the colony. In preliminary studies in our laboratory (W. E. JONES, J. K. GRACE and M. TAMASHIRO, unpubl.), 49–100% mortality resulted in groups of *C. formosanus* when fungal spores from three strains each of *B. bassiana* and *M. anisopliae* were applied as a dust to 20% of the worker population. All of these strains were also capable of growth and spore production on carton material. Selection of the most effective fungal strains is critical, since control of a field colony containing millions of termites is obviously much more difficult than laboratory results with small groups might indicate

(LAI, 1977). Building upon these previous studies, we investigated the possibility of a fungal bait system for subterranean termite control, concentrating on the potential repellency of fungal isolates, and the propensity of foraging termites to contact sporulating fungal cultures.

2 Materials and methods

2.1 Fungi and termites

Fungal isolates were obtained from the United States Department of Agriculture, Agricultural Research Service collection of Entomopathogenic Fungi (ARSEF) (USDA-ARS Plant Protection Research Unit, U.S. Plant, Soil, and Nutrition Lab., Ithaca, New York). ARSEF accession numbers and hosts were as follows: *Beauveria bassiana* (Balsamo) Vuillemin strain 3041, isolated from *Reticulitermes flavipes* (Isopt. Rhinotermitidae) (ZOBARI and GRACE, 1990b); *B. bassiana* strain 787 (Col., Tenebrionidae); *Metarhizium anisopliae* (Metsch.) Sorokin strain 472 (?*Dasyganthus* sp., Col., Scarabaeidae); *M. anisopliae* strain 346 (*Aphodius tasmaniae*, Col., Scarabaeidae); and *M. anisopliae* strain 2162 (Chafer larva, Col., Scarabaeidae).

Cultures of *B. bassiana* and *M. anisopliae* were maintained on Sabouraud dextrose agar (Difco)+0.5% yeast extract (SDAY) at pH 6.8 (after BAO and YENDOL, 1971), and grown in the dark at 25°C. Based on previous experience with cultures of different ages W. E. JONES, J. K. GRACE and M. TAMASHIRO, unpubl.), 14-day-old sporulating cultures were chosen as our standard for termite exposure. Strains were recultured every three weeks, and each strain was periodically re-isolated from inoculated workers to ensure virulence.

Formosan subterranean termites, *C. formosanus*, were collected from an active field colony on the Manoa campus of the University of Hawaii, in a method previously described by TAMASHIRO et al. (1973). Termites were collected immediately before their use in each laboratory bioassay.

2.2 Agar bait station

Rather than forcing termites into contact with the fungal pathogens, a choice test was designed that would better simulate field conditions by providing both a foraging arena and a fungus-free nesting area, or refugia. Two disposable polystyrene Petri dishes (150 × 25 mm) were connected by a glass tube (8 × 130 mm). Both dishes contained a moistened Whatman No. 2 filter paper disk covered by 200 ml of sterile silica sand (Silica S151—Fine Grain Silica Dioxide, Fisher Scientific, Fair Lawn, New Jersey) to which 50 ml of distilled water was added. The treatment dishes (nest refugia and foraging arena with agar bait) were constructed as follows: pieces of sterile tongue depressors (127 × 174 mm), serving as a food source, were inserted into small Petri dishes (35 × 10 mm) containing SDAY media only (refugia dish) or a 14-day culture of *B. bassiana* strain 3041 or *M. anisopliae* strain 472 on SDAY media (agar bait dish). The small dishes were inverted and placed in the center of the larger Petri dishes where the sand had been excavated to accommodate the bait station. Controls contained two refugia dishes only. A strip of corrugated cardboard was placed in the glass tube connecting the two dishes to facilitate movement between the two sides of the bioassay unit. Two hundred *C. formosanus* (180 workers [pseudergates, or undifferentiated individuals older than the third instar] and 20 soldiers) were added to the refugia dish of each bioassay unit. Five replications were prepared of each *B. bassiana* and *M. anisopliae* strain and the controls. Dishes

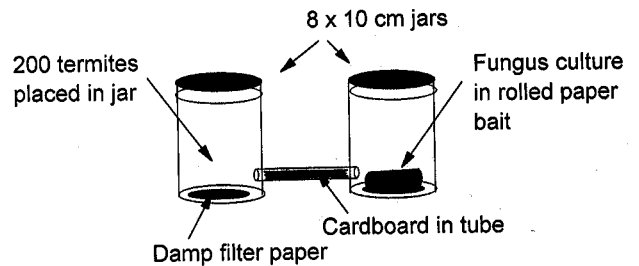


Fig. Bioassay apparatus for assessing termite foraging on paper baits containing fungi

were covered with standard Petri dish covers and placed in an unlighted temperature-controlled cabinet at 25°C.

Termite mortality was evaluated after 8 days. Percentage mortality data were transformed by the arcsine of the square root and subjected to analysis of variance (ANOVA), and means significantly different at the 0.05 level separated by the Ryan-Einot-Gabriel-Welsch multiple F-test (SAS INSTITUTE, 1987).

2.3 Rolled paper baits

2.3.1 14-day choice test

In this experiment, rolled paper baits containing sporulating fungus cultures were created to simulate a possible method of fungal delivery to termites in the field. Similar baits could be delivered to foraging subterranean termites by insertion into bait stations in the soil, such as the hollow wooden stakes described by EWART et al. (1992), or inserted into termite galleries in infested wood. *Beauveria bassiana* strain 3041 and *M. anisopliae* strain 2162 were used, 200 termites (180 workers and 20 soldiers) were placed in each experimental unit, and five replicates were prepared with each fungal strain and controls.

The rolled paper baits consisted of Whatman No. 2 filter paper disks. Each disk was placed flat in the bottom of a Petri dish, impregnated with 6 ml of SDAY media and inoculated with *B. bassiana* or *M. anisopliae*. After sporulation of the cultures (8 days), the disk was removed from the Petri dish, and rolled so that the sporulating fungal culture was on the inside of the paper tube with the agar underside of the filter paper presented to the termites.

Each experimental unit consisted of two wide-mouth plastic screw-top jars (8 × 10 cm), connected near the base by a glass tube (8 × 130 mm) containing corrugated cardboard (see fig.). One of the jars served as a refugia and contained two moistened Whatman No. 2 filter paper disks, while the other jar contained a rolled paper bait with either *B. bassiana* or *M. anisopliae*. In the control units, both jars contained two moistened Whatman No. 2 filter paper disks. Termites were placed on the refugia side of each unit, and the lids were loosely placed on the jars. The experimental units were placed in an unlighted cabinet at 25°C, and the paper disks were moistened with 0.5 ml of distilled water every other day. Termite mortality was evaluated after 14 days, and percentage mortality data were analysed as described above.

2.3.2 Post-exposure mortality

Fungal strains used in this experiment were *B. bassiana* strain 787 and *M. anisopliae* strain 346, and rolled paper baits were prepared as previously described. As described above, each experimental unit consisted of two connected plastic jars. However, instead of damp filter papers, the refugia jar in each unit contained a rolled Whatman No. 2 filter paper disk impregnated with 6 ml of SDAY media alone as a control.

Table 1. Mortality of *C. formosanus* termites exposed to fungus cultures in agar bait stations in a 8 day choice test

Treatment	Mortality (%)
<i>M. anisopliae</i> 472	51.9 ± 18.6a
<i>B. bassiana</i> 3041	50.5 ± 30.0a
Agar control	64.6 ± 21.4a

Each mean (±SD) represents 5 replicates of 200 termites each. Means followed by the same letter are not significantly different at the 0.05 level.

Five replicates were prepared for each *B. bassiana* and *M. anisopliae* strain and controls, each with 200 termites (180 workers and 20 soldiers). The experimental units were disassembled after 6 days incubation in an unlighted cabinet at 25°C, and termite mortality evaluated. To assess latent effects of fungus exposure, surviving termites in each unit were transferred to a jar containing a moistened paper disk, and mortality was assessed again after 7 days. Percentage mortality data were analysed as described above.

2.3.3 Sterile and unsterile conditions

An experiment was performed to assess the effect of sterile environmental conditions compared with unsterile conditions, such as are found in the field, on the efficacy of rolled paper fungus baits. As described above, two sterile disposable plastic jars (8 × 10 cm) were connected near the base by a glass tube (8 × 130 mm) containing corrugated cardboard. In the sterile treatment, one jar contained 75 g of sterile (autoclaved) silica sand with a rolled paper bait on the surface (*B. bassiana* strain 787), while the other refugia jar contained 30 g of autoclaved termite carton material. This carton was collected from termite trap boxes at our field sites, crushed to a fine matrix, sifted through a 12 cm mesh screen, and sterilized by autoclaving. In the unsterile treatment, the experimental units were similar, except that neither the sand nor carton were sterilized prior to initiation of the bioassay. Each jar received 15 ml of distilled water, and 200 termites (180 workers and 20 soldiers) were placed in the refugia jar prior to incubation in an unlighted cabinet at 25°C. Four replicates were prepared for the sterile units, unsterile units and the controls (rolled papers impregnated with agar alone). Termite mortality was evaluated after 15 days, and percentage mortality data were analysed as described above.

3 Results and discussion

In all experiments, termites moved readily from the refugia side of the bioassay units through the glass tube to the containers with the fungal baits. In our initial experiment with agar bait stations placed in sand, termites tunneled into the agar, but neither exposure to the *B. bassiana* nor the *M. anisopliae* culture for eight days elicited termite mortality exceeding that observed in the controls (table 1). In both the treatment and control replicates, however, the agar bait stations were in direct contact with the sand matrix, which created an environment conducive to competing bacterial growth (i.e., *Serratia* sp.). Within three days of initiation of this bioassay, many termites in both treatment and control replicates were visibly infected with *Serratia* sp., as evidenced by the high control mortality. Because of this contamination problem, and the lack of significant ter-

Table 2. Mortality of *C. formosanus* termites exposed to fungus cultures in rolled paper baits in a 14 day choice test

Treatment	Mortality (%)
<i>M. anisopliae</i> 2162	100 ± 0a
<i>B. bassiana</i> 3041	70.4 ± 19.0b
Agar control	33.6 ± 6.2c

Each mean (±SD) represents 5 replicates of 200 termites each. Means followed by the same letter are not significantly different at the 0.05 level.

mite mortality after eight days, we omitted the sand matrix in subsequent experiments and lengthened the exposure period to the fungal cultures.

In the experiments with agar-coated rolled paper baits, termites readily explored the rolled paper, and were observed feeding on the papers within two days of test initiation. After 14 days of exposure, significantly greater termite mortality was observed in the fungal units than in the control replicates, with *M. anisopliae* eliciting complete mortality within that period (table 2). Termites exposed to *M. anisopliae* strain 2162 appeared slow-moving on the second day of the bioassay, congregated on the rolled paper baits in the manner described by LEONG (1966) prior to their death, and died by the fourth day. Mortality occurred more slowly with both *B. bassiana* strain 3041 (table 2) and strain 787 (table 3). This slower mortality profile is generally characteristic of exposure to *B. bassiana* (GRACE, 1991; GRACE and ZOBBERI, 1992). Latent mortality from exposure to *B. bassiana* was particularly apparent in our second experiment with rolled paper baits, in which termite mortality continued to increase up to seven days after cessation of exposure to *B. bassiana* strain 787 (table 3).

A 15-day exposure to rolled paper baits containing *B. bassiana* strain 787 caused complete mortality in groups of *C. formosanus* termites (table 4). Although we had speculated that competition from other fungi or bacteria might inhibit effects of *B. bassiana* exposure under unsterile conditions, sterility of the sand-carton tunnelling matrix did not affect termite mortality. In previous work, we found that both *B. bassiana* and *M. anisopliae* were capable of growth and sporulation on sterile *C. formosanus* carton. In this experiment, we

Table 3. Mortality of *C. formosanus* termites exposed to fungus cultures in rolled paper baits in a 6 day choice test immediately after exposure and 7 days post-exposure

Treatment	Treatment mortality (%)	Post-treatment mortality (%)
<i>M. anisopliae</i> 346	100 ± 0a	100 ± 0a
<i>B. bassiana</i> 787	31.1 ± 11.9b	69.2 ± 16.1b
Agar control	18.4 ± 9.2b	38.7 ± 6.1c

Each mean (±SD) represents 5 replicates of 200 termites each. Means within a column followed by the same letter are not significantly different at the 0.05 level.

Table 4. Mortality of *C. formosanus termites* exposed to *B. bassiana* strain 787 in rolled paper baits in 15 day choice tests under sterile or unsterile conditions

Treatment	Mortality (%)
Sterile conditions	100 ± 0a
Unsterile conditions	100 ± 0a
Agar control (unsterile)	27.5 ± 3.1b

Each mean (±SD) represents 4 replicates of 200 termites each. Means followed by the same letter are not significantly different at the 0.05 level.

noted that individuals infected with *B. bassiana* strain 787 were left in the open, contrary to the typical termite response of burying infected individuals (KRAMM et al., 1982; ZOBBERI and GRACE, 1990b). This may indicate variation in termite response to different isolates of this pathogenic fungus, which could be manipulated by selection of appropriate isolates to achieve greater spread of the pathogen through the colony.

Several contradicting factors impact upon the potential use of insect pathogens for termite control. The social interactions of termites and other social insects (grooming and food sharing) facilitate the dissemination of inoculum throughout the colony (IGNOFFO, 1992). The humid, confined environment of the termite colony structure is also conducive to fungal growth and survival. Termite defensive and avoidance responses toward infected or otherwise abnormal individuals, however, would seem to inhibit the successful integration of spore-bearing termites in the colony (KRAMM et al., 1982; LOGAN et al., 1990; ZOBBERI and GRACE, 1990b). Our study suggests that fungal bait stations may circumvent these problems by providing a continuous and non-repellent source of sporulating cultures for foraging termites to contact. In these experiments, termites showed no propensity to avoid the rolled paper baits containing sporulating fungal cultures, and the normal microflora that termites carried into the laboratory did not interfere with the effects of *B. bassiana* and *M. anisopliae* on termite mortality.

Although LAI (1977) was unable to obtain control of *C. formosanus* in the field by treating foraging groups with conidia of either a *B. bassiana* strain or a *M. anisopliae* strain, inadvertent use of less-virulent strains or rapid isolation of the infected individuals in the absence of a continuing source of inoculum may have been contributing factors. Survival of *B. bassiana* spores and control of other insects in soil have been reported (MCCOY et al., 1984; WATT and LEBRUN, 1984), although STIMAC et al. (1993) reported less than optimum ant mortality when *B. bassiana* conidia were mixed with soil. Indeed, unsterilized aqueous extracts of soil were found to inhibit germination of *B. bassiana* conidia (CLERK, 1969). Microbial populations or chemical compounds from unsterilized soil may interfere with the germination and growth of insect pathogens (LINGG and DONALDSON, 1981; OSBORNE and BOUCIAS, 1985), due to the fungistatic properties of soil bacteria or actinomycetes. CLERK (1969), however, stated that conidia of insect-parasitizing fungi may lie dormant until stimu-

lated by the presence of an insect. Our results correspond to those of GRACE and ZOBBERI (1992), and indicate that delivery of a sufficient quantity of inoculum on living termite workers and transfer from those workers to nestmates, rather than the degree of microbial or chemical interactions in the surrounding soil, is the critical factor for eliciting mortality in the colony. We are continuing additional studies to identify promising fungal strains and develop methods of field application for subterranean termite control.

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Authors' addresses: Dr K. M. DELATE, Beaumont Experiment Station, University of Hawaii, 461 W. Lanikaula St., Hilo, HI 96720, USA; Prof. J. K. GRACE (for correspondence) and C. H. M. TOME, Department Entomology, University of Hawaii, 3050 Maile Way, Honolulu, HI 96822-2271, USA