

Experimental Evidence for Transmission of *Beauveria bassiana* by *Reticulitermes flavipes* Workers (Isoptera: Rhinotermitidae)¹

by

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ABSTRACT

Insect pathogens are potentially useful in bait systems for termite control if a significant portion of the colony can be infected without stimulating avoidance of the pathogen and infected individuals by healthy termites. In a laboratory study, *Reticulitermes flavipes* workers were exposed to a sporulating culture of a *Beauveria bassiana* strain originally isolated from *R. flavipes*. When the fungus-exposed workers were placed in nest containers with uninfected termite workers, transfer of conidia among the termites and growth of the pathogen caused significant mortality among the unexposed workers. However, exposure of as large a proportion as 50% of the test group to the fungal culture did not result in greater than 50% mortality of the unexposed workers during the 15-day assay period. We speculate that improved fungal culture and inoculation methods can infect termite workers with a greater number of conidia, leading to greater mortality from spore transfer. Our results confirm the potential value of fungi in termite control, but illustrate the problem of delivering a sufficient quantity of inoculum without stimulating colony defensive behaviors such as isolation of infected individuals.

INTRODUCTION

The efficacy of baiting systems for subterranean termite (Isoptera: Rhinotermitidae) control will depend upon successful delivery of a slow-acting toxicant to the colony. Insect pathogens would

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be ideal slow-acting toxicants, due to their self-replicating nature and safety to nontarget organisms. However, as pointed out by Logan *et al.* (1990) in a recent review of non-chemical termite control, the common termite behavior of isolating and avoiding infected colony members is a significant impediment to the transfer of pathogens within the colony.

Since avoidance of infected individuals depends upon recognition of the infection, vectoring of pathogen inoculum by unimpaired termites would be the most effective method of infiltrating the colony. Although nematodes have received the most attention as microbial control agents, largely due to their host-seeking abilities, their aggressive penetration of the host and the rapid rate of host mortality (Georgis *et al.* 1982; Trudeau 1989) may not be ideal for infiltrating a termite colony. On the other hand, the spores (conidia) of entomopathogenic fungi might be vectored passively on the termite cuticle and transferred to other individuals by mutual grooming behavior before infection is apparent, in a manner analogous to the transfer of insecticidal dusts (Grace 1991a). Promising, although not completely successful, field results were reported by Hanel & Watson (1983) in tests of *Metarhizium anisopliae* (Metsch.) Sorok. spores against *Nasutitermes exitiosus* (Hill). Recently, Fernandes (1991) reported successful control by inoculating mounds of *Cornitermes cumulans* (Kollar) with fairly large quantities of conidia of both *M. anisopliae* and *Beauveria bassiana* (Bals.) Vuill.

In the course of a survey of the mycoflora associated with eastern subterranean termite, *Reticulitermes flavipes* (Kollar), colonies in the Toronto area (Zoberi & Grace 1990a), we reported the isolation of a pathogenic strain of *B. bassiana* (Zoberi & Grace 1990b). In this paper, we describe the results of a laboratory experiment to determine the effectiveness of *R. flavipes* workers in transferring *B. bassiana* spores and inducing infection by such indirect exposure in colony mates.

MATERIALS AND METHODS

Eastern subterranean termites, *R. flavipes*, were collected from a site in Scarborough, Ontario, using a trapping technique described by Grace (1989). Collected termites were maintained on corrugated cardboard in plastic boxes in a dark cabinet at

27 ± 0.5 °C and 90 ± 5% RH. The *B. bassiana* culture was originally isolated from *R. flavipes* workers collected in Toronto, Ontario (Zoberi & Grace 1990b). This isolate is deposited in the Canadian Collection of Fungus Cultures (Biosystematic Research Centre, Agriculture Canada, Ottawa, Ontario), and in the USDA-ARS Collection of Entomopathogenic Fungi (Boyce Thompson Institute at Cornell University, Ithaca, New York) as ARSEF strain No. 3041.

Termite workers (pseudergates) that were not to be exposed to the fungus were marked by feeding on Whatman No. 3 filter paper impregnated with a 2% (weight/weight) concentration of the oil-soluble dye Fat Red 7B (Sigma Chemical Co., St. Louis, Missouri) (=Sudan Red 7B) for five days. This dye marker is clearly visible through the cuticle for 15 days, and is not passed in significant concentration by trophallaxis or cannibalism to interfere with recognition of marked individuals (Grace & Abdallay 1989; Grace *et al.* 1989). At the end of the five-day feeding period, three groups each of 40, 30, or 20 dyed workers were placed in 44.8ml polystyrene vials (60X36mm dia.) containing 7ml (ca 10g) washed silica sand, a ca 2X6.5cm strip of Whatman No. 1 filter paper as food, and 1.5ml deionized water. The vials were capped with polyethylene foam plugs and placed in the dark cabinet for 24 hours. After this acclimatization interval, termite workers that had been exposed to *B. bassiana* were added to the vials to form groups consisting of either 0:40, 10:30, 20:20, or 40:0, treated: untreated workers.

The *R. flavipes* workers were exposed to *B. bassiana* by placing groups of 40 workers into 44.8ml polystyrene vials containing an agar plug cut from an actively sporulating 2-week old culture of *B. bassiana* grown on water agar (Zoberi & Grace 1990b). After a one hour exposure to the fungus, the termites were gently transferred to the prepared incubation vials described above. Thus, each vial contained 40 termite workers, with 0, 25, 50, or 100% of each group (n = 3 replicates) having been exposed to sporulating *B. bassiana*.

Termite mortality was recorded 15 days after introduction of the fungus-inoculated workers. Percentage mortality was transformed by the arcsine of the square root and subjected to analysis of variance (ANOVA), with significantly different means separated by the Ryan-Einot-Gabriel-Welsch multiple F test at $\alpha \leq 0.05$ (SAS Institute 1987).

RESULTS AND DISCUSSION

Almost all (80–100%) of the *R. flavipes* workers exposed to the sporulating *B. bassiana* culture died within the 15 day test interval (Table 1). In comparison to the low mortality in the unexposed control groups ($8 \pm 6\%$), mortality was significantly greater among the unexposed termites in the groups consisting of either 25% or 50% *B. bassiana*-treated workers. Thus, living *R. flavipes* workers are effective vectors of *B. bassiana* spores, while the introduction of dead fungus-killed workers under similar conditions to groups in test vials did not result in sufficient spore transfer or mycelial growth to cause significant mortality (Zoberi & Grace 1990b). Similar conclusions were reached by Kramm *et al.* (1982) concerning transfer of *M. anisopliae* spores among *Reticulitermes* spp. Avoidance of dead individuals, grooming of active termites, and dissemination of fungal spores through the gallery system (due to motion and contact with the gallery walls) by active termites are important considerations and suggest that slow spore germination and/or slow disease development would be of value in encouraging spread of the pathogen through the termite colony.

Table 1. Mean (\pm SD) percentage mortality in groups of *R. flavipes* workers maintained in damp sand for 15 days. Prior to incubation, a proportion of each group was exposed to a sporulating *B. bassiana* culture.

Proportion Exposed	Percent Mortality ^a		
	Exposed	Unexposed ^b	Total Group
100%	98 \pm 3a	-	98 \pm 3a
50	83 \pm 21a	47 \pm 28a	65 \pm 24b
25	93 \pm 12a	38 \pm 9a	52 \pm 7b
0	-	8 \pm 6b	8 \pm 6c

^aMeans within each column followed by the same letter are not significantly different ($\alpha \leq 0.05$, ANOVA of transformed values, Ryan-Einot-Gabriel-Welsch multiple F test).

^bUnexposed workers were marked with the dye Fat Red 7B (= Sudan Red 7B).

Although significant (Table 1), the levels of mortality resulting from the presence of 25–50% *B. bassiana* exposed workers would not be considered sufficient to control a termite infestation in the field. The spore load vectored by *R. flavipes* workers was not determined in this study, and may have been less than optimal due to our method of inoculation and use of water agar as the growth medium. In recent studies of the potential of this fungal isolate to control *Coptotermes formosanus* Shiraki (Grace 1991b), Sabouraud dextrose agar with 0.5% yeast extract (after Bao & Yendol 1971) was found to be a preferential medium for *B. bassiana* growth (unpubl. data). Transmission tests in which *C. formosanus* workers were rolled in harvested spores, rather than allowed to walk over an agar culture, resulted in greater mortality (Grace, Tamashiro & Jones, in prep.) than in the current study.

Although the potential value of pathogens in termite control is apparent, successful application will depend upon our ability to overcome the chief difficulty faced in development of any bait-toxicant system: maximizing delivery of the non-repellent and slow-acting pest control agent throughout the colony to achieve the high mortality needed for effective termite control. Bait toxicants may be used in combination with chemical barriers (or nonchemical particle barriers) and remedial wood treatments, but the ultimate goal of bait development is the eradication of nuisance termite colonies. Pathogens are suited to this goal, if sufficient inoculum can be transmitted and the social defenses of the colony are not triggered by the pathogen or the infected termite vectors.

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