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Inhibition of Termite Feeding by Fungal Siderophores

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Inhibition of Termite Feeding by Fungal Siderophores[1,2,3]

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ABSTRACT

Siderophores are iron-chelating extracellular fungal metabolites which may be involved in initiating wood decay. A purified siderophore extract isolated from the brown-rot decay fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. (Basidiomycetes: Polyporaceae) was found to deter feeding by Formosan subterranean termites, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). This fungus has previously been associated with preferential feeding on decayed wood by subterranean termites, and solvent extracts have been reported to induce termite trail-following, arrestment, and/or aggregation. This is the first report of *G. trabeum* metabolites or fungal siderophores having a negative behavioral effect on subterranean termites.

KEYWORDS: siderophores, *Coptotermes formosanus*, *Gloeophyllum trabeum*, Rhinotermitidae

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1 INTRODUCTION

Many brown and white-rot wood decay fungi (Basidiomycetes: Polyporaceae) produce low molecular weight (500-1000 daltons) iron-chelating extracellular metabolites, or siderophores (Fekete et al. 1989; Jellison et al. 1990; Kim et al. 1990). Because cellulase enzymes are too large to diffuse freely through wood cell walls (Cowling and Brown 1969), a non-enzymatic degradative agent has been proposed to be active in the initial stages of fungal decay (Koenigs 1974). In addition to scavenging transition metals for fungal metabolism and extracellular metabolism, siderophores may play such a role in lignocellulose degradation by preceding enzymes into the wood cell wall (Jellison et al. 1990).

Fekete et al. (1989) demonstrated that the brown-rot decay fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. produced phenolate-type siderophores. This fungus also produces compounds inducing trail-following, arrestment and/or aggregation, and preferential feeding by subterranean termites (Isoptera: Rhinotermitidae) (Esenther et al. 1961; Allen et al. 1964; Grace 1989, 1990). The compound (cis,cis,trans)-3,6,8-dodecatrien-1-ol, originally isolated from *G. trabeum* infected wood (Matsumura et al. 1968), is considered to be the principal component of the trail pheromone of a number of Rhinotermitids, including the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Tokoro et al. 1990).

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In an ongoing investigation of termite-fungal interactions, we evaluated the effects of a purified siderophore extract from *G. trabeum* on *C. formosanus* feeding behavior. Although it is possible that siderophores may play a role in lignocellulosic degradation by both fungi and wood-feeding insects, filter papers treated with the *G. trabeum* extract were found to deter feeding by *C. formosanus*. Production of semiochemicals having positive effects on termite behavior varies with different *G. trabeum* isolates and culture conditions (Amburgey and Smythe 1977), but induction of negative behavioral effects by *G. trabeum* metabolites has not previously been reported.

2 MATERIALS AND METHODS

The purified siderophore extract was prepared as described by Jellison et al. (1990) from *G. trabeum* grown in low iron stationary liquid culture media. The spent culture media was separated from the mycelium by filtering through Whatman No. 2 filter paper, concentrated by rotary evaporation, and filtered through an Amicon ultrafiltration unit using a YM2 membrane (1000 MW cutoff). Phenolate siderophores were isolated by acidification of the supernatant to pH 2.5 with concentrated HCL, three-fold extraction with an equal volume of ethyl acetate, evaporation of the ethyl acetate extract to dryness, and resuspension of the residual in deionized water. Whatman No. 2 filter papers were soaked to saturation in the aqueous suspension, and air-dried in the dark at ambient laboratory conditions.

For bioassays with *C. formosanus*, the treated filter papers were cut into ca. 1.5 cm squares. Three bioassays were performed: (i) a no-choice (forced-feeding) assay in which only one food source (either a treated or untreated filter paper) was provided to the termite workers, (ii) a two-choice feeding assay in which each siderophore-treated paper was paired with an untreated control paper within the same termite container, and (iii) a siderophore degradation/leaching assay in which siderophore-treated papers were placed on damp sand without termites present for a period equivalent to that of the feeding assays.

In each assay, siderophore-treated and untreated filter papers were air-dried, weighed, and placed on ca. 5 ml dry crushed coral sand (sieved to pass a US 20-mesh screen) in a 3.5 cm diameter plastic petri dish, and 2.5 ml distilled water was added to the sand. *Coptotermes formosanus* workers were collected from traps on the Manoa campus of the University of Hawaii (Tamashiro et al. 1973), and 50 workers (pseudergates) were placed in each no-choice and two-choice replicate. In the no-choice feeding assay, 5 treated and 5 untreated papers were evaluated; the two-choice assay was performed with 10 replicates (each containing both a treated and untreated paper); and 5 treated papers were evaluated for weight loss without termites present in the siderophore degradation/leaching assay. After 3 days, surviving termites were counted, and the papers were removed, oven-dried (80°C, 24 hours), and weighed to determine mass loss due to termite feeding and/or siderophore degradation/leaching.

Due to the large paper weight losses observed in the siderophore degradation/leaching assay (without termites), weight loss data from all three assays were subjected to analysis of variance (unbalanced ANOVA, PROC GLM), and significantly different mean weight losses separated by Tukey's Studentized Range Test (SAS Institute 1987).

3 RESULTS AND DISCUSSION

Practically no termite mortality (4% maximum) was observed in either the no-choice or two-choice assays over the 3-day period. Termites were observed walking on both treated and untreated papers, and building their characteristic carton (consisting of sand, masticated paper, feces and salivary secretions) at the edges of both papers. However, the untreated papers were almost completely consumed in three days, while only slightly roughened edges, which could indicate very minor feeding, were noted on the siderophore-treated papers.

Termites fed equally on the untreated papers in the no-choice and two-choice assays (Table 1). The greater weight losses of the siderophore-treated papers in these assays was not due to termite feeding, as was confirmed by the equivalent mean weight loss of the treated papers when no termites were present. These weight losses are attributable either to leaching of the *G. trabeum* extract from the treated papers or siderophore-initiated chemical degradation of the cellulose.

Although this is the first report of *G. trabeum* metabolites deterring termite feeding, certain white-rot wood decay fungi have been reported to inhibit termite attack (Amburgey and Beal 1977). White-rot decay fungi also produce biological chelators (Fekete et al. 1989), which may initiate lignin oxidation as well as cellulose depolymerization (Jellison et al. 1990) as well as participate in some lignin degradation reactions. This suggests that the feeding deterrence observed in this study may be concentration dependent. Since termites and decay fungi compete for the same cellulosic resource, secretion of a termite feeding deterrent could benefit the fungus. Conversely, the presence of a high concentration of fungal siderophores could act as a chemosensory cue to termites that the substrate is excessively degraded and therefore nutritionally inadequate. This suggests an intriguing possibility for protecting wood from termite attack by artificial stimulation of this behavioral response. Studies are proceeding to clarify the mechanism of termite deterrence, and the role of fungal and siderophore-mediated lignocellulose degradation in feeding by the Formosan subterranean termite.

TABLE 1 Weight loss of siderophore-treated and untreated filter papers placed on damp sand for three days in no-choice (force-feeding) and two-choice (treated vs. untreated) assays with *Coptotermes formosanus* workers, and without termite workers present.

Assay type	Mean (± SEM) paper weight loss (mg)* Siderophore-treated	Untreated
No-choice feeding assay	17.42 ± 0.95 a	8.72 ± 0.62 b
Two-choice feeding assay	18.43 ± 0.83 a	8.22 ± 0.51 b
Without termites	17.34 ± 1.04 a	---

*Mean weight losses within each column are not significantly different (ANOVA, Tukey's Studentized Range Test, $\alpha \leq 0.05$). In no-choice and two-choice assays, termite feeding was observed only on the untreated papers.

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