

Fungi Isolated from Integument and Guts of *Coptotermes formosanus* and Their Antagonistic Effect on *Gloeophyllum trabeum*

POORNIMA JAYASIMHA AND GREGG HENDERSON¹

Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

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ABSTRACT In our earlier efforts to demonstrate the spread of a brown rot fungus, *Gloeophyllum trabeum* (Pers.) Murrill by Formosan subterranean termites, *Coptotermes formosanus* Shiraki, we found not only that *C. formosanus* does not spread *G. trabeum* but also that *G. trabeum* did not survive in the presence of *C. formosanus*. Further investigation of this antagonistic interaction between this termite and fungus led to a hypothesis that green-spored fungi may be carried by termites and that they play a role in suppressing the growth of *G. trabeum*. Fungal cultures were isolated from integument and guts of laboratory-maintained colonies of *C. formosanus* and groups of *C. formosanus* freshly collected from the field. Only the green-spored fungi were selected from the many fungi isolated. Green-spored fungi isolated from the integument were identified as *Aspergillus flavus* Link, *Trichoderma harzianum* Rifai, *Trichoderma virens* Miller et al., *Trichoderma asperellum* Samuels, Lieckfeldt & Nirenberg, and *Trichoderma ghanense* Y. Doi, Y. Abe & J. Sugiyama. A different set of fungi were isolated from the gut, which included *A. flavus*, *Hypocrea virens* Chavarri, Samuels and Steward, *T. asperellum* cultures along with *Penicillium janthinellum* Biourge and *Cladosporium cladosporioides* (Fres.) de Vries. *A. flavus* was associated with every laboratory maintained colony but was associated only with one replication of one of the field collected groups. Our results suggest that *A. flavus* may be contaminating the colonies that were maintained in the laboratory and the fungus may become proliferous as the colonies become weak. Dual culture tests showed that all the fungi isolated from the integument, and gut were parasites and/or antagonists and that they effectively controlled the growth of *G. trabeum*. We think termites may be using parasitic fungi to control a brown rot fungus.

KEY WORDS *Coptotermes formosanus*, *Gloeophyllum trabeum*, integument cultures, gut cultures, dual culture techniques

Interactions between fungi and termites have been the subject of many studies, and associations between fungi and termites were documented more than seven decades ago (Hendee 1933, 1934). Interactions between insects and fungi range from agonistic to mutualistic, and they include many spectacular examples of complex symbioses (Martin 1992). Fungi usually play an important role in termite nutrition by being a direct source of food or by modifying the wood to favor termite feeding (Beard 1974). In turn, termites may help the fungi by transporting and spreading them to new locations. The ability of termites to carry fungi was demonstrated by the isolation of fungi from the colony and from the gut of termites, and by the observation of hyphae, conidiophores, and conidia clinging to the bodies and appendages of termites (Hendee 1933).

Many studies focused on cultivation of fungi by mound-building higher termites in the family Termitidae (Zoberi 1979), but the relationship between lower

termites and fungi remains debatable (Zoberi and Grace 1990). Matsuura (2006) showed an extraordinary case of egg mimicry where *Fibularhizoctonia* sp. nov., a corticoid fungus, parasitically mimics the eggs of *Reticulitermes speratus* (Kolbe), *Reticulitermes flavipes* (Kollar), and *Reticulitermes virginicus* (Banks). Interactions between a brown rot fungus, *Gloeophyllum trabeum* (Pers.) Murrill, and subterranean termites gained importance when Esenther et al. (1961) showed that wood decayed by *G. trabeum* is attractive to *R. flavipes*, *R. virginicus*, and *Nasutitermes columbicus* Holmgren. It has been shown through many additional studies that wood decayed by the fungus *G. trabeum* produces an attractant for *R. flavipes* (Allen et al. 1964; Esenther and Coppel 1964; Smythe et al. 1965, 1967a, 1967b, 1971; Beard 1974) as well as other species of *Reticulitermes* (Becker 1965, Becker and Lenz 1975) and *Coptotermes* (Becker and Lenz 1975, Matsuo and Nishimoto 1974).

The same chemical responsible for trail forming, (Z,Z,E)-3,6,8-dodecatrien-1-ol, has been isolated from wood decayed by *G. trabeum* (Smythe et al. 1967b,

¹ Corresponding author, e-mail: grhenderson@agcenter.lsu.edu.

Table 1. Collecting date and location of the termites used to isolate fungi from integument and guts

Culture	Lab colonies	Date of collection	Collected from	Fresh field colonies	Date of collection	Collected from
Integument	Colony 1	4 Aug. 2004	Brechtel Park, New Orleans, LA	Collection 1	13 May 2005	Brechtel Park
	Colony 2	14 Oct. 2004		Collection 2	13 May 2005	
	Colony 3	24 Mar. 2005		Collection 3	13 May 2005	
				Collection 4	14 June 2005	Citrus Station, Port Sulfur, LA
				Collection 5	14 June 2005	
				Collection 6	14 June 2005	
Gut	Colony 4	9 Nov. 2005	Brechtel Park	Collection 7	7 Mar. 2006	Brechtel Park
	Colony 5	9 Nov. 2005		Collection 8	7 Mar. 2006	
	Colony 6	9 Nov. 2005		Collection 9	7 Mar. 2006	

Matsumura et al. 1968) and from whole body extracts of *R. virginicus* (Matsumura et al. 1969), and Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Tokoro et al. 1992). Observations of *R. flavipes* suggested that a fungal product might be helping termites locate the decaying wood; shelter tubes on buildings and trees invariably led directly to dead and decaying wood (Esenther et al. 1961). These lines of evidence indicate that *C. formosanus* benefits from *G. trabeum*.

In our earlier efforts to answer the questions 1) Does *C. formosanus* spread *G. trabeum* from infected wood to uninfected wood? and 2) What is the effect of *C. formosanus* on the growth of *G. trabeum*?, we found not only that *C. formosanus* does not spread *G. trabeum* but also that *G. trabeum* did not survive in the presence of *C. formosanus* (Jayasimha and Henderson 2007b). We also observed that when *G. trabeum*-infected wood pieces were exposed to Formosan subterranean termites, *G. trabeum* was suppressed and several green-spored fungi were introduced on to the wood pieces. This led us to hypothesize that these green-spored fungi may be carried by *C. formosanus* and act as fungistats controlling the growth of *G. trabeum*.

The environment in which termites live is particularly favorable for the growth of fungi (Beard 1974). With the exception of 25 fungal genera isolated by Hendee in 1933 from the western subterranean termite *Reticulitermes hesperus* Banks and termite-infested wood, little information is available on mycofloral communities associated with North American subterranean termite populations (Zoberi and Grace 1990). Hendee (1933) isolated *Penicillium* spp., *Trichoderma* spp., *Mucor* spp., *Tilachlidium* spp., *Mortierella* spp., *Catenularia* spp., *Oedocephalum* spp., *Lepidoglyphum* spp., *Oospora* spp., and *Acrostalagmus* spp. associated with colonies of *R. hesperus*. "Colony" in that study was defined as termites, fecal pellets, loose detritus, frass in their tunnels, and also wood incorporated into walls of the tunnels. Gouger and Kimbrough (1969) isolated a hyphomycete *Antennopsis gallica* Heim and Buchli, which grows on the bodies of *R. flavipes* and *R. virginicus*. Zoberi and Grace (1990) worked with *R. flavipes* and isolated several fungal cultures, such as *Mucor mucedo* (L.) Fr., *Mucor hiemalis* Weh., *Aspergillus niger* Van Tieg., *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., *Alternaria* spp., *Fusarium* spp., *Cladosporium* spp., and *Acremonium* spp. from their surfaces and their guts. Rojas et

al. (2001) isolated three fungal species associated with *C. formosanus*. This kind of mycofloral information is desirable from both a fundamental and applied view (Zoberi and Grace 1990). Our objectives were to isolate green-spored fungi present on the integument and guts of *C. formosanus* and to test the effect of these fungi on a brown rot fungus, *G. trabeum*.

Materials and Methods

Collection of Termites. *C. formosanus* used to isolate the fungi were collected from laboratory-maintained colonies or termite groups freshly collected from the field. Alcohol-sterilized gloves, forceps, and autoclaved glass jars wrapped in aluminum foil were used to collect the termites from the field to minimize contamination. Fungal isolation of field-collected groups was conducted within 24 h upon return to the laboratory.

Integument Cultures. Three laboratory maintained colonies (6 d–7 mo) were used to isolate fungi from the integument. Colony 1 was collected on 4 August 2004, colony 2 on 14 October 2004, and colony 3 on 24 March 2005 from Brechtel Park, New Orleans, LA, and the experiments were started on 30 March 2005 (Table 1). Additionally, six field-collected groups also were used for isolation of fungi from the integument of termites. Three of the six groups were collected from Brechtel Park on 13 May 2005, and they were used in the fungal isolations on 14 May 2005. The other three groups were collected from the LSU AgCenter Citrus Station, Port Sulfur, LA, on 14 June 2005, and they were used in the experiment on the same day (Table 1).

Gut Cultures. Three laboratory-maintained termite colonies used to isolate gut fungi were collected from Brechtel Park on 9 November 2005. Experiments were conducted on 13 February 2006 (Table 1). Three groups of field termites used to isolate gut fungi were collected from Brechtel Park on 7 March 2006, and they were used in the experiment on the same day (Table 1).

Isolation of Fungi from Termites. *Integument Cultures.* Seven termite workers from each colony were released into separate petri dishes containing potato dextrose yeast agar (PDYA) medium with 25 mg/liter streptomycin. Termites were allowed to walk on the medium for 1 min, their bodies were gently rubbed onto the medium, and then they were removed. The entire procedure was done under a laminar airflow

hood. Petri dishes were then sealed with parafilm, and they were incubated at 25°C for 4–7 d. Controls were handled in the same way as the treatments, except the termites were not added to the petri dishes. This procedure was repeated six times with three different laboratory-maintained colonies, and it was repeated three or five times with six different field-collected colonies. Any fungi growing were subcultured two or three times to obtain pure cultures. Only the green-spored fungi were selected from these pure cultures and rest of the fungi were discarded. These green-spored fungi were visually differentiated based on their color and colony growth characteristics. This resulted in 12–14 pure cultures from each laboratory maintained termite colonies and 11–15 pure cultures from each of field-collected termite groups. Subcultures were made thereafter every week to maintain the pure cultures.

Gut Cultures. Five percent NaClO (Acros Organics, Morris Plains, NJ) was diluted to 1% NaClO with sterile distilled water. Termites were surface sterilized using 1% NaClO, and they were subsequently rinsed three times with sterile distilled water. Each termite was held with sterile forceps, and the gut of the termite was gently pulled out with another sterile forceps. The gut was then gently streaked on the PDYA medium. Two termite guts were streaked onto PDYA medium in each petri dish. This procedure was repeated with three laboratory-maintained termite colonies and three field-collected termite groups. The entire experiment was replicated three times.

The procedure followed to obtain pure cultures and the selection and separation of green-spored fungi was similar to the procedure followed for the integument cultures. This resulted in two to eight pure cultures from each laboratory-maintained termite colony and one to three pure cultures from each field-collected termite group.

Selection and Identification of the Isolated Fungi. Based on the hypothesis that green-spored fungi may be carried by *C. formosanus* and act as fungivores controlling the growth of *G. trabeum*, we concentrated our work on the green-spored fungi only. Green-spored fungi isolated from the same termite colony were separated based on similar fungal colony characteristics. From the integument cultures, seven cultures of green-spored fungi in total were isolated from laboratory colonies 1, 2 and 3, and six cultures of green-spored fungi were isolated from fresh field collections 1, 2, 4, 5, and 6 (Table 2). From the gut cultures, five cultures in total of green-spored fungi from colony 4, 5, and 6, and six cultures from collection 7, 8, and 9 were isolated (Table 2). The purified cultures were identified by using morphological characters by Dr. Steven Carpenter (Abbey Lane Laboratory LLC, Philomath, OR) and in some cases genetic analysis by Dr. Gary Samuels (USDA-ARS, Systematic Botany and Mycology Laboratory, Beltsville, MD).

Dual Culture Techniques to Test the Antagonist Effects of Selected Fungi against *G. trabeum*. Fungi isolated from the integument were used in the dual culture studies. *Gloeophyllum trabeum* (Pers.) Murrill

Table 2. Number of green-spored fungal species isolated from each colony

Culture	Lab colonies	No. green-spored fungal species isolated	Fresh field termites	No. green-spored fungal species isolated
Integument	Colony 1	2	Collection 1	1
	Colony 2	2	Collection 2	1
	Colony 3	3	Collection 3	0
			Collection 4	2
			Collection 5	1
			Collection 6	1
Gut	Colony 4	3	Collection 7	2
	Colony 5	1	Collection 8	3
	Colony 6	1	Collection 9	1

isolate Madison 617 was purchased from American Type Culture Collection (Manassas, VA) (ATCC 11539). Integument cultures and *G. trabeum* were maintained on PDYA medium at the urban entomology laboratory at LSU AgCenter (Baton Rouge, LA). On the day of the experiment discs of the fungi on PDYA medium were cut out using a 0.75-mm-diameter cork borer. To determine whether selected fungi inhibited *G. trabeum* growth, a disc of *G. trabeum* was placed at one end of the petri dish containing PDYA medium. After 2 d, a disc of the selected fungi isolated from the termites was placed on the other end. Controls contained only *G. trabeum*. This procedure was replicated five times with 13 of the selected fungi. The total growth of *G. trabeum* and the identified green-spored fungi were measured after 15 d of incubation. Data were analyzed using analysis of covariance (ANCOVA) PROC MIXED version 9.0 (SAS Institute, Cary, NC).

Results

Identification of Selected Fungi. *Integument Cultures.* Fungi were identified as *Aspergillus flavus* Link, *Trichoderma harzianum* Rifai, *T. virens* Miller et al., *T. asperellum* Samuels, Lieckfeldt & Nirenberg, and *T. ghanense* Y. Doi, Y. Abe & J. Sugiyama. Of 13 cultures selected, six cultures were *T. harzianum* and four cultures were *A. flavus*. The final three cultures were identified as *T. virens*, *T. asperellum*, and *T. ghanense*.

Among the three laboratory colonies, *A. flavus* was present in all six replications of colony 1 and 2 and in four replications of colony 3 (Table 3). *T. harzianum* was present in two replications of colony 1 and 3 and *T. virens* in one replication of colony 2 (Table 3).

Among the field-collected termite groups, *T. harzianum* was isolated from one of five replications of collection 1 and 2, and *A. flavus* was not isolated from any of the replications of collection 1 and 2 (Table 3). No green-spored fungi were isolated from collection three (Table 3). *T. asperellum* was isolated from two of three replications of collection 4 and *A. flavus* was isolated from one replication (Table 3). *T. harzianum* was isolated from all the three replications of the collection 5 (Table 3). *T. ghanense* was isolated from two replications of collection 6. *A. flavus* was not isolated from collection 5 and 6 (Table 3).

Table 3. Fungi isolated from integument of laboratory-maintained termites vs. fungi isolated from field-collected termites

Fungal species	Laboratory-maintained colonies			Field-collected termites					
	Colony 1	Colony 2	Colony 3	Collection 1	Collection 2	Collection 3	Collection 4	Collection 5	Collection 6
<i>A. flavus</i>	+	+	+	—	—	—	+	—	—
<i>T. harzianum</i>	+	—	—	+	—	—	—	+	—
<i>T. virens</i>	—	+	—	—	—	—	—	—	—
<i>T. ghanense</i>	—	—	—	—	—	—	—	—	+
<i>T. asperellum</i>	—	—	—	—	—	—	+	—	—

+, presence of fungus; —, absence of fungus.

Gut Cultures. Fungi were identified as *A. flavus*, *Hypocrea virens* Chavarri, Samuels and Steward, *T. asperellum*, *Penicillium janthinellum* Biourge, and *Cladosporium cladosporioides* (Fres.) de Vries. *A. flavus* was isolated from all the laboratory maintained termite colonies, but it was not isolated from any of the field collections (Table 4). *P. janthinellum* was isolated from all of the three field collections, but it was not isolated from any of the laboratory-maintained termite colonies (Table 4).

Control of *G. trabeum* by Selected Fungi. Fungi isolated from the integuments of laboratory maintained termite colonies had a significant negative effect on the growth of *G. trabeum* in the dual cultures ($F = 1882.95$; $df = 1, 32$; $P < 0.0001$) (Fig. 1). The same species of fungi isolated from different laboratory maintained termite colonies did not significantly differ in their effect on *G. trabeum* ($F = 227.07$; $df = 3, 32$; $P > 1.00$). A similar pattern was observed with fungi isolated from field-collected termite colonies. All of the fungi isolated from the integuments of field-collected termite colonies had a significant negative effect on the growth of *G. trabeum* in the dual cultures ($F = 67.07$; $df = 1, 27$; $P < 0.0001$) (Fig. 2). Also, the same species of fungi isolated from different field-collected termite colonies did not significantly differ in their effect on *G. trabeum* ($F = 0$; $df = 2, 27$; $P > 1.00$). All of the fungi isolated from integuments of laboratory-maintained and field-collected termite colonies were antagonistic to *G. trabeum*, and they suppressed the growth of *G. trabeum* (Fig. 3).

Discussion

We isolated *Aspergillus flavus* and four *Trichoderma* spp. from the integument of the *C. formosanus*. We also isolated *A. flavus* from gut cultures of termites along with *Trichoderma asperellum*, *Penicillium janthinellum*, *Hypocrea virens*, and *Cladosporium cladospori-*

oides. *Trichoderma*, *Aspergillus*, and *Penicillium* have been previously isolated from different termite species in many studies; but the use of these fungi by termites to control a feeding competitor has not been suggested previously. For example, Hendee (1933) isolated cultures from the integument and guts of *R. hesperus*; *Kaloterme minor* Hagen; and Pacific dampwood termite, *Zootermopsis angusticollis* (Hagen). Hagen found that *Penicillium* and *Trichoderma* were the most common and widespread fungi among these termite species. Zoberi and Grace (1990) isolated 40 species of fungi from *R. flavipes*, including *Aspergillus*, *Penicillium*, and *Trichoderma*. Rojas et al. (2001) isolated two species of *Aspergillus* from the bodies of *C. formosanus*. *Aspergillus* spp. also has been isolated from colonies of *Kaloterme minor* Hagen by Hendee (1933).

A. flavus was predominant in all the external cultures and gut cultures made from laboratory-maintained colonies, but it was found in only one external culture from a field collection. *A. flavus* was not isolated from any of the gut cultures made from field-collected termites. Laboratory conditions, especially air exposure to carton nest and termites may be favorable for *A. flavus* allowing it to overtake other fungi.

Aspergillus can be very dangerous to termites (Becker and Kerner-Gang 1964). Several strains of *A. flavus* are known to be toxic to termites (Smythe and Coppel 1966, Lenz 1969). When *R. flavipes* and *R. virginicus* were infected with *A. flavus*, 80% mortality was observed (Beal and Kais 1962). There is a correlation between the contents of aflatoxin compounds produced by *A. flavus* and toxicity to termites (Becker et al. 1969). Termite colonies might get contaminated with *A. flavus* in the laboratory, and if the colony is weak and declining, *A. flavus* may become parasitic to *C. formosanus* and cause its demise. Beal and Kais (1962) isolated *A. flavus* from dying laboratory colonies of *R. flavipes*. Zoberi and Grace (1990) observed

Table 4. Fungi isolated from guts of laboratory-maintained termites vs. fungi isolated from field-collected termites

Fungal species	Laboratory-maintained colonies			Field-collected termites		
	Colony 4	Colony 5	Colony 6	Collection 7	Collection 8	Collection 9
<i>A. flavus</i>	+	+	+	—	—	—
<i>H. virens</i>	—	—	+	—	—	—
<i>T. asperellum</i>	—	—	—	+	+	—
<i>P. janthinellum</i>	—	—	—	+	+	+
<i>C. cladosporioides</i>	—	—	—	—	+	—

+, presence of fungus; —, absence of fungus.

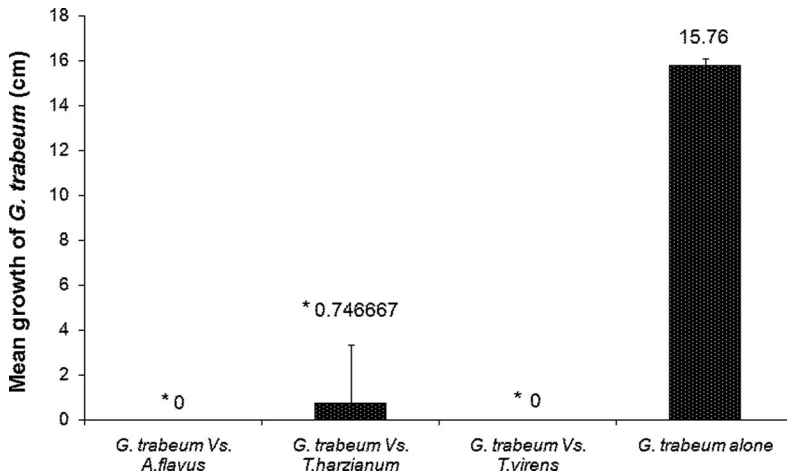


Fig. 1. Growth of *G. trabeum* alone and in dual cultures with fungi isolated from laboratory maintained termites. Asterisk (*) indicates values are significantly different from control ($\alpha = 0.0001$).

that many fungi that were isolated from healthy *R. flavipes* were absent on weak and dead termites. Jayasimha and Henderson (2007a) showed that *A. flavus* was toxic to *C. formosanus*, and as the spore concentration of *A. flavus* increased, the mortality of the termites also increased.

Interestingly, some of these fungi, in addition to helping termites by suppressing a competing cellulose consumer, may directly benefit termite fitness. For example, *T. viride* inoculated wood increased the number of gut protozoa in the Pacific dampwood termite (Mankowski et al. 1998). Whether *Trichoderma* spp. directly benefits *C. formosanus*, however, still needs to be investigated. Honey bees, *Apis mellifera* L., were used to deliver powdered form of *T. harzianum* to strawberry (*Fragaria* L. spp.) flowers to control Botrytis fruit rot. It effectively controlled Botrytis, and it did not affect honey bees (Brownold and Flanders 2005). Jayasimha and Henderson (2007a) showed that *T. harzianum* was not toxic to *C. formo-*

sanus at 10^4 , 10^5 , 10^6 spores per ml. Our studies suggested that *Trichoderma* spores are present on the *C. formosanus* and that they parasitize brown rot fungus when the termites come in contact with a brown rot fungus.

Although *P. janthinellum* was isolated from all the gut cultures made from field-collected termite groups, none were isolated from the cultures made from laboratory-maintained termite colonies. The significance of this result is not known at this point. *P. janthinellum* produces chitinases (Di Giambattista et al. 2001) and carboxypeptidases (Yokoyama et al. 1974), which might aid in the digestion of the wood. Zettler et al. (2002) observed that *P. janthinellum* was one among the two most common fungi in the mounds of red imported fire ants, *Solenopsis invicta* Buren, and it was not very common in the nonmound soil. They also observed that nonmound soil had higher fungal diversity than the ant-occupied mound soils that they attribute to antibiotics and antifungal agents produced

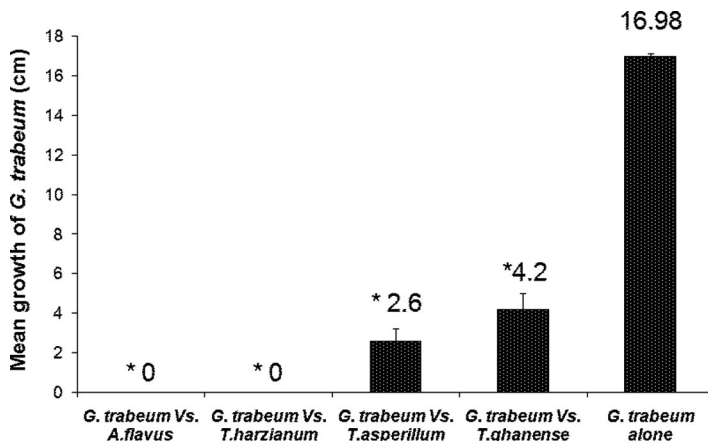


Fig. 2. Growth of *G. trabeum* alone and in dual cultures with fungi isolated from field collected termites. Asterisk (*) indicates values are significantly different from control ($\alpha = 0.001$).

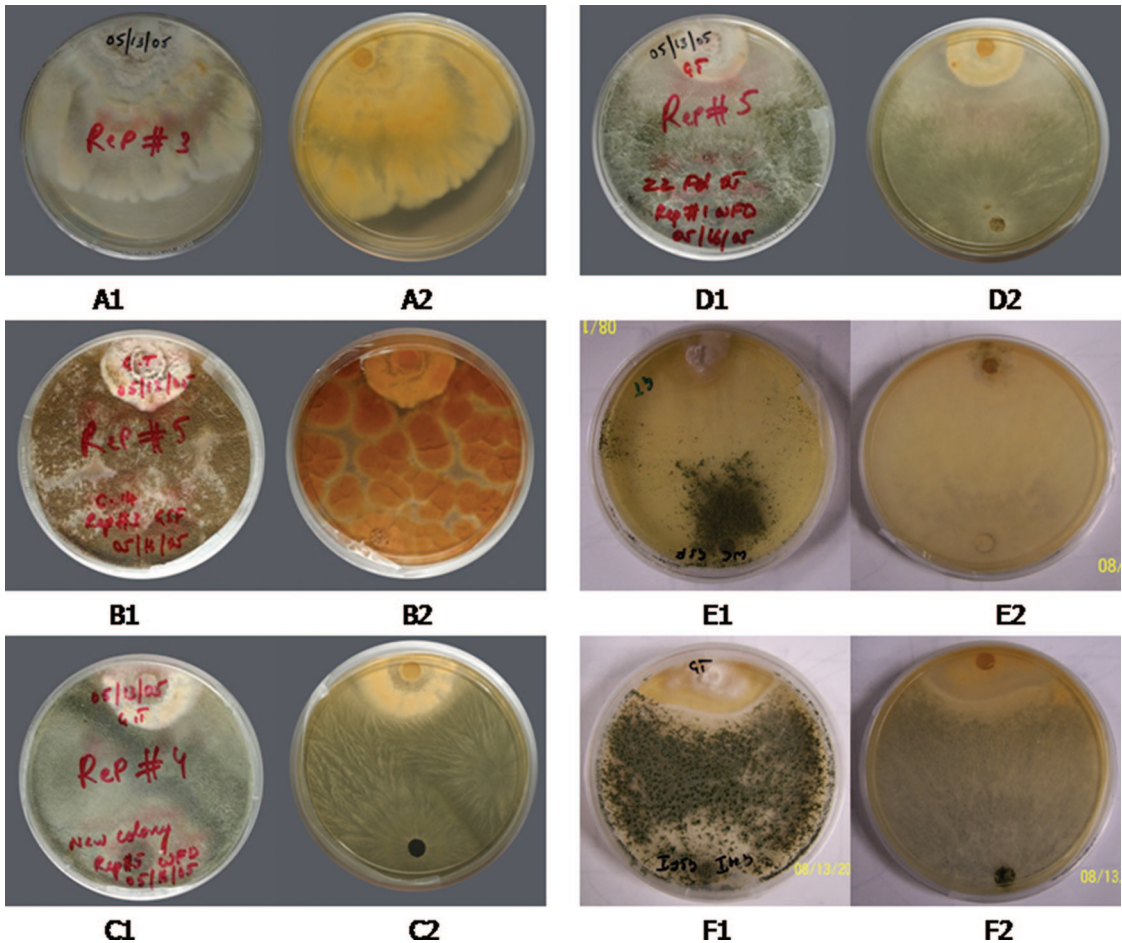


Fig. 3. Dual cultures of *G. trabeum* and antagonistic fungi isolated from integument of termites. Pictures to the left are front views and pictures to the right are back views of the dual cultures. (A1, A2) Growth of *G. trabeum* in controls. (B1, B2) *A. flavus* against *G. trabeum*. (C1, C2) *T. virens* against *G. trabeum*. (D1, D2) *T. harzianum* against *G. trabeum*. (E1, E2) *T. ghanense* against *G. trabeum*. (F1, F2) *T. asperellum* against *G. trabeum*.

by *P. janthinellum*. *P. janthinellum* may have similar function in the guts of Formosan subterranean termites; it may be controlling other fungi and bacteria in the guts by producing antibiotics and antifungal substances. If *P. janthinellum* is passed through the insect's feces, it may contribute as an antibiotic in the nest and termite galleries. A similar idea was investigated by Rosengaus et al. (1998), and they showed that gram-negative bacteria found on fecal pellets of *Z. angusticollis* significantly reduced the spore germination of *Metarhizium anisopliae* (Metchnikoff) Sorokin.

C. cladosporioides is a known pathogen of the clitoria tree psyllid *Euphalerus clitoriae* Burckhardt & Guajara (Marques et al. 2002, Gondim et al. 2005), but its effect on termites and *G. trabeum* needs to be investigated. *T. asperellum* produces an enzyme called beta laminarinase, a kind of cellulase (G. J. Samuels, personal communication). As such, *T. asperellum* may have a partial role in the digestion of cellulose in the guts of *C. formosanus*. *T. virens* was isolated from the integument but not from the guts, whereas its telo-

morph *Hypocrea virens* was isolated from the guts but not from the integuments. Does *T. virens* transform to its telomorph stage in the guts of *C. formosanus*? A relationship of this sort might suggest an obligatory association.

Under our laboratory conditions, dual culture tests of the fungi isolated from the integument of *C. formosanus* showed that these isolates were parasites and/or antagonists and that they effectively controlled the growth of *G. trabeum*. Highley (1997) found that *T. virens* completely inhibited the growth of several white rot and brown rot fungi, including *G. trabeum* and that it prevented decay in pine (*Pinus* spp.) blocks. *T. harzianum* also was found to kill *G. trabeum* and six other decay fungi grown on malt agar medium (Highley and Ricard 1988). In dual cultures of *T. asperellum* against *G. trabeum*, a zone of inhibition was observed (Fig. 3). Zone of inhibition is an area showing no obvious growth that can be detected by the unaided eye (Carter et al. 1994). A zone of inhibition is usually observed when antibiotics pro-

duced by one fungus diffuse through the medium inhibiting the growth of the susceptible fungus. Therefore *T. asperellum* might be producing mycotoxins that act as fungistatic substances inhibiting the growth of *G. trabeum*. These studies raised several interesting questions, such as what happens if mixed cultures of green-spored fungi were used in dual cultures instead of single culture and how would these fungi interact with each other under different circumstances (different temperatures, different substrates, and so on). Studies are required to investigate these aspects.

The mold fungi (e.g., *A. flavus*, *Trichoderma* spp., and *Penicillium* spp.) can colonize the wood and produce enzymes that decompose various storage products, such as fats and sugars, found primarily in special ray cells in sapwood (Kirk and Cowling, 1984, Amburgey 2000). As the hyphae colonize, the sapwood does not lose strength, but it might become more permeable (Amburgey 2000). In contrast, decay fungi such as brown rot fungi exude enzymes that break down cellulose and/or lignin and cause the wood to lose its strength (Amburgey 2000). The common surface molds such as *Aspergillus* spp. and *Penicillium* spp. cause discolorations in the wood that are so superficial that they can be removed by brushing, planning, or sanding (Kirk and Cowling 1984). Chung et al. (1999) showed that the weight loss caused by *Trichoderma* spp., *Penicillium* spp., and *Aspergillus* spp. is significantly low compared with the decay caused by a white rot and a brown rot fungus. Because these fungi effectively controlled a brown rot fungus, *G. trabeum* under our laboratory conditions, and they have negligible effect on the wood itself, they have potential to be used as biocontrol agents against *G. trabeum*.

In conclusion, to test the hypothesis that green-spored fungi may be carried on or in the body of *C. formosanus* and be the cause of observed *G. trabeum* suppression, we isolated fungi from the surface and from the guts of *C. formosanus*. Fungi isolated from integument of *C. formosanus* included *A. flavus*, *T. harzianum*, *T. virens*, *T. asperellum*, and *T. ghanense*. In the intestinal tracts of *C. formosanus*, a different complex of fungi were present, including *A. flavus*, *T. asperellum*, and *H. virens* cultures along with *P. janthinellum* and *C. cladosporioides*. *A. flavus* was more commonly isolated from laboratory maintained colonies than field-collected termites. Dual culture tests of fungi isolated from the integument against *G. trabeum* showed that several isolates were parasites and/or antagonists and effectively controlled the growth of *G. trabeum*.

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