

**INTERACTIONS BETWEEN FORMOSAN SUBTERRANEAN TERMITES, BROWN
ROT FUNGUS (*GLOEOPHYLLUM TRABEUM*) AND SOME OF THE FUNGI PRESENT
ON THE TERMITE INTEGUMENT AND GUT**

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ABSTRACT

Although the brown rot fungus, *Gloeophyllum trabeum* (Pers.:Fr.) Murrill, has been shown to be attractive and beneficial to subterranean termites, including *Coptotermes formosanus* Shiraki, to date no research has been conducted to determine if the association is mutualistic. We first set out to determine if the fungus could be spread by termites. This would represent an obvious benefit to the fungus. We found that *C. formosanus* does not spread *G. trabeum*. Unexpectedly, Formosan subterranean termites were found to suppress the growth of *G. trabeum*. To further investigate this finding, Formosan subterranean termite workers were released into Petri dishes with wood chips inoculated with *G. trabeum*. To serve as controls, an equal number of Petri dishes received all components as above but without termites. Growth of *G. trabeum* was measured on the 6th day of incubation and a significant suppression of fungus growth was observed in treatments with termites. In follow up studies *G. trabeum* inoculated wood chips were placed on to potato dextrose yeast agar medium and measured for *G. trabeum* growth after 7 days. *G. trabeum* did not grow but, many green-spored fungi were predominant in all the cultures. We hypothesized that these green-spored fungi may be carried on or in the body of *C. formosanus* and were the cause of the observed *G. trabeum* suppression. Dual culture tests of fungi isolated from the external surface of Formosan subterranean termites showed that several isolates were parasites and/or antagonists and effectively controlled the growth of *G. trabeum*. These fungi included *Aspergillus flavus* Link, *Trichoderma harzianum* Rifai, *Trichoderma virens* (Miller et al.), *Trichoderma asperillum* Samuels, Lieckfeldt & Nirenberg and *Trichoderma ghanense* Y. Doi, Y. Abe & J. Sugiyama. In the intestinal tracts of *C. formosanus* a different complex of fungi were present, some of which were antagonistic to *G. trabeum*. *Aspergillus flavus*, *Hypocrea virens* Chavarri, Samuels and Steward, *T. asperillum*, along with *Penicillium*

janthinellum Biourge and *Cladosporium cladosporioides* (Fres.) de Vries were the fungi isolated from the guts. *A. flavus* was commonly isolated from external surface and gut of laboratory maintained termite colonies when compared to freshly collected field termite colonies. When these fungi were tested against *C. formosanus*, only *A. flavus* was found to be toxic to termites. Our study is the first to show that fungi present on the termite exoskeleton control the growth of a competing cellulose consumer, *G. trabeum*.

CHAPTER 1

INTRODUCTION

Even though there are more than 2700 termite species in the world only a small minority of them are of economic importance (Culliney and Grace 2000). Not the least among them is *Coptotermes formosanus* Shiraki, the Formosan subterranean termite. Forty to fifty species of termites are present in the United States, most of which are native to this country (Mempe 1990). *C. formosanus* was introduced in to United States from the Far East (Coaton and Sheasby 1976). The Formosan subterranean termite has been a serious pest in China and Japan for centuries (Tamashiro et al. 1987). These termites are native to southern China (Kistner 1985) and moved in to some of the pacific islands in the late 19th century. Since then they have expanded their distribution into the southern United States in the last 60 years (Henderson 2001; Su and Tamashiro 1987). In the United States Formosan termites are currently distributed in Alabama, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee and Texas (Atkinson et al. 1993; Haagsma et al. 1995, Su and Tamashiro 1987). Discovered in Louisiana around 1966 (Spink 1967), they are believed to have entered Louisiana through ships returning with cargo from Asian lands during World War II (Beal 1987, La Fage 1987). Spink (1967) found Formosan termites in Baton Rouge, Houma, Lake Charles, West Lake, New Orleans and adjoining areas in Louisiana. Currently Formosan termites are found are found in Alexandria, Baton Rouge, Grand Isle, Houma, Lake Charles, Lafayette, Monroe, Natchitoches, New Orleans and adjoining areas, Noble, Shreveport, Thibodaux, and West Lake in Louisiana (Messenger et al. 2002).

C. formosanus has its greatest impact in North America and is currently one of the most destructive pests in the USA. (Su and Tamashiro 1987; Lax and Osbrink 2003). Ninety five percent of damage to wood and wood products caused by termites in the United States is due to subterranean termites (Mauldin 1986). The costs associated with damage and control of

subterranean termite damage and control are estimated to approach \$2 billion annually in the United States (Culliney and Grace 2000). In the city of New Orleans the control and repair costs due to Formosan termites was estimated at \$300 million annually (Suszkiw 1998).

Although their distribution in the United States is more restricted than other subterranean termites such as *Reticulitermes* species, *C. formosanus* cause substantial economic loss in infested areas. In China, Formosan termites have been reported to undermine dykes and cause flooding (Gao 1987). Formosan termites attack a variety of wood products and cause significant damage within a short time period. They can attack houses, telephone poles, boats, underground electrical and telephone cables, other finished goods (e.g. books, paper, and fabric) and also living plants, including valuable crops and ornamentals (Lai et al. 1983; Tamashiro et al. 1987; Felix and Henderson 1995). The diet of subterranean termites primarily consists of wood infected by fungi (Esenther et al. 1961). Fungus or fungal products may help termites to locate the decaying wood (Esenther et al. 1961).

Interactions between insects and fungi range from agonistic to mutualistic associations and include many spectacular examples of complex symbioses (Martin 1992). It is well documented that fungi are associated with termites (Hendee 1934) and are even attracted to wood infected with fungi (Hendee 1933). The literature is replete with evidence showing that termites are attracted to wood decayed by certain fungi and avoid wood decayed by certain other fungi. Esenther et al. (1961) observed that shelter tubes built by *Reticulitermes flavipes* (Kollar) on the bark of living trees invariably led directly to a dead, decaying branch stub. However, many of the interrelations between termites and fungi remain unknown.

There is evidence that a mutual beneficial relationship exists between termites and fungi. The nutritional value of wood to termites is increased when the wood is deteriorated by certain

basidiomycetes of the brown rot type (Light and Weesner 1947; Becker 1965; Ruyooka 1979; Williams 1965). Wood decaying fungi may furnish nitrogen, vitamins, and other substances beneficial to termites (Becker 1976); they may also break down toxic volatile materials in wood (Ebeling 1975). Williams (1965) observed that the heart wood of *Pinus caribaea* Morelet is made suitable for *Coptotermes niger* Snyder by the brown rot fungus *Lentinus pallidus* Berk and Curt and the diet of the termites is improved by this modification. *C. niger* attacks only the heartwood of *P. caribaea*, where it is infected with brown rot fungus *L. pallidus* (Perry et al. 1985). Becker (1971) showed that consumption of fungus infected wood by termites is greater than non-infected wood.

Interactions between *Gloeophyllum trabeum* (Pers. ex. Fr.) Murr. and subterranean termites gained importance when Esenther et al. (1961) showed that wood decayed by brown rot fungus is attractive to *R. flavipes*, *R. virginicus* (Banks), and *Nasutitermes columbicus* (Holmgren). Since then it has been shown in many studies that wood decayed by the fungus *G. trabeum* produces an attractant for *R. flavipes* (Esenther et al. 1961; 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). Pine wood decayed by the fungus *G. trabeum* is attractive to *C. formosanus* (Matsuo and Nishimoto 1974). The compound (Z, Z, E)-3, 6, 8-dodecatrien-1-ol isolated from *G. trabeum* (Smythe et al. 1967b; Matsumura et al. 1968) is the same compound isolated from whole body extracts of *R. virginicus*, and *C. formosanus* (Matsumura et al. 1969; Tokoro et al. 1992).

It is not only the termites that are benefited from this association, fungi also benefit in many ways. Termites may help the fungi by transporting and spreading them to new areas. Termites were found to be capable of transporting fungus spores and hyphae (Hendee 1934). The

ability of termites to carry fungi was demonstrated by the isolation of fungi from the exterior 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). Termites accumulated large tufts of hyphae on their legs while walking across cultures of nonspore bearing Basidiomycetes (Hendee 1934). Cultures made from the guts of the termites yielded abundant growths of the fungi on which the termites had been feeding (Hendee 1934). As a result, galleries formed by termites in perfectly sound wood are soon infected with fungi (Ebeling 1975).

Contrary to all of the above evidence fungi may not always be beneficial to termites. They may produce toxic metabolites and repellents (Hendee 1933; Lund 1962, 1965; Becker and Kerner-Gang 1964; Lenz 1969; Amburgey and Beal 1977). The behavior of termite species in relation to fungus-decayed wood is affected not only by fungal species but also by fungal strain, type of wood, age of the fungus and decay rate (Rouland-Lefevre 2000).

The feeding stimulus produced varies with the amount of the decay of wood (Amburgey and Smythe 1977). The attractiveness of *G. trabeum* is reduced or not present at all when cottony mycelia are present (Esenther and Coppel 1964). Roessler (1932) remarked that an excessive growth of fungi on the decayed wood caused the death of many termites. *C. formosanus* does not prefer heavily decayed wood but does prefer moderately decayed wood over undecayed wood (Lenz et al. 1991). The greater the weight loss of decayed wood the less suitable it is to the termites probably because of breakdown of celluloses (Becker 1976). The stage of fungal decay may be very important to termites because it changes the nutritional composition of the wood and also detoxifies the wood (Matsuura 2003). Wood extracts decayed by *Lentinus lepideus* Fr. had repellent activity at high concentrations of the fungus extract (Amburgey 1979). Smythe et al. (1971) have shown that wood containing dead mycelium is more readily eaten and supports more

termites than the wood with living mycelium. Amburgey (1979) showed that *R. tibialis* and *C. formosanus* could differentiate between their pheromone and attractant present in wood decayed by *G. trabeum*.

The overall goal of this research program was to evaluate the unique association between Formosan subterranean termites and *Gloeophyllum trabeum* (strain Madison 617).

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CHAPTER 2

**EVALUATION OF MUTUAL BENEFICIAL RELATIONSHIP BETWEEN
GLOEOPHYLLUM TRABEUM AND *COPTOTERMES FORMOSANUS* BY TESTING IF *C.*
FORMOSANUS CAN SPREAD *G. TRABEUM* FROM INFECTED WOOD TO
UNINFECTED WOOD**

INTRODUCTION

Interactions between insects and fungi range from agonistic to mutualistic and include many spectacular examples of complex symbioses (Martin 1992). Many interrelations between termites and fungi, however, remain unknown. The environment in which termites live is also favorable for growth of fungi (Beard 1974). Fungi usually play an important role in nutrition of termites 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 areas. The ability of termites to carry fungi was demonstrated by the isolation of fungi from the exterior 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).

Formosan subterranean termites, *Coptotermes formosanus* Shiraki, are native to southern China (Kistner 1985). The Formosan subterranean termite is currently one of the most destructive pests in the USA (Su and Tamashiro 1987; Lax and Osbrink 2003). The costs associated with damage and control of subterranean termites is estimated to approach \$2 billion annually in the United States (Culliney and Grace 2000). In the city of New Orleans alone the control and repair costs due to Formosan termites was estimated at \$300 million annually (Suszkiw 1998).

The brown rot fungus, *Gloeophyllum trabeum* (Pers. ex. Fr.) Murr. belongs to the phylum Basidiomycota (Gilbertson and Lindsey 1975). It is characterized as a brown rot fungus because it degrades and depolymerizes lignocelluloses of the wood and leaves the pigmented lignin biopolymers oxidized but intact (Highley et al. 1994). This process results in the darkening of the affected substrate in addition to the deterioration of its mechanical properties (Highley et al. 1994).

Interactions between *G. trabeum* and subterranean termites gained importance when Esenther et al. (1961) showed that wood decayed by this fungus is attractive to *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks), and *Nasutitermes columbicus* (Holmgren). Since then, it has been shown through many studies that wood decayed by the fungus *G. trabeum* produces an attractant for *R. flavipes* (Esenther et al. 1961; Allen et al. 1964; Esenther and Coppel 1964; Smythe et al. 1965, 1967a, 1967b, 1971; and Beard 1974) as well as other species of *Reticulitermes* (Becker 1965; Becker and Lenz 1975) and *Coptotermes* (Becker and Lenz 1975).

G. trabeum infected wood has been shown to be attractive to *C. formosanus* (Matsuo and Nishimoto 1974). The chemical responsible for trail following, (Z, Z, E)-3, 6, 8-dodecatrien-1-ol was isolated from the whole body extracts of *R. virginicus*, and *C. formosanus* (Matsumura et al. 1969; Tokoro et al. 1992) and the same compound was isolated from *G. trabeum* decayed wood (Smythe et al. 1967b; Matsumura et al. 1968). Clearly, indicates that *C. formosanus* is benefited by the brown rot fungus, *G. trabeum*. However, no studies have been conducted to examine whether the brown rot fungus is benefited by this association with Formosan subterranean termites. Our objective was to demonstrate the spread of *G. trabeum* by *C. formosanus* from *G. trabeum* infected wood to uninfected wood, which would represent an obvious benefit to the fungus.

MATERIALS AND METHODS

Gloeophyllum trabeum (Pers. ex. Fr.) Murr. isolate Madison 617 was purchased from ATCC, Manassas, Virginia (ATCC # 11539) and was maintained on PDYA (Potato Dextrose Yeast Agar) medium at the urban laboratory at LSU AgCenter. An autoclaved wet spruce pine fir wood chip was placed in each Petri dish and a loopful of *G. trabeum* was inoculated on the wood chip and was incubated for 20 days at 25°C to get *G. trabeum* infected wood chips. In a six-

chambered polypropylene container the first chamber was filled with 25 g of moistened sand. The rest of the chambers were filled with 70 g of moistened sand. *G. trabeum* infected wood was placed in the first chamber and uninfected autoclaved wood was placed in the last chamber. Holes were made at the bottom of each chamber to allow the termites to move from one chamber to another. Fifty worker termites and 5 soldiers were released in to the first chamber. Termites were not added to the controls. Containers were placed in an incubator at 25°C for 20 days. The entire experiment was replicated on 2 termite colonies (Colony 1 was collected from Signette Park, New Orleans on 08/11/04 and Colony 2 was collected from Brechtel Park, New Orleans on 08/04/04) and repeated 4 times with each colony. Observations were made daily to check for visible fungus growth on uninfected wood chips. After 20 days of incubation cultures were made from the original *G. trabeum* infected wood chips, previously uninfected wood chips, and the integument and guts (digestive tracts) of termites to determine if *G. trabeum* is carried on the surface and in the gut, and whether the termites spread the fungus to uninfected wood chips. Cultures of fungus infected wood chips and uninfected wood chips were made by placing each wood chip on PDYA medium in a Petri dish. Integument cultures were made by allowing 2 termites to walk on PDYA medium for about a minute and then gently rubbing their bodies on to the medium and removed. All the Petri dishes were sealed with Parafilm® and incubated at 25°C for 2-3 days. For external cultures, 6 termites were used from each bioassay replication. To prepare gut cultures termites were surface sterilized by means of 1% NaClO and then rinsed 3 times with sterile distilled water. Guts of the termites were pulled out gently with sterile forceps and then streaked on to PDYA medium. Gut cultures of 6 termites were made from each bioassay replication.

RESULTS

G. trabeum was not isolated from any of the cultures made from treatments with termites; however several other fungi were isolated from these cultures (Table 2.1). *G. trabeum* was isolated from *G. trabeum* infected wood chips from treatments without termites (Table 2.1). Surprisingly, *G. trabeum* was not isolated even from the original *G. trabeum* infected wood chips from treatments with termites (Table 2.1). No fungi were isolated from uninfected wood chips from treatments without termites. This latter result showed that our attempt to keep all extraneous fungi out of the experiment except for that which might be on the termites or infected wood chip was successful.

Table 2.1. Presence or absence of *G. trabeum* in the cultures made from treatments with termites and in treatments without termites. Yes = presence of fungus; No = absence of fungus.

	With termites				Without termites	
	Fungus infected woodchip	Uninfected woodchip	External cultures	Gut cultures	Fungus infected woodchip	Uninfected woodchip
<i>G. trabeum</i> growth	No	No	No	No	Yes	No
Other fungi	Yes	Yes	Yes	Yes	No	No

DISCUSSION

G. trabeum was not isolated from the integument or gut cultures indicating that termites were not carrying *G. trabeum* on their surface or in their guts. *G. trabeum* also was not isolated from the uninfected wood chips from treatments with termites. This confirmed that termites did not spread *G. trabeum* from infected wood chip to uninfected wood chip. To our surprise, *G. trabeum* was not isolated even from the originally infected *G. trabeum* wood chips in

treatments with termites but was isolated in treatments without termites, which indicated that for some reason *G. trabeum* did not survive in the presence of Formosan subterranean termites. Although *G. trabeum* was not isolated from any of the cultures made from treatments with termites, several other fungi were isolated. We did not identify these other fungi at this time though we did note that many were green in color. We suspected that these fungi were carried and transferred by the termites themselves. Factors that were inhibiting the growth of *G. trabeum* are not known. Rosengaus et al. (1998) stated that fecal pellets of *Zootermopsis angusticollis* Hagen significantly reduced the spore germination of *Metarhizium anisopliae* (Metchnikoff) Sorokin. Work done in our lab by Wiltz et al. (1998) suggests that Formosan subterranean termites might be using naphthalene to inhibit the growth of microorganisms. Our experiment led us to hypothesize that termites carry parasitic fungi that attack *G. trabeum* on their integument and guts. This idea led to a series of experiments that are reported in the next chapters.

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CHAPTER 3

GROWTH OF *GLOEOPHYLLUM TRABEUM* IN PRESENCE AND ABSENCE OF *COPTOTERMES FORMOSANUS*

INTRODUCTION

There is a great amount of evidence showing a mutual beneficial relationship between termites and fungi. Nutritional value of wood to termites is increased when the wood is deteriorated by certain basidiomycetes of the brown rot type (Light and Weesner 1947; Becker 1965; Ruyooka 1979; Williams 1965). Wood decaying fungi may furnish nitrogen, vitamins, and other substances beneficial to termites (Becker 1976); they may also break down toxic volatile materials in wood (Ebeling 1975). Williams (1965) observed that the heartwood of *Pinus caribaea* Morelet is made suitable for *Coptotermes niger* Snyder by the brown rot fungus *Lentinus pallidus* Berk and Curt and the diet of the termites is improved by this modification. *C. niger* attacks only those areas of heartwood of *P. caribaea*, where timber is infected with brown rot fungus *L. pallidus* (Perry et al. 1985). Becker (1971) showed that termite consumption of fungus infected wood is higher than non-infected wood.

It is not only the termites that are benefited from this association, fungi also benefit in many ways. Termites may help the fungi by transporting and spreading them to new areas. Termites were found to be capable of transporting fungus spores and hyphae (Hendee 1934). The ability of termites to carry fungi was demonstrated by the isolation of fungi from the integument and from the gut of termites, and by the observation of hyphae, conidiophores, and conidia clinging to the body and appendages of termites (Hendee 1933). Fungi belonging to the class Basidiomycetes were isolated were isolated from colonies of *Kaloterms minor* Hagen, *Reticulitermes hesperus* Banks, and *Zootermopsis angusticollis* Hagen (Hendee 1934). "Colony" in that study was defined as termites, fecal pellets, loose detritus, frass in their tunnels and also wood incorporated in to walls of the tunnels. Termites were shown capable of accumulating large tufts of hyphae on their legs while walking across cultures of nonspore bearing

Basidiomycetes (Hendee 1934). Cultures made from the guts of the termites yielded abundant growths of the fungi on which the termites had been feeding (Hendee 1934). The galleries formed by termites in perfectly sound wood are soon infected with fungi (Ebeling 1975).

Interactions between *Gloeophyllum trabeum* (Pers. ex. Fr.) Murr. and subterranean termites gained importance when Esenther et al. (1961) showed that wood decayed by the brown rot fungus is attractive to *R. flavipes*, *R. virginicus* (Banks), and *Nasutitermes columbicus* (Holmgren). It was also found that wood decayed by the fungus *G. trabeum* produces an attractant for *R. flavipes* (Esenther et al. 1961; Allen et al. 1964; Esenther and Coppel 1964; Smythe et al. 1965, 1967a, 1967b, 1971; and Beard 1974) as well as other species of *Reticulitermes* (Becker 1965; Becker and Lenz 1975) and *Coptotermes* (Becker and Lenz 1975). Pine wood decayed by the fungus *G. trabeum* is attractive to *C. formosanus* (Matsuo and Nishimoto 1974). The compound (Z, Z, E)-3, 6, 8-dodecatrien-1-ol was isolated from *G. trabeum* (Smythe et al. 1967b; Matsumura et al. 1968). The same compound has been isolated from whole body extracts of *R. virginicus*, and *C. formosanus* (Matsumura et al. 1969; Tokoro et al. 1992). Clearly, *C. formosanus* benefits from the brown rot fungus, *G. trabeum*. Our previous studies revealed that *C. formosanus* did not spread the brown rot fungus, and surprisingly, *G. trabeum* did not survive in the presence of *C. formosanus*. This led us to study the effect of Formosan subterranean termite on the growth of *G. trabeum*.

MATERIALS AND METHODS

Four laboratory maintained termite colonies were used to evaluate the growth of *G. trabeum* in the presence and absence of termites. Colony 1 was collected from Signette Park, New Orleans, Louisiana on 8/11/04. Colonies 2, 3, and 4 were collected from Brechtel Park,

New Orleans, Louisiana on 8/18/04, 8/4/04 and 8/18/04 respectively. Termites were maintained in the laboratory in 150 l containers with pine wood until the day of the experiment (10/15/04).

Spruce pine fir wood chips of 3.5×2×0.5 cm were soaked in double distilled water for 15 min and then autoclaved. An autoclaved, wet wood chip was placed in each sterile Petri dish. Brown rot fungus, *G. trabeum* (Madison 617) was purchased from ATCC, Manassas, Virginia (ATCC # 11539) and was maintained on PDYA (Potato Dextrose Yeast Agar) medium. A loop full of *G. trabeum* was inoculated on to the autoclaved wet wood chip. Fifty worker termites were then released into each Petri dish. No termites were added to controls. The entire procedure was done under a laminar air flow chamber (Edgegard, The Baker Company, Sanford, Maine). Petri dishes were then sealed with Parafilm® and were incubated at 25⁰C for 6 days. The entire experiment was replicated on 4 different termite colonies and repeated 7 or 14 times with each colony. Length and breadth of the fungal growth were measured with a ruler scale on the 6th day of incubation to obtain the total growth of the fungus. Data were analyzed using analysis of variance proc mixed (SAS 2002, Cary, NC. Version 9.0). Wood chips from termite treatments were then placed on PDYA medium to determine the survival of the inoculated fungus. Growth of *G. trabeum* was recorded after 7 days.

RESULTS

Growth of *G. trabeum* was significantly suppressed in treatments with termites when compared to treatments without termites (F= 69.53; df = 1, 76; P<0.0001) (Figs 3.1& 3.2). There was no significant difference in the growth of fungus between colonies (F= 1.18; df = 3, 76; P>0.3218). There was no interaction between colonies and treatments (F= 0.05; df = 3, 76; P>0.9839). Suppressed *G. trabeum* on treatments with termites did not grow when cultured on PDYA, but several fungi with green colored spores were predominant.

DISCUSSION

Though the brown rot fungus *Gloeophyllum trabeum* (Pers.: Fr.) Murrill. is attractive and beneficial to most subterranean termites including *Coptotermes formosanus* Shiraki, Formosan subterranean termites are controlling the growth of *G. trabeum*. There may be several reasons why Formosan subterranean termites suppress the growth of *G. trabeum*. Ebeling (1975) stated that wood decayed by *G. trabeum* might be attractive to termites but, the fungus itself may not be useful to the termites. *G. trabeum* also may not always be attractive to the termites. Esenther and Coppel (1964) observed that *G. trabeum* is not attractive when cottony mycelia are present. Excessive growth of fungi on decayed wood caused increased mortality in termites (Roessler 1932). Although these results were unexpected the competitive exclusion principle indicates that two species cannot coexist when they have identical needs of a limited resource.

Factors that were inhibiting the growth of *G. trabeum* are not known. Batra and Batra (1966) observed that many fungi were present in the immediate vicinity of the termitarium of *Odontotermes gurdaspurensis* Holmgren & Holmgren but were absent in the termitarium, fungal combs and royal chambers which led to thought that fungistatic substances might be present in the termitarium.

Rosengaus et al. (1998) said that fecal pellets of *Zootermopsis angusticollis* Hagen significantly reduced the spore germination of *Metarhizium anisopliae* (Metchnikoff) Sorokin. Work done in our laboratory by Wiltz et al. (1998) suggests that Formosan subterranean termites might be using naphthalene to inhibit the growth of microorganisms. Formosan subterranean termites may be using any or all of these mechanisms to control the growth of *G. trabeum*. In our experiments, when wood chips from treatments with termites were cultured on PDYA, green-spored fungi were predominant in all of the cultures. These green-spored fungi were not isolated

from treatments without termites. This led us to hypothesize that these green-spored fungi may be associated with termites and act as fungistats controlling the growth of *G. trabeum*.

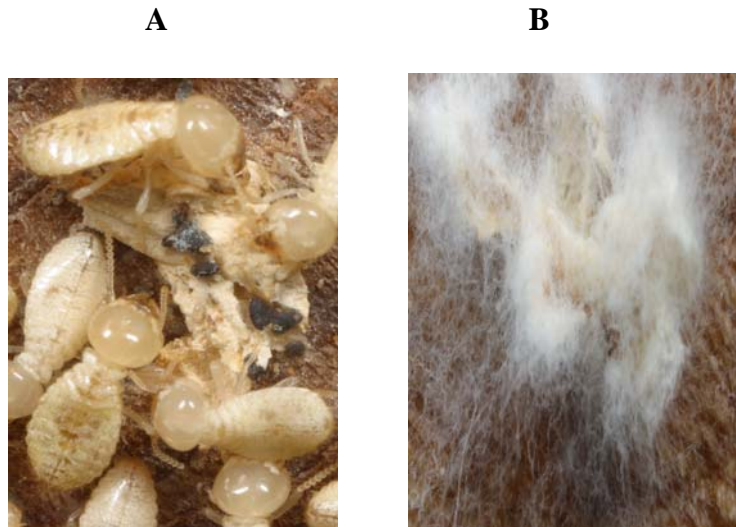


Fig. 3.1 Growth of *G. trabeum* in presence and absence of termites. (A) Growth in presence of termites. (B) Growth in absence of termites.

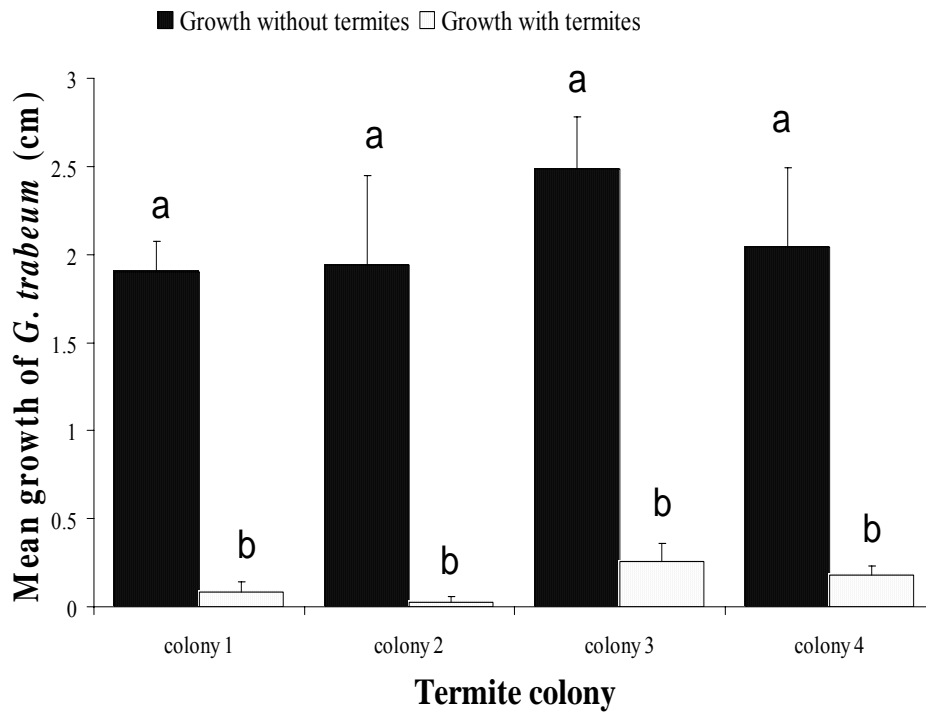


Fig. 3.2 Comparison of mean growth of *G. trabeum* on wood chips from treatments with termites and treatments without termites. Same letters are not significantly different ($\alpha= 0.05$).

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CHAPTER 4

FUNGI ISOLATED FROM INTEGUMENT AND GUT OF *COPTOTERMES FORMOSANUS* AND THEIR ANTAGONISTIC EFFECT ON *GLEOPHYLLUM TRABEUM*

INTRODUCTION

In our earlier efforts to demonstrate the spread of *G. trabeum* by *C. formosanus*, we found not only that Formosan subterranean termite do not spread *G. trabeum* but also *G. trabeum* does not survive in the presence of *C. formosanus*. Though the brown rot fungus, *G. trabeum*, is attractive and beneficial to most subterranean termites including *C. formosanus*, this suggests that there is an antagonistic relationship between Formosan subterranean termites and *G. trabeum* rather than mutual beneficial relationship. We also observed that when *G. trabeum* infected wood pieces were exposed to termites, *G. trabeum* was somehow 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*.

Very little information is available on the mycofloral communities of subterranean termites. Hendee (1933) studied fungi associated with *Reticulitermes hesperus* Banks, and Zoberi and Grace (1990) worked with *R. flavipes* and isolated several fungal cultures from their integument and guts. Rojas et al. (2001) studied fungi associated with *C. formosanus* and isolated three fungal species. Our objective was to evaluate isolated fungi present on the integument and in the guts of Formosan subterranean termites and to test the effect of these fungi on the brown rot fungus, *G. trabeum*.

MATERIALS AND METHODS

Collection of Termites

Formosan subterranean termites used to isolate the fungi were collected from both laboratory maintained colonies and 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. Field collected groups were maintained under sterile conditions and fungal isolation was conducted within 24 hrs upon return to the laboratory.

Integument Cultures: Three laboratory maintained colonies (6 days – 7 months) were used to isolate fungi from the integument. Colony 1 was collected on 08/4/04, colony 2 on 10/14/04, and colony 3 on 03/24/05 from Brechtel Park, New Orleans, Louisiana and the experiments were started on 03/30/05 (Table 4.1). Additionally, 6 field collected groups were also used for isolation of fungi from the integument of termites. Three of the 6 groups were collected from Brechtel Park, New Orleans, Louisiana on 05/13/05 and were used in the fungal isolations on 05/14/05. The other 3 groups were collected from the Citrus Station, Port Sulfur, Louisiana on 06/14/05 and were used in the experiment on the same day (Table 4.1).

Gut Cultures: Three laboratory maintained termite colonies used to isolate gut fungi were collected from Brechtel Park, New Orleans, Louisiana on 11/9/05. Experiments were conducted on 02/13/06 (Table 4.1). Three groups of fresh field termites used to isolate gut fungi were collected from Brechtel Park, New Orleans, Louisiana on 3/7/06 and were used in the experiment on the same day (Table 4.1).

Isolation of Fungi from Termites

Integument Cultures: Seven termite workers from each colony group were released into each Petri dishes containing PDYA medium with streptomycin (25mg/l). Termites were allowed to walk on the medium for 1 minute and their bodies were gently rubbed on to the medium and were then removed. The entire procedure was done under a laminar air flow chamber. Petri dishes were then sealed with Parafilm® and were incubated at 25⁰C for 4 to 7 days. Controls were handled in the same way as the treatments only termites were not added to the Petri dishes. This

procedure was repeated 6 times with 3 different laboratory maintained colonies and 3 or 5 times with 6 different field collected colonies. Any fungi growing were visually differentiated based on their color and colony growth characteristics. These fungi were subcultured 2 or 3 times to obtain pure cultures. Subcultures were made thereafter every week to maintain the pure cultures. This resulted in 12 to 14 pure cultures from each laboratory maintained termite colonies and 11-15 pure cultures from each of fresh field collected termite groups.

Table 4.1. Date and location from which the termite colonies used to isolate fungi from integument and guts were collected.

	Lab colonies	Date of collection	Collected from	Fresh field colonies	Date of collection	Collected from
Integument cultures	Colony 1	08/04/04	Brechtel park, New Orleans, Louisiana	Collection 1	05/13/05	Brechtel park, New Orleans, Louisiana
	Colony 2	10/14/04		Collection 2	05/13/05	
	Colony 3	03/24/05		Collection 3	05/13/05	
				Collection 4	06/14/05	Citrus Station, Port Sulfur, Louisiana
				Collection 5	06/14/05	
				Collection 6	06/14/05	
Gut cultures	Colony 4	11/09/05	Brechtel park, New Orleans, Louisiana	Collection 7	03/07/06	Brechtel park, New Orleans, Louisiana
	Colony 5	11/09/05		Collection 8	03/07/06	
	Colony 6	11/09/05		Collection 9	03/07/06	

Gut Cultures: Five percent NaClO was purchased from Acros Organics, Morris Plains, NJ 07950 and diluted to 1% NaClO with sterile distilled water. Termites were surface sterilized using 1% NaClO and subsequently rinsed 3 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 3 laboratory maintained termite colonies and 3 field collected termite groups. The entire experiment was replicated 3 times.

Any fungi growing were visually differentiated based on their color and colony growth characteristics. These fungi were subcultured 2 or 3 times to obtain pure cultures. Subcultures were made thereafter every week to maintain the pure cultures. This resulted in 2-8 pure cultures from each laboratory maintained termite colony and 1-3 pure cultures from each fresh field collected termite group.

Selection and Identification of the Isolated Fungi

Based on the observations of from an earlier experiment, we decided to concentrate our work with only the green-spored fungi. Green-spored fungi isolated from the same termite colony were separated based on having the same fungal colony characteristics. From the integument cultures, a total of 7 cultures of green-spored fungi were isolated from laboratory colonies 1, 2 and 3, and 6 cultures of green-spored fungi were isolated from fresh field collections 1, 2, 4, 5 and 6 (Table 4. 2). From the gut cultures, a total of 5 cultures of green-spored fungi from colony 4, 5 and 6, and 6 cultures from collection 7, 8 and 9 were isolated (Table 4. 2). The purified cultures were sent for identification to Dr. Steven Carpenter, Abbey Lane Laboratory LLC, Philomath, Oregon 97370.

Dual Culture Techniques to Test the Antagonistic Effects of Selected Fungi against *Gloeophyllum trabeum*

Fungi isolated from the integument were used in the dual culture studies. Fungi isolations and *G. trabeum* were maintained on PDYA medium. On the day of the experiment discs of the fungi on PDYA medium were cut out using a cork borer of 0.75mm diameter. To determine if selected fungi inhibited *G. trabeum* growth, a disc of *G. trabeum* was placed at one end of the

Petri dish containing PDYA medium. After two days 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 5 times with 13 of the selected fungi. The total growth of the *G. trabeum* and the isolated fungi were measured after 15 days of incubation. Data were analyzed using analysis of covariance proc mixed (SAS 2002, Cary, NC, version 9.0).

Table 4.2. Number of green-spored fungi isolated from each colony.

	Lab colonies	Number of green-spored fungi isolated	Fresh field termites	Number of green-spored fungi isolated
Integument cultures	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 cultures	Colony 4	3	Collection 7	2
	Colony 5	1	Collection 8	3
	Colony 6	1	Collection 9	1

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 and of the 13 cultures selected 6 cultures were

Trichoderma harzianum and 4 cultures were *Aspergillus flavus*. The final 3 cultures were identified as *T. virens*, *T. asperellum* and *T. ghanense*.

Among the 3 colonies collected from the laboratory *A. flavus* was present in all 6 replications of Colony 1 and 2 and in 4 replications of Colony 3 (Table 4.3). *T. harzianum* was present in 2 replications of Colony 1 and 3 and *T. virens* in 1 replication of Colony 2 (Table 4.3).

Among the field collected colonies, *T. harzianum* was isolated from 1 out of 5 replications of collection 1 and 2 and *A. flavus* was not isolated from any of the replications of collection 1 and 2 (Table 4.3). No green-spored fungi were isolated from collection 3 (Table 4.3). *T. asperellum* was isolated from 2 out of 3 replications of collection 4 and *A. flavus* was isolated from 1 replication (Table 4.3). *T. harzianum* is isolated from all the 3 replications of the collection 5 (Table 4.3). *T. ghanense* was isolated from 2 replications of collection 6. *A. flavus* was not isolated from collection 5 and 6 (Table 4.3).

Table 4.3. Fungi isolated from integument of laboratory maintained termites vs. fungi isolated from fresh field collected termites. + = presence of fungus; - = absence of fungus.

	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>Aspergillus flavus</i>	+	+	+	-	-	-	+	-	-
<i>Trichoderma harzianum</i>	+	-	+	+	+	-	-	+	-
<i>Trichoderma virens</i>	-	+	-	-	-	-	-	-	-
<i>Trichoderma ghanense</i>	-	-	-	-	-	-	-	-	+
<i>Trichoderma asperellum</i>	-	-	-	-	-	-	+	-	-

Gut Cultures: Fungi were identified as *A. flavus* Link, *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 4.4). *P. janthinellum* was isolated from all the 3 field collections but it was not isolated from any of the laboratory maintained termite colonies (Table 4.4).

Table 4.4. Fungi isolated from guts of laboratory maintained termites vs. fungi isolated from fresh field collected termites. + = presence of fungus; - = absence of fungus.

	Laboratory maintained colonies			Field collected termites		
	Colony 4	Colony 5	Colony 6	Collection 7	Collection 8	Collection 9
<i>Aspergillus flavus</i>	+	+	+	-	-	-
<i>Hypocrea virens</i>	-	-	+	-	-	-
<i>Trichoderma asperellum</i>	-	-	-	+	+	-
<i>Penicillium janthinellum</i>	-	-	-	+	+	+
<i>Cladosporium cladosporioides</i>	-	-	-	-	+	-

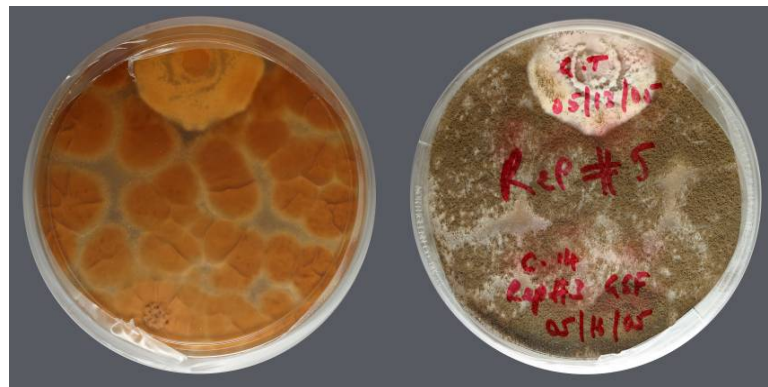
Control of *Gloeophyllum trabeum* by Selected Fungi

All of the fungi selected from integument cultures suppressed *G. trabeum* in the dual cultures studies (Fig. 4.1 A1-F2). Fungi isolated from laboratory maintained termite colonies (*A. flavus*, $t = 24.21$; $df = 32$; $P < 0.0001$; *T. harzianum*, $t = 24.97$; $df = 32$; $P < 0.0001$; *T. virens*, $t = 24.12$; $df = 32$; $P < 0.0001$), and field collections (*A. flavus*, *T. harzianum*, $t = 4.39$; $df = 27$; $P = 0.0002$; *T. ghanense*, $t = 4.81$; $df = 27$; $P < 0.0001$; and *T. asperellum*, $t = 5.00$; $df = 27$; $P < 0.0001$) significantly suppressed *G. trabeum* growth in dual culture studies (Figs. 4.2 and 4.3).



A1

A2



B1

B2



C1

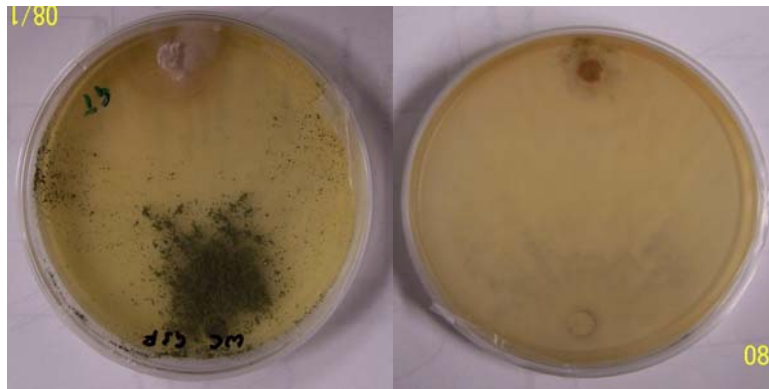
C2

Fig. 4.1 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) *Aspergillus flavus* against *G. trabeum*. (C1, C2) *Trichoderma virens* against *G. trabeum*. (D1, D2) *Trichoderma harzianum* against *G. trabeum*. (E1, E2) *Trichoderma ghanense* against *G. trabeum*. (F1, F2) *Trichoderma asperellum* against *G. trabeum*. (Fig cont'd)



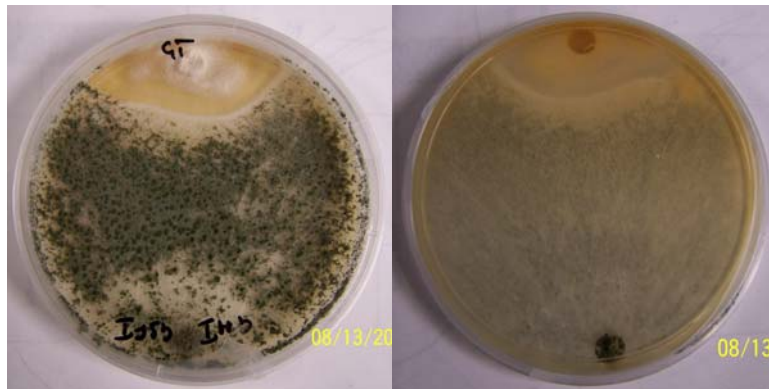
D1

D2



E1

E2



F1

F2

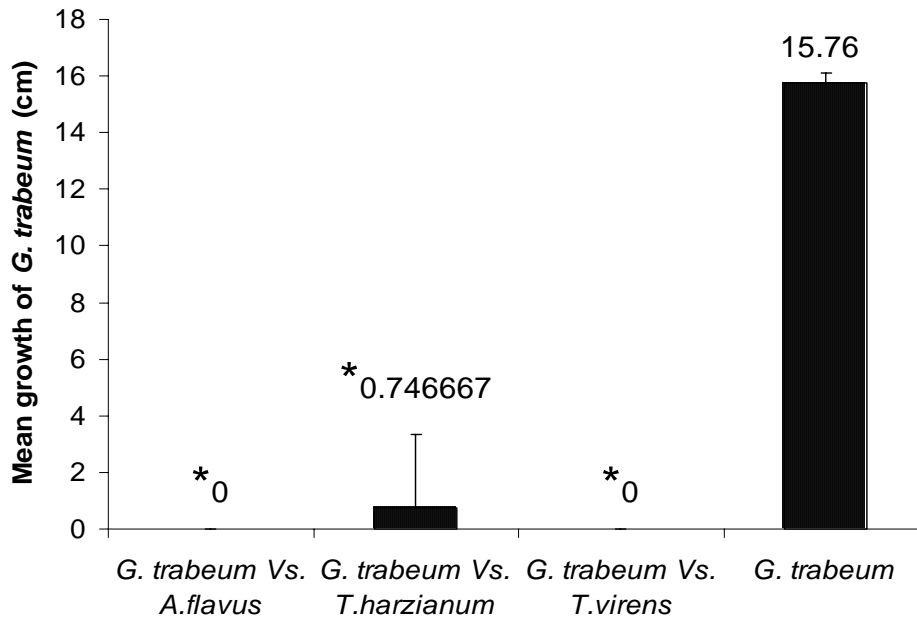


Fig. 4.2 Growth of *G. trabeum* in dual cultures with fungi isolated from laboratory maintained termites. * Values are significantly different ($\alpha= 0.001$).

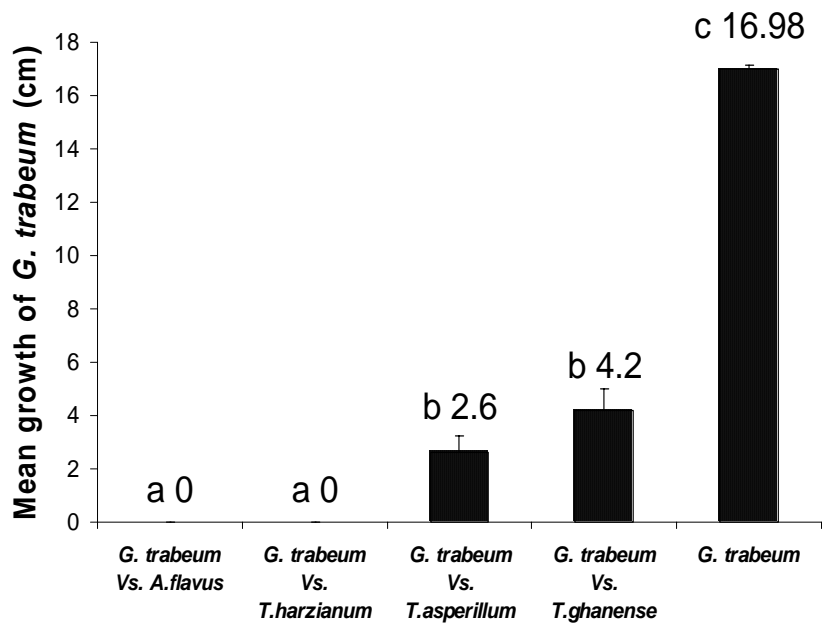


Fig. 4.3 Growth of *G. trabeum* in dual cultures with fungi isolated from fresh field collected termites. Same letters are not significantly different ($\alpha= 0.001$).

DISCUSSION

Dual culture tests of these fungi isolated from the integument of Formosan subterranean termites showed that these isolates were parasites and/or antagonists and 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 prevented decay in pine blocks. *T. harzianum* also was found to kill *G. trabeum* and 6 other decay fungi grown on malt agar medium (Highley and Ricard 1988). In dual cultures of *T. asperellum* against *G. trabeum* zone of inhibition was observed (Fig. 1). A zone of inhibition is an area showing no obvious growth that can be detected by the unaided eye (Carter et al. 1994) usually observed when antibiotics produced by one fungus diffuses into the medium inhibiting the growth of the susceptible fungus. Therefore *T. asperellum* might be producing mycotoxins which act as fungistatic substances inhibiting the growth of *G. trabeum*.

In our experiments we isolated *Aspergillus flavus* and 4 *Trichoderma* spp. from the integument of the Formosan subterranean termites. We also isolated *A. flavus* from gut cultures of termites along with *Trichoderma asperellum*, *Penicillium janthinellum*, *Hypocrea virens* and *Cladosporium cladosporioides*. *Trichoderma*, *Aspergillus*, and *Penicillium* have been previously isolated from different termite species in many studies but the association we found had not been investigated. For example, Hendee (1933) isolated cultures from the integument and guts of *R. hesperus*, *Kaloterme minor* Hagen and *Zootermopsis angusticollis* Hagen and found that *Penicillium* and *Trichoderma* were the most common and widespread fungi among the 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 Formosan subterranean termites. *Aspergillus* spp. has also been isolated from colonies of *Kalotermes minor* Hagen by Hendee (1933).

A. flavus was predominant in all the external cultures and gut cultures made from laboratory maintained colonies, but 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 termite groups. Laboratory conditions may be favorable for *A. flavus* allowing it to overtake other fungi.

Aspergillus can be detrimental 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 Formosan subterranean termites and take over the colony. Beal and Kais (1962) isolated *A. flavus* from dying laboratory colonies of *R. flavipes*. Zoberi and Grace (1990) observed that many fungi that were isolated from healthy *R. flavipes* were absent on weak and dead termites.

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 damp wood termite, *Zootermopsis angusticollis* (Mankowski et al. 1998). Whether *Trichoderma* spp. directly benefits *C. formosanus* however still needs to be investigated. Honey bees were used to deliver powdered form of *T. harzianum* to strawberry flowers to control Botrytis fruit rot. It effectively controlled Botrytis and did not affect honey bees (Brownold 2005). We believe that *Trichoderma* spores

may always be present on the *C. formosanus* and parasitize brown rot fungus when the termites come in contact with the fungus.

Though *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 (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 and it was not very common in the non-mound soil. They also observed that non-mound soil had higher fungal diversity than the ant-occupied mound soils which they attribute to antibiotics and antifungal agents produced by *P. janthinellum*. *P. janthinellum* may have similar function in the guts of Formosan 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. There is little information available on *C. cladosporioides*. It is a known pathogen to the *Clitoria* tree psyllid, *Euphalerus clitoriae* Burckhardt and Guajara (Marques et al. 2002). Further studies are necessary to study the effect of these fungi on *G. trabeum* and on termites themselves. *T. asperellum* produces an enzyme called beta laminarinase, a kind of cellulase (G. J. Samuels, personal communication). *T. asperellum* may have a partial role in the digestion of cellulose in the guts of Formosan subterranean termites. *T. virens* was isolated from the integument but not from the guts whereas its telomorph *Hypocrea virens* was isolated from the guts but not from the integuments. Does *T. virens* transform to its telomorph stage in the guts of Formosan subterranean termites? Further

studies are necessary to learn more about the association of these fungi with Formosan subterranean termites, their effect on wood rot fungi and also on the termites themselves.

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CHAPTER 5

EFFECT OF *ASPERGILLUS FLAVUS* AND *TRICHODERMA HARZIANUM* ON SURVIVAL OF *COPTOTERMES FORMOSANUS*

INTRODUCTION

Current methods to control termites emphasize placing a chemical barrier between infested or threatened wood upon the soil (Beard 1974). Application of large quantities of persistent insecticides around and under a building poses an environmental threat because of possible contamination of water resources (Beard 1974). They may also have an effect on non-target organisms. The necessity of alternative control measures is great (Beard 1974, Henderson 2001, Su & Scheffrahn 1998).

Biological control rather than persistent insecticides may provide long-lasting insect control and also seems to be ecofriendly (Grace 1997, Hokkanen & Lynch 1995, Howarth 1991, Khetan 2001). Laboratory cultures of termites often succumb to the fungal infections in spite of the practice of nest sanitation (Beard 1974). Laboratory studies have demonstrated the possibility of using pathogens to control subterranean termites (Lund 1971, Sun et al. 2003, Wells et al. 1995). Therefore, for several reasons, the role of fungi in termite control needs to be studied more thoroughly.

Fungi have the ability to replicate and spread in a termite population if introduced through attractant-baited traps (Fuxa et al. 1998, Fuxa & Tanada 1987). The soil environment is humid, and is protected from ultraviolet radiation, highly favorable conditions for sustaining infection and promoting epizootics (Culliney & Grace 2000). Grooming behavior and proctodeal trophallaxis could inhibit the spread of fungal diseases but many also hasten the spread of fungal disease in a termite colony (Grace & Zoberi 1992, Kramm et al. 1982, Rosengaus & Traniello 1997). *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) when experimentally infected with *Aspergillus flavus* Link showed 80% mortality (Beal & Kais 1962). *A. flavus* was also found to be toxic to *Heterotermes indicola* and *Coptotermes amanii* (Lenz

1969). *A. flavus* has been isolated from dying laboratory colonies of *R. flavipes* (Beal & Kais 1962). Whether *A. flavus* is toxic to *C. formosanus* is unknown.

Trichoderma spp. were found to be toxic to *Kaloterms flavicollis* (Fabr.), *Heterotermes indicola* (Wassmann), *Reticulitermes lucifugus* (Rossi) var. *santonensis* Feytaud and *Nasutitermes ephrate* (Holmgren) (Becker & Kerner-Gang 1964). All the temperate and tropical soils contain 10^1 to 10^3 culturable propagules of *Trichoderma* per gram of soil (Harman et al. 2004). Formosan subterranean termites are in constant touch with the soil but the effect of *Trichoderma* spp. on the Formosan termites is not known.

Our previous experiments showed that on PDYA medium *A. flavus* and *T. harzianum* controls the brown rot fungus, *G. trabeum*, which competes with *C. formosanus* for the same limited cellulose resource. Our goal was to test the effect of *Aspergillus flavus* and *Trichoderma harzianum* on the survival of *Coptotermes formosanus*.

MATERIALS AND METHODS

Termites

Three termite colonies were used for the experiment. Group of termites from each colony were marked by feeding them 0.1% Nile blue solution treated filter paper for 3 days. 5% NaClO is purchased from Acros Organics, Morris Plains, NJ 07950 and is diluted to 1% NaClO with sterile distilled water. Marked termites and unmarked termites are surface sterilized with 1% NaClO for 1 minute and then they were rinsed 3 times with sterile distilled water. Termites were then allowed to dry on a sterile filter paper. Five marked termites and 15 unmarked termites were released in to each Petri dish. A total of 12 Petri dishes were prepared for each colony.

Fungal Suspensions

Fungi were isolated from the integument of Formosan subterranean termites. Seven cultures of *A. flavus* and 5 cultures of *T. harzianum* were isolated from 15 colonies used for fungal isolation. Cultures were maintained in Petri dishes on PDYA medium until the day of the experiment. A Culture of *Trichoderma harzianum* and *Aspergillus flavus* was selected haphazardly from the cultures isolated from the integument of Formosan termites. Spores were harvested by flooding the plate with sterile distilled water and then gently scraping the plate with a spatula. Spores were stirred into a suspension of distilled water which was then filtered through triple layered cheese cloth. The concentration of spores in the suspension was determined by means of a hemacytometer (Spencer®, Bright-Line, Reichert Scientific instruments, Buffalo, NY). Spore concentration was adjusted to 10^4 , 10^5 , 10^6 spores/ ml.

Bioassay

Termites were anesthetized with a frozen refrigerant pack by placing the pack under the Petri dish with the termites for one minute and a 0.5µl of suspension was applied to the ventral surface of each marked termite with a micro-dispenser. Controls were treated with sterile distilled water. The bioassay included 3 doses of fungal suspension and a control. The entire bioassay was replicated on three termite colonies and repeated three times with each colony. Petri dishes were sealed with Parafilm® and incubated at 25°C. Mortality of the termites was recorded after 12 days of incubation. Data were analyzed using t-tests proc GLM (SAS 2002, Cary, NC, version 9.0).

RESULTS

Mortality of termites treated with *T. harzianum* at 10^4 , 10^5 , 10^6 spores/ml was not significantly different from controls ($t = 1.99$; $df = 62$; $P > 0.05$) (Fig. 5.1). Mortality of *A. flavus*

treated termites was significant at 10^5 , 10^6 spores/ml over controls and also significant compared to termites treated with *T. harzianum* ($t = 1.99$; $df = 62$; $P < 0.05$) (Fig. 5.1).

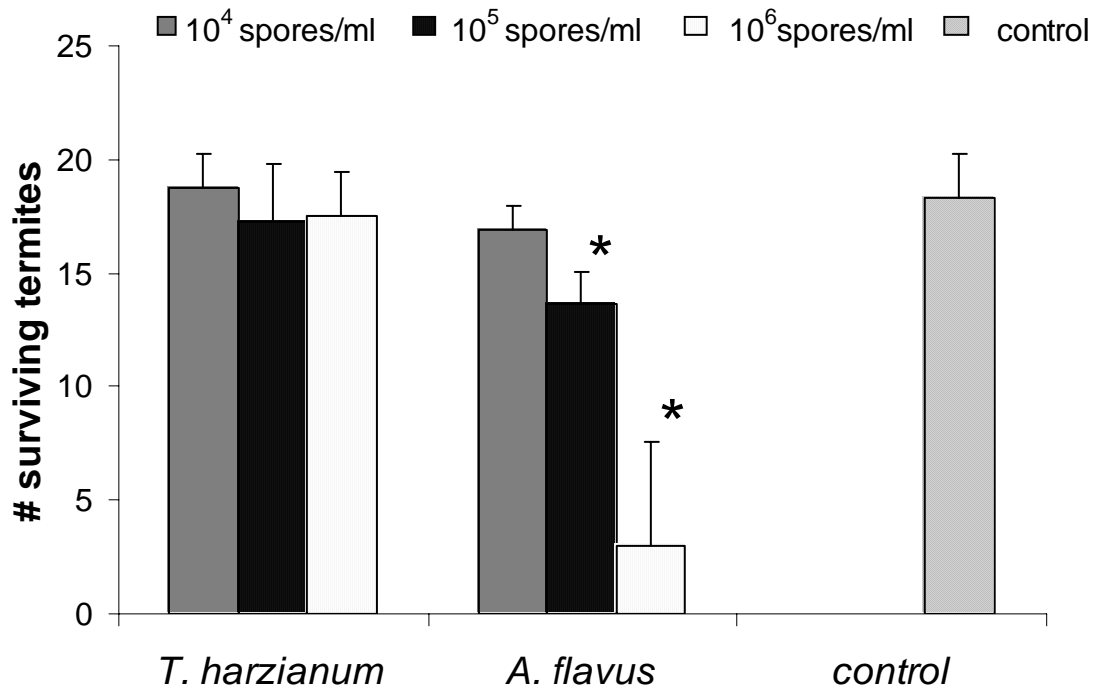


Fig. 5.1 Survival of *C. formosanus* when treated with different doses of *T. harzianum*, *A. flavus*.
* Bars are significantly different from controls ($\alpha = 0.05$)

DISCUSSION

Our study showed that *A. flavus* was toxic to *C. formosanus*. As the spore concentration of *A. flavus* increased the mortality of the termites also increased. *A. flavus* was also found to be toxic to *R. flavipes* and *R. virginicus*, *H. indicola*, *C. amanii* (Beal & Kais 1962, Lenz 1969). LD₅₀s of *Beauveria bassiana* (Balsamo) Vuillemin ranged from 4.5×10^3 to 4.96×10^5 conidia/termite and LD₅₀s of *Metarhizium anisopliae* (Metsch.) ranged from 7.89×10^3 to 1.22×10^5 conidia/termite (Sun et al. 2003). We used concentrations much less than these concentrations to test the toxicity of *A. flavus* but even at these low concentrations *A. flavus* was toxic to *C. formosanus*.

Some of the *Trichoderma* species were found to be toxic to *K. flavicollis*, *H. indicola*, *R. lucifugus* and *N. ephrate* (Becker & Kerner-Gang 1964). But *T. harzianum* was not found to be toxic to *C. formosanus* at 10^4 , 10^5 , 10^6 spores/ml concentrations. *T. harzianum* was used on strawberry flowers to control *Botrytis* fruit rot, honey bees were used to deliver *T. harzianum* (Brownold 2005). Powdered forms of *T. harzianum* sprayed on to honey bees effectively controlled *Botrytis* and honey bees themselves were not adversely affected (Brownold 2005). This again shows that *T. harzianum* is not toxic to some insects.

A. flavus has potential to be used as biocontrol agent to control brown rot fungus, *G. trabeum* and also to control Formosan termites. *T. harzianum*, on the other hand benefits the termites by controlling brown rot fungus which is a competitive cellulose consumer. In turn, termites spread *T. harzianum* from one place to another (Jayasimha and Henderson, unpublished) with no adverse affects to the termites themselves. The association between Formosan termites and the *T. harzianum* appears to be a mutual beneficial relationship.

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CHAPTER 6

SUMMARY AND CONCLUSIONS

The original goal of our research was to evaluate the association between brown rot fungus *Gloeophyllum trabeum* (Pers. ex. Fr.) Murr. and Formosan subterranean termite, *Coptotermes formosanus* Shiraki. We hypothesized it to be mutual beneficial relationship and we performed experiments to demonstrate the spread of *G. trabeum* by *C. formosanus* from *G. trabeum* infected wood to uninfected wood, which would represent an obvious benefit to the fungus. Unexpectedly our results indicated that *C. formosanus* does not spread *G. trabeum* and *G. trabeum* does not survive in the presence of Formosan subterranean termites. To further test this finding we conducted an experiment to test the effect of *C. formosanus* on growth of *G. trabeum*. This test also indicated that *G. trabeum* hardly grows in the presence of *C. formosanus*. We also isolated several green-spored fungi which might be potentially parasitic/ antagonistic fungi that were associated with termites. We hypothesized that these green-spored fungi may be carried on or in the body of *C. formosanus* and be the cause of observed *G. trabeum* suppression. In conclusion of these two experiments *G. trabeum* does not survive in the presence of *C. formosanus*. Potentially parasitic/antagonistic fungi associated with termites might be the cause of observed *G. trabeum* suppression.

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 Formosan subterranean termites. Fungi isolated from integument of *C. formosanus* included *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. In the intestinal tracts of *C. formosanus* a different complex of fungi were present. *Aspergillus flavus* and *Trichoderma* cultures along with *Penicillium janthinellum* Biourge and *Cladosporium cladosporioides* (Fres.) de Vries were

the fungi isolated from the guts. Dual culture tests of fungi isolated from the integument of Formosan subterranean termites showed that several isolates were parasites and/or antagonists and effectively controlled the growth of *G. trabeum*. In conclusion of this test there were several fungi are parasites and/or antagonists to brown rot fungus, *G. trabeum* found. We selected several fungi isolated from the integument of termites to test against *G. trabeum*. There were several other fungi that need to be studied.

Our final objective was to test the effect of different concentrations of these fungi on *C. formosanus*. Our tests showed that *A. flavus* is toxic to *C. formosanus*. Mortality of termites increases the concentration of the fungus increases. *Trichoderma harzianum* was not found to be toxic to *C. formosanus*. *A. flavus* has potential to be used as biocontrol agent to control brown rot fungus, *G. trabeum* and also to control Formosan termites. *T. harzianum* benefits the termites by controlling brown rot fungus which is a competitive cellulose consumer. In turn termites spread *T. harzianum* from one place to another and also *T. harzianum* was not found to be toxic to Formosan termites. Therefore the association between Formosan termites and the *T. harzianum* appears to be mutual beneficial relationship.

We selected several fungi isolated from the integument and guts of termites. Among those fungi several isolates were parasitic/antagonistic to a destructive wood fungus, brown rot fungus. We also found a fungus antagonistic/parasitic to both brown rot fungus and Formosan subterranean termites. There are several other fungi that are present on the surface and guts of *C. formosanus* and several of them might be parasitic/antagonistic to these destructive wood pests and pathogens. Further studies are necessary to discover them.

VITA

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