FUNGI ASSOCIATED WITH THE SUBTERRANEAN TERMITE *RETICULITERMES FLAVIPES* IN ONTARIO

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ABSTRACT

In a survey of the mycoflora associated with *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) subterranean termite populations in Ontario, Canada, forty species of fungi were isolated. These included both cellulolytic fungi and potential pathogens. Twenty-one fungal species were cultured from living termites. Observations of isolates from field collections, and the low diversity of fungi in declining laboratory termite colonies, suggest that interactions among fungi may suppress pathogenic effects and promote termite survival.

Key Words: Insect-fungus interactions, fungal ecology, termite ecology, biological control

Interactions between fungi and soil-dwelling termites (Isoptera) have been considered by numerous authors, and reviewed by Amburgey (**1979**), Becker (**1976**) and Sands (**1969**). Although much interest has focused on the cultivation of fungi by mound-building termites in the higher family Termitidae (*cf.* Martin and Martin, **1978**; Zoberi, **1979**), the nature of the relationship between lower termites and fungi is subject to debate.

Unlike the Termitidae, subterranean termites in the family Rhinotermitidae do not build mounds, but nest in or around partially buried wood and forage over large areas through an extensive gallery system (Grace *et al.*, **1989**). Several species in the holarctic genus *Reticulitermes* Holmgren are widely distributed in North America, and are serious economic pests (Mauldin, **1986**). This genus is frequently found in partially decayed wood, and compounds extracted from wood decayed by isolates of *Gloeophyllum trabeum* (Pers.: Fr.) Murr. (Esenther *et al.*, **1961**) and *Oligoporus balsameus* (Pick) (Grace and Wilcox, **1988**) elicit positive behavioural responses in laboratory bioassays. Waller *et al.* (**1987**) isolated 30 basidiomycetes causing white-rots and one ascomycete from logs infested by *Reticulintermes* spp. and *Coptotermes formosanus* Shiraki, and observed *Reticulitermes* spp. feeding on basidiocarps.

As celluloytic decay fungi may play a positive role in termite nutrition or orientation to food materials, pathogenic fungi may act as biological control agents. Kramm and West (**1982**) and Yendol and Paschke (**1965**) reported mortality in *Reticulitermes flavipes* (Kollar) exposed to stock cultures of several pathogenic fungi, but reports of naturally occurring fungal infections of *Reticulitermes* are rare. Gouger and Kimbrough (**1969**) isolated the entomogenous hyphomycete *Antennopsis gallica* Heim & Buchli from both *R. flavipes* and *Reticulitermes virginicus* Banks, and *Aspergillus flavus* Link. (Beal and Kais, **1962**) and *Absidia coerulea* Bainier (Lund and Engelhardt, **1962**) were isolated from dying *R. flavipes* laboratory colonies.

With the exception of Hendee's (**1933**) isola-

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tion of 25 fungal genera (plus one basidiomycete and several unidentified cultures) from the western subterranean termite *Reticulitermes hesperus* Banks and termite-infested wood, little information is available on the mycofloral communities associated with North American subterranean termite populations. Such information is desirable from both a fundamental and an applied viewpoint. Our study was initiated as a survey of the mycoflora associated with *R. flavipes* colonies in southern Ontario, Canada. *Reticulotermes flavipes* was first reported in Ontario province in 1929 (Kirby, **1965**), and is considered to be an introduced species spread primarily by man's movement of infested wood and soil (Urquhart, **1953**). Thus, at the northern boundary of the species' distribution in North America, *R. flavipes* might encounter pathogens endemic to its new habitat, or, conversely, act as a vector for the introduction of beneficial fungal associates.

MATERIALS AND METHODS

Insect collections.—The eastern subterranean termite, *R. flavipes,* has been reported in 29 southern Ontario municipalities (Grace, **1987**). For this study, termites were collected from four locations: two in the City of Scarborough, one in the City of Toronto, and one in the Town of Kincardine. Termites were collected in surface wood (cut tree stems and branches), in a corrugated cardboard trap placed on top of a Manitoba maple (*Acer negundo*) stump, and in similar traps placed in the soil. The trap on the stump was based on the design of La Fage *et al.* (**1983**), and consisted of single-faced corrugated cardboard, dampened with deionized water, and rolled within a 15 cm length of 10 cm ID plastic (ABS) pipe. This was capped with a plastic pipe (test) cap, and secured to the top of the stump with three metal L-brackets. The soil traps consisted of two 15 cm lengths of 4 cm ID plastic pipe, each containing a roll of cardboard, placed within a 10 cm ID plastic pipe which was capped and buried slightly below the soil surface (Grace, **1989**). Collected termits were kept in the laboratory in plastic boxes within an unlighted temperature (27 ± 0.5 C) and humidity (90 ± 5% RH) cabinet (Constant Temperature Control, Ltd., Weston, Ontario). This cabinet was also used in some fungal incubations.

Fungal isolations.—Fungi were isolated from the following substrates: live termites from field-collected wood and traps placed in the soil (T1) and from the trap placed on a stump (T2); shavings from field-collected wood (W) containing termites; soil carried by foraging termites into the pipes within the soil traps (S1) and the stump trap (S2); corrugated paper (P) from the soil traps after feeding by termites; dead termites (TD), soil (SD), and Whatman No. 1 filter paper (PD) from a laboratory container with a declining termite population; termite shelter tubing (SH) constructed up the side of a laboratory container containing apparently healthy and active termites; and corrugated paper before exposure to termites.

The following natural media (Difco) were used for fungal isolation and culture: malt extract agar, potato dextrose agar, nutrient agar, Bacto yeast malt extract agar, starch agar, agar agar, cellulose agar, and filter paper malt extract agar. To prepare cellulose agar medium, 40 g of alpha-cellulose was mixed with 20 g of agar in 1 L deionized water and autoclaved. For filter paper malt extract agar, 1.52 g shredded Whatman No. 1 filter paper was mixed with 45 g of malt extract agar in 1 L of water, and autoclaved.

Eight living termites, a single dead termite, or small samples (either *ca* 3 mg or a *ca* 3 × 3 mm piece) of the other substrates were transferred aseptically in sterile Petri dishes containing a suitable medium. Three replicates were prepared with each of the media and experiments with each sample were repeated at least three times. Only those fungi that appeared commonly in several replicates were catalogued.

To determine the extent of fungal growth on the corrugated paper before exposure to termites, small samples were aseptically cut from a newly-purchased roll of paper. These were moistened with sterile water and incubated at room temperature (22–24 C) in a sterile Petri dish, and also plated on malt extract agar. No fungal growth resulted under either condition.

Only termite workers and nymphs with small wingpads were selected for isolations. To exclude mites and other contaminant organisms, termites were either transferred several times from one sterile culture plate to another or were placed in a freezer at −10 C for 3 minutes before culturing. Any plates showing mite contamination were discarded and the results excluded.

Petri dishes containing samples were incubated in the unlighted chamber at 27 ± 0.5 C and 90 ± 5% RH. Observations of fungi appearing in Petri dishes were made daily, and the species

isolated and identified. Selected isolates were deposited in the Herbarium, Department of Botany, University of Toronto (TRTC), or in the National Herbarium, Biosystematics Research Centre, Agriculture Canada, Ottawa, Ontario (DAOM). Isolation of some species in pure culture was not possible, and slides (Zoberi, **1967**) were prepared for reference purposes.

RESULTS AND DISCUSSION

Forty species of fungi were isolated from *R. flavipes* and associated substrates (TABLE I). Many of these are common saprophytic soil organisms (Griffin, **1972**; Barnett and Hunter, **1972**; Gilman, **1957**; Raper and Thom, **1949**; Thom and Raper, **1945**). Termite foragers passing through the soil could easily become contaminated with fungal propagules, and pass them to other members of the group through body contact and grooming behaviour (Preston *et al.*, **1982**). Hendee's (**1934**) fungal isolations from termite guts and fecal pellets indicate that propagules ingested during soil movement or grooming retain their viability.

Several of the fungal species isolated in this study, such as *Mucor mucedo* (L.) Fr. and *Aspergillus niger* Van Tieg. (Steinhaus, **1949**), have been reported to be facultative insect pathogens. *Mucor hiemalis* Weh., isolated from most of our field and laboratory sources, was reported by Burnside (**1935**) as a pathogen of bees. *Arthrobotrys oligospora* Fres., isolated from the soil sample collected from the traps set into the soil, is a common predacious hyphomycete that produces trapping mechanisms in the presence of appropriate prey (Duddington, **1951**). To determine the effect of this isolate upon *R. flavipes*, eight vigorous termite workers removed from a soil trap were aseptically transferred to a Petri dish containing a culture of the sporulating fungus grown on soil taken from the trap. These termites appeared moribund after 24 hours, and died within a five-day exposure period while no mortality was observed among termites placed in similar Petri dishes with sterile soil. Replication of the experiment gave similar results, suggesting that *A. oligospora* is deleterious to *R. flavipes*. Similar preliminary studies suggest that *Rhizopus stolonifer* Ehrenb.: Fr. and *Cunninghamella echinulata* Thaxter may also be detrimental to termite survival, and further investigations of these associations are in progress.

A number of celluloytic fungi were isolated, including species of *Aspergillus* (Thom and Raper, **1945**), *Penicillium, Alternaria, Fusarium, Cladosporium, Acremonium,* and *Stachybotrys* (Hawker and Linton, **1971**). *Trichoderma* species have both celluloytic (Papavizas, **1985**) and antifungal activity (Widden and Scattolin, **1988**). In laboratory cultures, we have noted that the growth of fungi deletarious to termite survival, such as *R. stolonifer,* is inhibited by *Trichoderma* spp., suggesting that interfungal interactions may benefit *R. flavipes.*

A striking feature of this survey was the absence on dead termites, and associated soil and paper, of many of the fungi cultured from field materials and healthy laboratory collections. Only 35% of the species isolated from healthy field or laboratory materials were also found on dead termites or materials from declining laboratory populations. However, these represented 14 (78%) of the 18 fungi isolated from the unhealthy substrates. Only three species (*Stachybotrys atra* Corda: Fr., *Dictyostelium* sp., and *Stemonitis* sp.) were found exclusively under unhealthy conditions. Again, this suggests that competitive or parasitic interactions among fungi promote termite survival, an hypothesis supported by the relatively large number of species (21) actually carried by the living termites. Zoberi (**1979**), in isolating 27 fungal species from a mound of *Macroteermes natalensis* Haviland, also suggested that multi-species fungal interactions promoted termite survival.

Eight of the 25 fungal genera identified by Hendee (**1933**) from the western subterranean termite, *R. hesperus,* were represented in our survey: *Absidia, Mucor, Mortierella, Cunninghamella, Penicillium, Acremonium, Trichoderma,* and *Alternaria.* No comparable information has been reported for populations of *R. flavipes* from other geographic regions or other *Reticulitermes* species, although Waller *et al.* (**1987**) recently reported associations between termites and wood decay fungi in Louisiana.

Hopefully, information on associated mycoflora will be developed for other *R. flavipes* populations. Past work on the role of fungi in *Reticulitermes* diet and survival has emphasized wood decay fungi (Carter *et al.*, **1972**; Smythe *et al.*, **1971**), but other cellulolytic fungi may affect feeding as well. We are currently studying effects of mixed fungal cultures on *R. flavipes* behaviour and survival. Perturbations of the ecological balance within the mycofloral community could explain the fungus-induced mortality observed in

TABLE I

FUNGI ASSOCIATED WITH *RETICULITERMES FLAVIPES*

Fungal isolate	Field sources[a]						Laboratory sources[a]			
	T1	T2	W	S1	S2	P	TD	SD	PD	SH
Mucorales										
Absidia fusca Linnemann	+[b]	−	+	+	+	−	−	+	−	+
Actinomucor elegans Benj. & Hesseltine	+	−	+	−	−	−	+	−	+	−
Circinella minor Lendner	+	−	+	−	−	−	−	−	−	−
Circinella muscae Berlese & De Toni	−	+	−	−	−	−	+	−	−	−
Cunninghamella echinulata Thaxter	+	−	−	−	+	+	+	+	+	−
Mortierella sp.	−	−	−	−	−	−	−	−	−	+
Mucor circinelloides Van Tiegh.	−	−	−	−	−	−	−	−	−	+
Mucor hiemalis Wehmer	+	+	+	+	−	+	+	+	+	+
Mucor mucedo (L.) Fresenius	+	−	−	−	−	−	+	−	−	−
Mucor plumbeus Bon	+	−	+	−	−	+	+	+	+	+
Mucor pusillus Lindt.	+	−	−	−	−	−	−	−	−	+
Mucor racemosus Fresenius	+	−	+	−	−	+	+	−	−	−
Pirella circinans Bainier	−	−	+	−	−	−	−	−	−	−
Rhizopus stolonifer Ehrenb. ex Fr.	+	−	+	+	−	−	−	−	−	+
Hyphomycetes										
Acremonium sp.	−	+	−	−	−	−	−	−	−	−
Alternaria sp.	−	−	−	+	−	+	−	−	−	−
Arthrobotrys oligospora Fresenius	−	−	−	+	−	−	−	−	−	−
Aspergillus niger Van Tieghem	−	−	−	−	−	−	−	+	−	+
Aspergillus terreus Thom.	+	−	−	−	−	−	−	−	−	−
Aspergillus zonatus Kwon & Fennell	+	+	−	−	−	−	−	−	−	−
Aspergillus sp.	+	+	−	−	−	−	−	−	−	+
Aspergillus sp.	−	−	−	−	+	−	−	−	−	−
Cladosporium cladosporiodes (Fres.) de Vries	−	−	−	−	−	−	−	−	−	+
Fusarium oxysporum Schlecht.	−	−	−	−	−	−	+	−	−	−
Fusarium solani (Martius) Sacc.	+	−	−	−	−	−	−	−	−	+
Gliomastix sp.	−	−	−	−	−	+	−	−	−	−
Penicillium brevi-compactum Diercks	−	−	−	−	−	+	−	−	−	−
Penicillium sp.	+	+	−	−	+	−	−	+	−	+
Stachybotrys atra Corda: Fr.	−	−	−	−	−	−	−	−	+	−
Trichoderma coningii aggr. *sensu* Rifai	−	−	+	−	+	−	−	+	−	+
Trichoderma harzianum Rifai aggr.	+	−	+	−	−	+	−	−	−	−
Trichoderma viride aggr. *sensu* Rifai	+	−	+	+	+	+	−	+	−	+
Dictyosteliales										
Dictyostelium sp.	−	−	−	−	−	−	+	−	−	−
Dictyostelium sp.	+	−	−	−	−	−	+	−	−	−
Polysphondylium sp.	−	−	−	−	−	−	−	−	−	+
Stemonitales										
Stemonitis sp.	−	−	−	−	−	−	−	+	+	−
Unidentified Isolates										
Unidentified sp. (Actinomycetes)	+	−	−	−	−	−	+	−	−	−
Unidentified sp. (Actinomycetes)	−	−	−	−	−	−	−	−	−	+
Unidentified sp. (Basidiomycotina)	−	−	+	−	−	−	−	−	−	−
Unidentified sp. (Hyphomycetes)	−	−	−	−	−	+	−	−	−	−

[a] Field Sources: T1 = live termites from wood and soil traps, T2 = live termites from stump trap, W = shavings from wood containing termites, S1 = soil carried by termites into soil traps, S2 = soil carried by termites into stump trap, P = corrugated paper from soil traps; Laboratory Sources: TD = dead termites from rearing container, SD = soil in container with dying/dead termites, PD = filter paper in container with dying/dead termites, SH = termite shelter tubing constructed in container with healthy termites.

[b] + = present, − = absent.

groups of subterranean termites in the laboratory (Beal and Kais, **1962**; Lund and Engelhardt, **(1962)**, and be applicable in the development of new biological control strategies to manage subterranean termite populations.

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LITERATURE CITED

Amburgey, T. L. 1979. Review and checklist of the literature on interactions between wood-inhabiting fungi and subterranean termites: 1960–1978. *Sociobiology* **4**: 279–296.

Barnett, H. L., and B. B. Hunter. 1972. *Illustrated Genera of Imperfect Fungi.* 3rd Ed. Burgess, Minneapolis. 241 p.

Beal, R. H., and A. G. Kais. 1962. Apparent infection of subterranean termites with *Aspergillus flavus* Link. *J. Insect Pathol.* **4**: 488–489.

Becker, G. 1976. Termites and fungi. *Mater. Org. Beiheft.* **3**: 465–478.

Burnside, C. E. 1935. A disease of young bees caused by a *Mucor. Amer. Bee J.* **75**: 75–76.

Carter, F. L., L. A. Dinus, and R. V. Smythe. 1972. Effect of wood decayed by *Lenzites trabea* on the fatty acid composition of the eastern subterranean termite, *Reticulitermes flavipes. J. Insect Physiol.* **18**: 1387–1393.

Duddington, C. L. 1951. The ecology of predacious fungi. *Trans. Brit. Mycol. Soc.* **34**: 322–331.

Esenther, G. R., T. C. Allen, J. E. Casida, and R. D. Shenefelt. 1961. Termite attractant from fungus-infected wood. *Science* **134**: 50.

Gilman, J. C. 1957. *A Manual of Soil Fungi.* 2nd Ed. Iowa State Univ. Press, Ames. 450 p.

Grace, J. K. 1987. Termites in eastern Canada: a brief review and assessment. *Inter. Res. Group Wood Preserv.* Doc. No. IRG/WP/1333. 6 p.

———. 1989. A modified trap technique for monitoring *Reticulitermes* subterranean termite populations (Isoptera: Rhinotermitidae). *Pan-Pac. Entomol.* (In press).

———, **A. Abdallay, and K. R. Farr.** 1989. Eastern subterranean termite (Isoptera: Rhinotermitidae) foraging territories and populations in Toronto. *Canad. Entomol.* **121**: 551–556.

———, **and W. W. Wilcox.** 1988. Isolation and trail-following bioassay of a decay fungus associated with *Reticulitermes hesperus* Banks (Isoptera: Rhinotermitidae). *Pan-Pac. Entomol.* **64**: 243–249.

Gouger, R. J., and J. W. Kimbrough. 1969. *Antennopsis gallica* Heim and Buchli (Hyphomycetes: Gloeohaustoriales) an entomogenous fungus on subterranean termites in Florida. *J. Invert. Pathol.* **13**: 223–228.

Griffin, D. M. 1972. *Ecology of Soil Fungi.* Chapman and Hall, London. 193 p.

Hawker, L. E., and A. H. Linton. 1971. *Microorganisms: Function, Form, and Environment.* Edward Arnold Ltd., London. 391 p.

Hendee, E. C. 1933. The association of the termites *Kalotermes minor, Reticulitermes hesperus,* and *Zootermopsis angusticollis* with fungi. *Univ. Calif. Publ. Zoo.* **39**: 111–134.

———. 1934. The association of termites and fungi. Pp. 105–116. *In: Termite and Termite Control.* 2nd Ed. Ed., C. A. Kofoid. Univ. Calif. Press, Berkeley.

Kirby, C. S. 1965. The distribution of termites in Ontario after 25 years. *Canad. Entomol.* **97**: 310–314.

Kramm, K. R., and D. F. West. 1982. Termite pathogens: effects of ingested *Metarhizium, Beauveria,* and *Gliocladium* conidia on worker termites (*Reticulitermes* sp.). *J. Invert. Pathol.* **40**: 7–11.

La Fage, J. P., N.-Y. Su, M. J. Jones, and G. R. Esenther. 1983. A rapid method for collecting large numbers of subterranean termites from wood. *Sociobiology* **7**: 305–9.

Lund, A. E., and N. T. Engelhardt. 1962. Subterranean termites and *Absidia coerulea* Bain. (Mucorales). *J. Insect Pathol.* **4**: 131–132.

Martin, M. M., and J. S. Martin. 1978. Cellulose digestion in the midgut of the fungus-growing termite *Macrotermes natalensis*: the role of acquired digestive enzymes. *Science* **199**: 1453–1455.

Mauldin, J. K. 1986. Economic importance and control of termites in the United States. Pp. 130–143. *In: Economic Impact and Control of Social Insects.* Ed., S. B. Vinson. Praeger, Toronto.

Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. *Ann. Rev. Phytopath.* **23**: 23–54.

Preston, A. F., F. H. Erbisch, K. R. Kramm, and A. E. Lund. 1982. Developments in the use of biological control for wood preservation. *Proc. Amer. Wood-Preserv. Assoc.* **78**: 53–61.

Raper, K. B., and C. Thom. 1949. *A Manual of the Penicillia.* Williams and Wilkins, Baltimore. 875 p.

Sands, W. A. 1969. The association of termites and fungi. Pp. 495–524. *In: Biology of Termites.* Vol. 1. Eds., K. Krishna and F. M. Weesner. Academic Press, New York.

Smythe, R. V., F. L. Carter, and C. C. Baxter. 1971. Influence of wood decay on feeding and survival of the eastern subterranean termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Ann. Entomol. Soc. Amer.* **64**: 59–62.

Steinhaus, E. A. 1949. *Principles of Insect Pathology.* McGraw-Hill, New York. 757 p.

Thom, C., and K. B. Raper. 1945. *A Manual of the*

Aspergilli. Williams and Wilkins, Baltimore. 373 p.

Urquhart, F. A. 1953. The introduction of the termite in Ontario. *Canad. Entomol.* **85**: 292–293.

Waller, D. A., J. P. La Fage, R. L. Gilbertson, and M. Blackwell. 1987. Wood-decay fungi associated with subterranean termites (Rhinotermitidae) in Louisiana. *Proc. Entomol. Soc. Wash.* **89**: 417–424.

Widden, P., and V. Scattolin. 1988. Competitive interactions of *Trichoderma* species colonizing spruce litter. *Mycologia* **80**: 795–803.

Yendol, W. G., and J. D. Paschke. 1965. Pathology of an *Entomophthora* infection in the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). *J. Invert. Pathol.* **7**: 414–422.

Zoberi, M. H. 1967. A simple technique for preparing and preserving slide cultures. *Trans. Brit. Mycol. Soc.* **50**: 157–158.

———. 1979. The ecology of some fungi in a termite hill. *Mycologia* **71**: 537–545.

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