THREE FUNGAL SPECIES ISOLATED FROM *COPTOTERMES FORMOSANUS* (ISOPTERA: RHINOTERMITIDAE) BODIES, CARTON MATERIAL, AND INFESTED WOOD

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The Formosan subterranean termite (FST), *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), is one of the most destructive termites in the U.S., causing millions of dollars in damage annually to wood products and living trees (Beal 1987, Edwards & Mill 1986, La Fage 1987, Su & Tamashiro 1987).

Termite-fungus associations have been extensively studied (Alasoadura 1966, Batra and Batra 1966, Bose 1923, Bottomley and Fuller 1921, Fletcher 1921, Lund 1960a, 1960b, 1962, Roonwal 1960, Sands 1970). The objective of this study was to isolate and identify fungi associated with *C. formosanus* that might help in their nutrition.

Collections were made in the metropolitan New Orleans, LA, area. The search was limited to fungi associated with wood decay, and fungal spores present externally on the body of alates, workers and soldiers, since these can be ingested during grooming of nestmates. Alates were collected by the use of UV (22-watt circle black light, BioQuip, Gardena, CA) light traps placed in three different locations during the 1998 swarming season (May-August). The collected alates were transferred into plastic boxes lined with wet paper and placed into a Percival® environmental chamber at 25°C ± 1°C until they lost their wings. Then, 100 pairs (male and female) were transferred to sterile glass vials containing 3 ml of sterile media composed of yellow pine dust: agar (Difco, Detroit, MI,) (1:3) (King et al. 1974). The vials were closed with sterile cotton and incubated at 27 ± 1°C and 70% RH for 30 d. Twenty mated pairs were randomly selected for extraction of fungi.

Workers and soldiers were extracted from logs and debris found in three locations. Thirty groups of 10 termite workers and two soldiers were transferred to sterile plastic Petri dishes containing 10 g of the media (10 dishes per location) described above. The edges of the dishes were sealed with Parafilm® to maintain humidity. The dishes were kept in the chamber as above.

Samples of wood and carton material from seven wind-fallen FST infested trees were collected following a storm (Hurricane Georges, September 1998). Ten samples from each tree were individually transferred to sterile Petri dishes and humidified with sterile ionized-Q water to favor fungal growth. The dishes were incubated for 30 d and fungi were isolated and identified using the methods described below.

Ten dishes of each group and 20 mated pairs were selected for isolation of fungi. The samples consisted of three small pieces of media collected from the inside of the nuptial chamber of the alates where mycelia growth was evident. Samples were transferred to sterile Petri dishes containing 15-ml sterile media, to favor the isolation of rapid growth fungi, which are more likely to be an abundant source of supplementary food.

Two media were used: 1) PDA (potato-dextrose-agar, Difco, Detroit, MI) prepared according to the label and 2) water: agar: yellow pine dust (97.5:1.5:1). Both media were autoclaved for 20 min at 120°C, cooled to 55°C in a 50°C water bath, and acidified with lactic acid to a 5.6 ± 0.2 pH. The edges of the inoculated dishes were also sealed with Parafilm® to maintain humidity. These dishes were incubated at 27 ± 1°C for 1 and 2 weeks, respectively.

The dishes were then examined under a stereo microscope to find conidia and mycelia. One technique to isolate pure cultures was designed to favor the isolation of fungi associated with wood decay. Seven sterile 2 × 2 × 0.5 cm pieces of woods: sweetgum, *Liquidambar styraciflua* L. (Hamamelliaceae), pecan, *Carya illinoensis* (Wangenh.) K. Koch (Juglandaceae), and yellow birch, *Betula alleghaniensis* Britton (Betulaceae) were individually placed into Petri dishes containing sterile PDA. Each piece of wood was inoculated on one of the edges with a small piece of agar and fungal mixture. The dishes were sealed and incubated as above for 7 d. Small pieces of agar with mycelia from each culture was transferred to sterile PDA to obtain pure fungal cultures. This was repeated 2 times for each culture.

The second technique was designed to isolated saprophytic fungi, 1-ml spore suspensions of the mixed fungal cultures were made by scraping culture plates containing 10 ml of 0.01% Triton X-100 (Amresco, Solon, OH). Spore suspensions were enumerated using a Levy hemacytometer (Hausser, Horsham, PA), and diluted with 0.01% Triton X-100 to a final concentration of approximately 1×10^3 spores/ml. A 100 µl aliquot of each suspension was spread, with a sterile glass hockey stick onto an agar plate containing 10 ml of the above PDA. Plates were incubated at 25 ± 1°C for 2-4 d, until small colonies were visible. Each colony was individually transferred by sterile loop to one section of a quadrant Petri dish con-

TABLE 1. PERCENT OF SAMPLES OF DIFFERENT SUBSTRATES WITH SPECIES OF FUNGI ASSOCIATED WITH *COPTOTERMES FORMOSANUS* IN NEW ORLEANS, LA.

Substrate	Species		
	Af	An	Cl
Infested trees[a]			
Ulmaceae			
American elm, *Ulmus americana* L.	80	80	50
Chinese elm, *Ulmus parvifolia* Jacq.	80	80	44
Fagaceae			
Live oak, *Quercus virginiana* Mill.	78	78	60
Aceraceae			
Red maple, *Acer rubrum* L.	65	65	70
Platanaceae			
Sycamore, *Platanus occidentalis* L.	60	60	55
Salicaceae			
Willow, *Salix babylonica* L.	60	60	80
Taxodiaceae			
Bald cypress, *Taxodium distichum* (L.)	45	45	50
Termites			
Cartoon material[b]	25	25	100
Alates[c]	100	100	100
Workers[c]	100	1001	100

Percentage of successful isolation from 30 samples per substrate. *Af* = *Aspergillus fumigatus* Fresenius, *An* = *Aspergillus nomius* Kurtzman, and *Cl* = *Curvularia lunata* (Wakker).

[a]By *C. formosanus*.

[b]From nests of *C. formosanus* found in . . .

[c]From external body parts.

taining the PDA as above, incubated at 25± 1°C for 7 d until sporulation, and transferred by sterile loop to new PDA plates.

Plates containing pure cultures of the fungi were incubated at 25 ± 1°C for 7 d to induce sporulation. Identification was accomplished after growth on standard media using Ellis (1971) (for *Curvularia)*, and Klich and Pitt (1988) (for *Aspergillus)* taxa keys. Identification of *Aspergillus nomius* was confirmed using the methods of: Kurtzman et al. (1987) and Singh et al. (1991).

Species of fungi isolated from the reproductive and worker termites placed in the pine medium, carton material, and infested trees were identified as *Curvularia lunata* (Wakker) Boedijn (Pleosporales: Pleosporaceae), *Aspergillus fumigatus* Fresenius, and *Aspergillus nomius* Kurtzman, Horn, and Hesseltine (Eurotiales: Trichocomaceae) (Table 1).

Curvularia lunata is a facultative pathogen of mainly monocotyledonous plants (Bhale et al. 1982, Bisen 1983, Domsch et al. 1980, Gadage & Patil 1977, Kore & Bhide 1981, Pearson & Mukiu 1982).

Aspergillus fumigatus is ubiquitous (Cutler et al. 1996, Dorner et al. 1984, Ekundayo 1983, Klich & Pitt 1988, Pal et al. 1986, Rath et al. 1997).

Aspergillus nomius has been isolated from insects (Kurtzman et al. 1987, Ito et al. 1997) and plant substrates (Kurtzman et al. 1987; Feibelman et al. 1998).

We thank A. Morgan, D. Daigle, S. Boue, and C. Carter (USDA-ARS-Southern Regional Research Center) for the review of this manuscript.

SUMMARY

Three species of imperfect fungi (*Curvularia lunata*, *Aspergillus fumigatus* and *Aspergillus nomius*) were isolated from the body of *Coptotermes formosanus* alates and workers from 6 different locations around the Greater New Orleans area. Samples from 7 species of trees infested by *C. fomosanus* as well as their carton material also presented these fungi. *C. lunata* growth was favored in the carton material while the *Aspergillus* species growth was favored in the wood. The possibility of a termite-fungi association is discussed.

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