Mildew fungi found in termites (*Reticulitermes lucifugus*) and their nests

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Abstract

This paper presents the results of observation of mould growth in laboratory colonies of termites. It also attempts to determine the species of mould fungi present in the research laboratory and the main colonies and their entomopathogenic for the termites. The following four species were found in test termite colonies: *Trichoderme viride*, *Mucor himeralis*, *Rhizopus nigricans*, *Aspergillus* sp., *Aspergillus flavus*, *Alternaria* sp., *Penicylium verucosum* and *Fusarium* sp. were recognisable in test colonies with domestic and exotic wood. Morphological observations of the fungi were carried out using a microscope with a 40x magnification. The growth of mould fungi in test containers caused death of whole termite colonies.

Introduction

During the years 2010-11, at the Division of Wood Protection of Faculty of Wood Technology of WULS, a series of tests were performed, on rate of selected domestic and exotic woods and selected wood-based materials degradation caused by a European termite *Reticulitermes lucifugus* (Rossi). Test samples were infected by mildew fungi. The fungi propagated on termites, causing extinction of all or most termites.

It is necessary to remark high mortality rate of termites in test colonies. The cause of that should be taken into consideration; was it the weakening of insects separated from main nest and bred in 200 mL and 500 mL beakers or high pathogenicity of fungi strains? Another question is how sterile colonies, prepared in accordance to Anonymous (2005, 2008) were infected by fungi? In order to answer these questions, research was undertaken. It was focused on determining mildew fungi species causing high mortality in test colonies as well as identifying fungi appearing in the main termite nest.

Termites feed on cellulose-rich plant tissue. However, these insects do not have enzymes necessary for cellulose and hemicelluloses digestion. Digestion of these polysaccharides occurs through microorganisms present in the alimentary tract of the insects. In intestines of the insects, the following fungi were found: *Alternaria alternata* (Fr) Keissel, *Paecilomyces fusisporus* (Saksena), *Rhizopus stolonifer* (Ehrenb.), as well as some species of *Aspergillus* and *Cladosporium* families (Grzywnowicz, 2002).

Some termite species provide their nests with special chambers, called fungi garden, in which special fungi are bred and then used as food for larvae. In nests of fungi breeding species the following moulds were found: *Fomes* sp., *Acremonium* sp., *Fusarium* sp., *Stachybotrys* sp., *Penicylium* sp. and *Trichoderma* sp. (Grzywnowicz, 2002). Other research isolated worker and soldier castes of Formosan subterranean termite *Coptotermes formosanus* (Shiraki) (Isoptera: Rhinotermitidae) with spores of three mould fungi: *Aspergillus fumigatus* (Fresenius), *Aspergillus nomius* (Kurtzman, Horn & Hesselt) and *Curvularia lunata* (Boedijn). In the aforementioned works the possibility of insects and fungi coexisting and the possibility of their diet being supplemented with fungal proteins was not excluded. The possibility of a termite-fungi association is discussed by Rojas *et al.* (2001).

Nests may become the growth place for not only the feeding termite species, but also the rival ones. Jayasimha and Henderson (2007) showed that all fungal structures isolated from intestines and skin of the *C. formosanus* were antagonistic towards *Gloeophyllum trabeum* (Persoon), efficiently controlling growth of this *Basidiomycete*. Termites then can use parasitic fungi to control *G. trabeum,* the fungi competing with these community insects (Jayasimha & Henderson, 2007).

Over 700 species of entomopathogenic fungi are known (Milner, 2000; Lenz, 2005). Species such as *Paecilomyces farinosus* (Holmskjold) and *Paecilomyces fumosoroseus* (Wize) are most common (Piontek, 1999; Mazurkiewicz-Zapałowicz, 2006; Zimmerman, 2008). *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin are also regarded as harmful for ground termites (Lenz, 2005; Yanagawa *et al*., 2010, Sawicki, 2010). *Metarhizium anisopliae* (Metchnikoff) Sorokin fungus produces as many as 17 paralysing substances. Its hyphae contain large amounts of endoprotease responsible for adhesion of fungus' hyphae to the cuticle of the insect. This fungus belongs then to specialised invasive species (Mazurkiewicz-Zapałowicz, 2006). The pathogenic properties of *Aspergillus flavus* (Link), fungi produce aflatoxin causing mortality in various termite species (Becker *et al*., 1969; Zoberi & Grace, 1990). Worldwide literature also describes mutual protection of worker-termites against translocation of harmful spores

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such as *Beauveria brongniartii* (Saccardo) on their bodies. Through mutual nursing, worker-termites remove spores of pathogenic fungi from their bodies. Insects recognise dangerous fungi species by their sense of smell (Yanagawa *et al*., 2010).

On the other hand, some termites have high resistance against fungal epizootic diseases (Boucias & Pendland, 1996; Rosengaus *et al*., 1998; Myles, 2002; Shimizu & Yamaji, 2003; Meikle *et al*., 2005). Such differences are caused by variations of research methods, like infection methods, concentration of spores in lab wares, quantity of tested insects, etc.

Interactions between termite and insecticidal fungi species are not fully determined and require further investigation.

Materials and methods

There were 80 beakers (250 or 500 mL) with 250 termites workers per beaker. Samples of selected domestic woods: *Pinus sylvestris* (L.) and *Quercus robur* (L.) and exotic woods: *Hopea pieeri* (Hanse) and *Erythrophleum fordii* (Oliver) and as well as wood-based materials such as fibreboard and medium density fibreboard were used.

Mycelium and spore samples of mould fungi were acquired from the infected annual rings of wood. Biological material was incubated on nutrient agar at 27°C. Pure fungi cultures were obtained by inoculation on MEA (Malt Extract Agar) in Petri dish. After the incubation, macroscopic and microscopic morphology using a 40x microscope was performed to identify fungi (Thom & Raper, 1945; Raper *et al*., 1949; Piontek, 1999).

Results and discussion

The following mould fungi were discovered: *Penicylium verucosum* (Stolk et Hadlok), *Mucor himeralis* (Wehmer), *Trichoderma viride* (Pers. ex Gray), mould of *Aspergillus* (*A. ustus* or *A. flavus*) genre, as well as *Rhizopus* sp., *Paecilomyces* sp. and *Alternaria* sp. fungi. *Fusarium gramirenorum* (Petch) and others of *Fusarium sp.* genre were possibly observed as well but these species could not be isolated. Fungi of the nest appeared to be similar to fungi in test colonies of the experiment. Six mould species were bred from the nest: *Aspergillus ustus* (Thom et Church) (Figure 1) and probably *Aspergillus flavus* (Link), *Fusarium* sp. (possibly *F. gramirenorum*, *T. viride*, *Paecilomycetes sp.*, and *Alternaria sp*. In the nest, there was intensive growth of *Coniophora puteana* (Karsten) fungus and spores of the fungus *Alternaria* sp. and *Fusarium* sp. It was also possible to determine the *Penicillium verucosum* species after the construction of conidia occurred as well as to find conidia of *Trichoderma viride*, and hülle cells characteristic of the mould *Aspergillus* sp.

Part of the selected insects came from the nest infected with *Coniophora puteana* (Karst.) fungus. Termites originating from this nest showed lighter shade of the cuticle, varying from the natural colour. Discoloration could give some indication about initial fungi infection, caused by a weakened immunological system. Weakened and infected individual insects could then transfer fungi spores on wood samples. Fungi, rapidly growing in optimal conditions (moisture and temperature factors), quickly spread to the weakest termites, isolated from their home nest. After the mycelium appeared on the termite bodies, necrobiosis occurred (Figures 1 and 2).

Some fungi species isolated from the test colonies produce toxic compounds. Numerous microtoxins are produced by *Alternaria sp.* fungi. Some dead insects were overgrown with hyphae of this species (Figures 3 and 4). The presence of *Aspergillus flavus* (Link), producing dangerous aflatoxin, probably caused high mortality. The aforementioned fungus is described in numerous articles as toxic to various insects (Becker *et al*., 1969; Zoberi & Grace, 1990). Aflatoxin and other microtoxins are as well produced by *Penicilium verucosum* (Stolk et Hadlok) while *T. viride* produces trichodermin. Brownold and Flanders (2005) describe another mould species - *Trichoderma harzianum* (Rifai) as a known pathogen for western honeybee *Apis mellifera* L. *Ophiocordyceps sphecocephala* (Klotzsch et Berk) (Lohmeyer & Künkele, 2006) is a parasite affecting wasps and other *Hymenoptera* sp.

Isolated *Mucor himeralis* (Wehmer) fungus infests insects through their damaged cuticle (Piontek, 1999). Their pathogenic properties are widely known, especially against younger bees (Piontek, 1999). *Mucor* sp. was isolated from *C. formosanus* nests. Damage to termite cuticle may have occurred during sampling for the tests. Similar damages may have also occurred during other activities related to moving the insects to test colonies, weighting or raking the soil in the main nest. As it is commonly known, damaged cuticle is one of the most important infection causes of fungi pathogens. Fungi of *Mucor* sp. belong to most common ground species. Strong toxic properties are also shown by *Rhizopus* sp. (Piontek, 1999). Other moulds isolated from the main

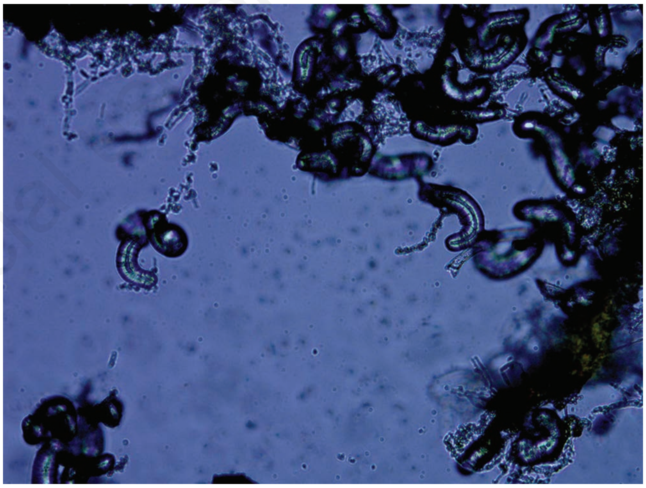

Figure 1. *Aspergillus ustus*.

Figure 2. Beaker with termites reared on mould.

nest or test colonies such as *Paecilomyces* sp. fungi are widely known as insect parasites and they are used as biological insecticides. As an example of such application, *Paecilomyces fumosoroseus* (Wize) may be used against whiteflies (Homoptera: Aleyrododea) and roundworms (Nematodes) (Mazurkiewicz-Zapałowicz, 2006; Zimmerman, 2008).

The growth of mould fungi in test containers caused death of whole termite colonies. Some fungi species isolated from colonies were found also in the nest. In test containers other fungi species were also found, but were not found in the main nest.

Appearance of the mould fungi in the isolated test colonies was probably caused by introduction of the already infected termites with pathogens. The possibility that termites infected sterile environments with spores attached to the cuticles cannot be excluded. In test colonies the following species were found: *Trichoderma viride*, *Mucor himeralis* (Wehmer), *Rhizopus nigricans* (Ehrenberg) and *Aspergillus* sp. In test colonies with domestic and exotic wood, *T. viride*, *Aspergillus flavus* (Link), *Alternaria* sp., *P. verucosum* and *Fusarium* sp. were recognisable. One of the exotic wood samples showed probable appearance of *Aspergillus ustus* (Thom et Church) (Figure 5). Test colonies containing samples of wood-based materials showed the following species: *T. viride*, *M. himeralis*, *R. nigricans* and *Aspergillus* sp. Except for the species coming from the main nest, common mould species appeared probably during preparations or later maintenance and observations of the colonies. Most frequently repeating species were *T. viride* and *Aspergillus* sp. fungi. These fungi were present in the main nest; colonies were probably infected by the insects.

Figure 3. Initial stage of mould growth on termite body.

Figure 4. Advanced stage of mould growth on termite body.

Figure 5. Very advanced stage of mould growth on termite body.

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