Histopathology of *Metarhizium anisopliae*, an Entomopathogenic Fungus, Infection in the Termite, *Coptotermes curvignathus*

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ABSTRAK

Anai-anai pekerja, Coptotermes curvignathus *(Isoptera: Rhinotermitdea), serangga perosak pokok buah-buahan dan pokok ladang hutan, telah diinokulasi dengan kulat* Metarhizium anisopliae *dengan mendedahkan anai-anai tersebut kepada konidia di dalam piring petri. Ontogeni kulat diteliti dengan cara histologi. Konidia yang terlekat pada kutikel anai-anai bercambah dalam masa 24 jam selepas inokulasi. Tiub germa menembusi kutikel dan hifa kemudian menyerang tisu mengikut urutan seperti berikut: tisu lemak, otot, tisu saraf, sel epitelia usus dan hempedal. Anai-anai yang dijangkiti kulat mati dalam masa 36-48 jam selepas inokulasi. Walau bagaimanapun, secara histologi jangkitan sepenuhnya, ternyata berlaku 72 jam setelah tiub germa menembusi kutikel. Setelah 100 jam selepas inokulasi miselia mula keluar dari kutikel anai-anai. Miselia ini membentuk jisim konidiofora mampat yang mengeluarkan konidia berwarna hijau selepas 120 jam inokulasi.*

ABSTRACT

Workers of the termite, Coptotermes curvignathus *(Isoptera: Rhinotermitidae), a pest of many tree crops including fruit and plantation trees, were inoculated with the entomopathogenic fungus,* Metarhizium anisopliae *by exposing the termite to viable conidia in a petri dish. The ontogeny of the fungus was followed histologically. Conidia which landed on the cuticle germinated within 24 h. The germ tube penetrated the cuticle and the hyphae subsequently invaded the tissues in the following order: fat body, muscles, neural, gut epithelial cells and gizzard. Infected termites died between 36 - 48 h post-inoculation. Complete colonization of the termite, however, was not histologically evident until 72 h post-inoculation. At this stage, all parts of the insect's internal organs were infected. At 100 h, whitish mycelia began to emerge from the cuticle. Compacted masses of conidiophore-producing-green conidia were formed 120 h post-inoculation.*

INTRODUCTION

The subterranean termite, *Coptotermes curvignathus* (Isoptera: Rhinotermitidae), is a pest of many tree crops including forest plantation species and structural timber in Malaysia. It is a major pest of *Pinus caribaea* plantations and a potential pest of a newly introduced forest plantation species, *Acacia mangium.* Heretofore, neither the biology or ecology was clearly understood nor was there an effective method of controlling this termite other than using chemicals. To date, only chlorinated hydrocarbon insecticides have been recommended to control this termite.

The use of persistent insecticides to control soil insects, like termites, is known to cause groundwater contamination and destruction of soil fauna (Thompson and Edwards 1974; Edwards 1975). These problems therefore warrant the search for alternatives to chemical control which are effective and safe to the environment. In recent years, many trials using entomopathogens to control insect pests have been carried out.

Among these pathogens, the fungi, *Beauveria bassiana, Metarhizium anisopliae* and *Entomophthora* sp., have been tested against termites with considerable success (Yendol and Paschke 1965; Hanel 1981; Lai et al. 1982; Hanel and Watson 1983). A recent study by Ahmad Said and Phang (1990), has also shown that *C. curvignathus* is susceptible to *B. bassiana* and *M. anisopliae*. This study was undertaken with the objective of examining histologically the development of *M. anisopliae* in infected *C. curvignathus*.

MATERIALS AND METHODS

Termites

Termites were collected from an infested *P. caribaea*. The infested tree was cut into short billets and kept in a damp fiberglass tank measuring 90 cm x 72 cm x 60 cm. In this study, only worker termites were used. These termites were extracted from the billets and kept in petri dishes containing wet filter paper for 24 h before inoculation.

Inoculation

Twenty healthy worker termites were placed in a petri dish containing *M. anisopliae* conidia. This fungal isolate, ATCC No. 1, was obtained from Dr. Heinz Hanel of Hoechst Aktiengesellschaft, Frankfurt, West Germany. A total of 200 termites were exposed to the conidia. The termites were allowed to move around in the petri dish for 5 min and were then transferred into clean petri dishes containing wet filter paper. The petri dishes were kept in a dark chamber at room temperature $(28 \pm 2°C)$.

Histology

At successive intervals of 12 h, until the appearance of conidiophore on the termite's cuticle (120 h after inoculation), 10 termites were removed from the petri dish and fixed in an alcoholic Bouin's fluid. Two termites from each batch (i.e. at 0, 12, 24 and 36 h post-inoculation) were dissected. Small pieces of the cuticle were mounted on glass slides and stained with lactophenol blue (Hanel 1982). The remaining termites were dehydrated in a graded series of ethyl alcohol, cleared in benzene, embedded in paraffin, sectioned at 8 μm, stained with Delafield's hematoxylin and counterstained with eosin Y

(Humason 1972). Photomicrographs were taken with a Carl-Zeiss Jenaval microscope.

RESULTS AND DISCUSSION

The conidia germinated on the cuticle and the germ-tube penetrated into the cuticle of the termite within 24 h post inoculation *(Fig. 1)*. As in the *Nasutitermes exitiosus*, penetration occurred anywhere on the cuticle (Hanel 1982). The elongated germ tube formed an appressorium at the point of fungal entry. This appressorium apparently attached the fungus firmly to the cuticle while the hypha penetrated into the integument. It remained intact even after molting had occurred in the infected termite (Hanel 1982). After the fungus had penetrated the integument, the hypha began to ramify inside the termite. The penetration of the integument by the fungus involves both an enzymatic activity and mechanical pressure (Zacharuk 1973). At this stage, the infected termite became sluggish and failed to coordinate locomotory response. A similar symptom of pathogenesis has also been observed in *Reticulitermes flavipes* infected with *Entomophthora coronata* and *Entomophthora virulenta* (Yendol and Paschke 1965).
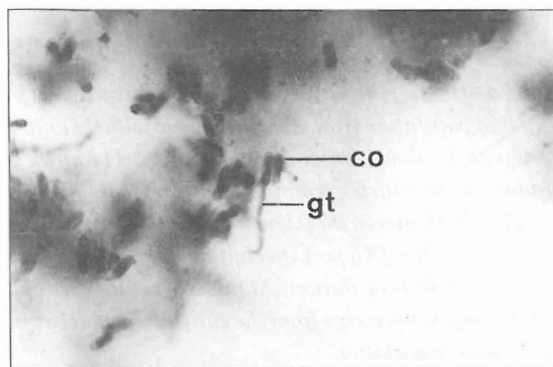

Fig 1.: A conidium germinating on the cuticle of Coptotermes curvignathus. co = conidium; gt = germ tube (500X)

At 36 h post inoculation, some of the infected termites were already in the moribund stage. The death of the termite, which occurred between 36 - 48 h post inoculation, might be due to the toxin metabolites produced by the fungus (Ferron 1978 and Roberts 1980). At this stage, hyphae were seen invading the fat body underlying the cuticle *(Fig. 2)*. The fat body in the uninfected termite, like in most insects, is the most
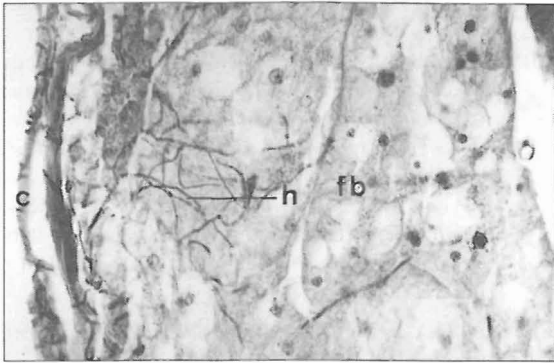
Fig 2.: Hyphae invading the fat body underlying the cuticle. c = cuticle; fb = fat body; h = hypha (450X)

conspicuous tissue filling the space between the vital organs. Other tissues such as muscle tissue, gut ephitelium, nervous tissue and gizzard were still relatively free from fungal invasion.

At 48 h post inoculation, the hyphal bodies were abundant in the hemocoel and the fungus had started penetrating the muscle tissue. *Fig. 3* shows hyphae in the muscle tissue. The termite muscle primarily consists of long bundles of striated fibres with oval nuclei. By this time, the hyphae were seen in almost all of the fat body.
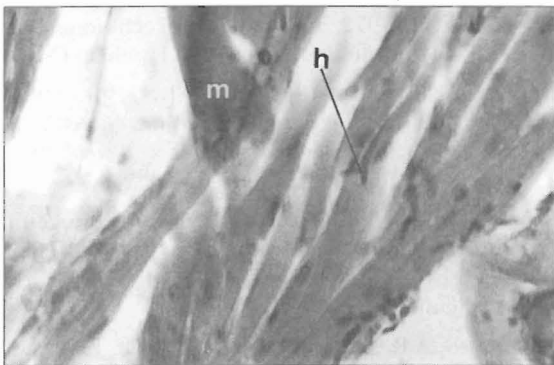

Fig 3.: Hyphae invading the muscle. h = hypha; m = muscle (900X)

At 60 - 72 h post inoculation, the fat body and the muscle tissues were extensively invaded by the fungus. Extensive cytological changes had occurred in the fat body cells. The cytoplasm and membranes of these cells began to disintegrate. As the infection proceeded, the fat body cells further deteriorated, culminating with the nuclei of the cells being no longer visible. Hyphae ramified within the muscle and split the muscle fibres into shorter and smaller bundles. Some of

the muscle tissues were no longer identifiable. At this stage, the digestive tract and the nervous tissue began to show signs of fungal invasion *(Fig. 4)*. The fungal hyphae colonized the intestinal musculature and subsequently penetrated through the epithelial cells into the gut lumen completely colonizing all organs in the termite.
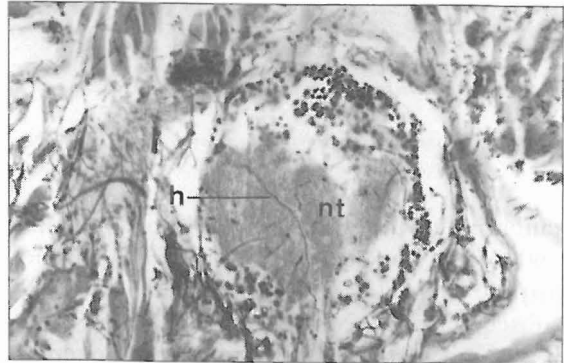

Fig 4.: Hyphae invading the nervous tissue. h = hypha;nt = nervous tissue (450X)

As mycosis progressed in the gut, the crypts of the midgut and the gizzard were attacked. *Fig. 5* shows hyphae in the circular muscle of the gizzard at 72 h post inoculation. The gizzard of this termite has a distinctive musculature which forms a series of folds provided with a very well-differentiated cuticular armature and well-developed chitinous intima. The presence of chitinous structure in the gizzard could possibly make the tissue of this organ the least susceptible to infection compared with other tissues of the termite.

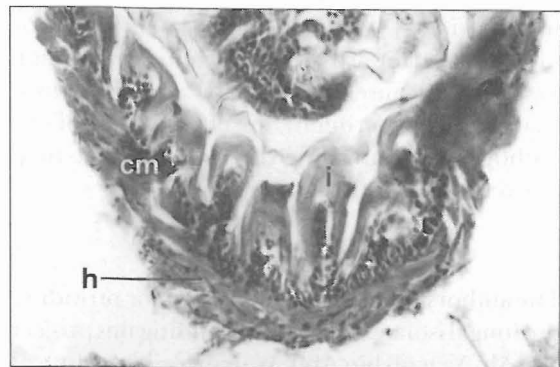Even though some termites died between 36 to 48 h after inoculation, mycelia were not seen

Fig 5.: Hyphae invading the circular muscle of the gizzard. h = hypha; i = intima; cm = circular muscle (450X)
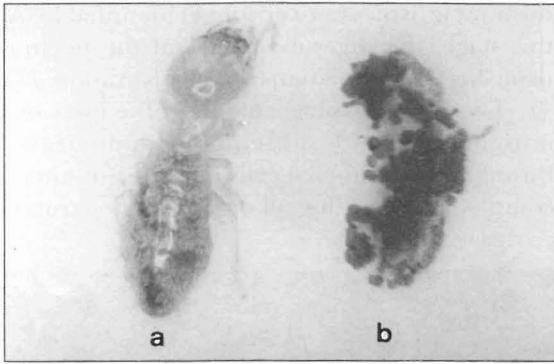
Fig 6.: A worker termite cadaver completely covered with conidiophores bearing conidia. a = a healthy termite; b = an infected termite (10X).

emerging from the cuticle until approximately 100 h post inoculation. The mycelia, which appeared pale yellowish white initially subsequently turned olive-green in colour at 120 h post inoculation. By this time conidiogenesis had begun. *Fig. 6* shows a compact mass of mycelia growing on the infected termite. At this stage of infection, the termite was virtually filled with the fungus. The cuticle was no longer visible and all the internal organs had been completely destroyed. A prominent layer of closely packed conidiophores with their interwining branches was formed around the termite.

CONCLUSION

Histological evidence shows that *C. curvignathus* is susceptible to infection by *M. anisopliae*. The complete developmental cycle of the pathogen, which took about 120 h paralleled that of this fungus in other termites (Hanel 1982). As noted in *N. exitiosus* (Hanel 1982), *C. curvignathus* apparently does not possess any defense mechanisms, haemocytic reactions or melanization towards the pathogen. Even though *M. anisopliae* causes mortality to *C. curvignathus* in a laboratory experiment, the effectiveness of this pathogen in controlling the termite in the field has yet to be confirmed.

ACKNOWLEDGEMENTS

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