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HISTOPATHOLOGY OF *Metisa plana* (LEPIDOPTERA: PSCYHIDAE) INFECTED WITH *Beauveria bassiana* (DEUTEROMYCOTINA: HYPHOMYCETES)

Keywords: *Metisa plana*; *Beauveria bassiana*; mode of infection; histopathology; oil palm and *Elaeis guineensis*

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Fourth larval instars of Metisa plana *Walker were sprayed with conidial suspensions of* Beauveria bassiana *(Balsamo) Vuillemin containing* 6.25×10^6 *conidia/ml. The mode of fungal infection was determined histologically. At 48 hours after inoculation, fungal hyphae penetrated the integument inside the trachea and via the nuclei of epidermal cells. Fungal hyphae infiltrated the fat bodies underlying the integument. At 72 hours after inoculation, the fat tissues were damaged by progressive colonization with hyphae. Subsequently, the hyphae invaded the muscle tissues, neural tissues, germ cavities, Malpighian tubules, gut musculature and epithelial cells, and finally colonized the gut lumen. Between 96 and 120 hours post inoculation, all internal organs of the bagworm were heavily colonized with hyphae, and the infected bagworms were already dead. Twenty-four hours after death, whitish mycelia began to emerge from the cuticle of the bagworm cadavers.*

INTRODUCTION

The bagworm, *Metisa plana* Wlk., is the most serious insect pest of oil palms (*Elaeis guineensis* Jacq.) in Malaysia (Wood, 1968). Outbreaks of *M. plana* have been reported since 1956, and between 1975–1980 (Wood, 1982) and 1981–1985 (Basri *et al.*, 1988). Currently, narrow spectrum and systemic insecticides are being applied to control this pest effectively by either spraying or trunk

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injection (Wood *et al.*, 1974; Chung, 1988). However, application of chemical insecticides has potential hazards to the environment and results in insect resistance. Therefore, alternative control of this pest ought to be attempted, in particular by the use of entomopathogenic fungi. Recently, the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. was isolated from *M. plana* (Ramlah *et al.*, 1994) and the bioassay results showed that it could possibly be exploited to control *M. plana* in the field (Ramlah *et al.*, 1993). Before field application more study is needed, for example on the histopathological aspects of this fungus.

Investigation on pathogenesis in bagless insects showed that *B. bassiana* infection was via the respiratory system (Clark *et al.*, 1968), the alimentary tract (Gabriel, 1959; Bao and Yendol, 1971; Broome *et al.*, 1976; Yanagita, 1987) and the external integument (Vey and Fargues, 1977; Pekrul and Grula, 1979). The pathogenesis of *B. bassiana* on insects which have bags, such as *M. plana*, is still unknown. The presence of bags might prevent the penetration of hyphae through the integument, which is the most common route of the fungal invasion (Atuahene and Doppelreiter, 1982; Pekrul and Grula, 1979; Vey and Fargues, 1977).

The objective of the present study was to investigate the mode of infection of *B. bassiana* on larvae of *M. plana* using common histopathological techniques.

MATERIALS AND METHODS

Insects

Insects were reared as described by Basri and Kevan (1995). The mated females of *M. plana* collected from an estate called Teluk Merbau in Selangor were allowed to hatch in plastic vials measuring 4.5 cm in diameter × 8.5 cm in height. Newly hatched larvae were transferred onto a leaflet segment of oil palm 22.5 cm long. Leaflet segments were obtained from fronds number 17 of the hybrid *tenera*. The leaflet segments were sterilized with 75% alcohol, rinsed twice in distilled water and air dried in laminar flow. About 4.0 cm of the lower part of the leaflet segments was inserted through an opening made in the cap of a vial, and submerged in the distilled water it contained. The plastic vials were then transferred into a clean transparent plastic cylinder 13.5 cm in diameter × 25.5 cm in height. The plastic cylinders were maintained at an average temperature of 24 ± 3°C with relative humidity of 65%–75%. Leaflet segments were changed weekly.

Fungus

The strain of *B. bassiana* used in this study was originally isolated from *M. plana* (Ramlah *et al.*, 1993; 1994). It was maintained on malt extract agar (Oxoid) and incubated in darkness at 25 ± 3°C.

Conidia suspensions

Conidia were harvested from two-week old cultures using sterilized distilled water containing 0.2% Tween 80 (SDWT80). Ten millilitres of SDWT80 were poured into a culture plate, and the conidia were scraped from the medium using a sterilized needle. The spore suspension was transferred into a universal bottle and vortexed at nine revolutions per minute for five minutes and then filtered through sterilized glass wool. The concentration of conidial suspensions was determined using a haemocytometer.

Inoculation

Active larvae of fourth instars of *M. plana* placed on leaflet segments were sprayed with conidial suspensions containing 6.25×10^6 conidia/ml using a hand sprayer in a fumehood. To ensure a homogenous spread of conidia, both sides of leaflet segments were sprayed.

Histology

Ten larvae were removed at 0, 24, 48, 72, 96 and 120 hours after inoculation. Six larvae were fixed in an alcoholic Bouin's fixative, dehydrated in a graded series of ethyl alcohol, cleared in methyl benzoate and embedded in paraffin with a high melting point. The entire larvae were sectioned to obtain sections 6 µm thick using a Leitz, 1512 rotary microtome. Sections were placed on clean glass slides containing Mayer's albumin adhesive and dried at 40°C. Specimens were then stained with Delafield's hematoxylin and counterstained

with eosin Y (Humason, 1972). Stained sections were mounted in DPX. Photomicrographs were taken with a Carl Zeiss Jenaval microscope.

RESULTS

Fungal penetration was observed as early as 48 hours after inoculation. At this stage most of the larvae were still alive. Fungal infection occurred only at the ventral and dorsal integument of the thoracic segments, where fungal hyphae penetrated the integument through the nucleus of an epidermal cell (*Figure 1A*). Transverse sections showed that penetration also occurred inside the trachea (*Figure 1B*). The fat bodies were the earliest tissues to be infected by the fungus soon after the hyphae penetrated through an insect's integument (*Figure 1A* and *B*). The hyphae penetrated the cuticle at the abdominal regions of an insect. During this stage, germinated conidia or hyphae were not visible in the gut lumen and all tissues of the abdomen appeared uninfected.

At 72 hours after inoculation, infected bagworms were already moribund. Extensive fungal growth was evident in fat body tissues and muscle tissues underlying the cuticle at the thoracic and abdomen regions (*Figure 2*). The hyphae also invaded the Malpighian tubules (*Figure 3*). Although the infection progressed within fat body tissues surrounding the midgut and hindgut, both of the gut lumina appeared uninvaded. At this stage, the Malpighian tubules, germ cavities, neural tissues, muscle tissues and some of the fat bodies were still intact.

Between 96 and 120 hours post inoculation, the fat body tissues and muscle tissues at the thorax and abdomen were extensively invaded by the fungus, and drastic cytopathological changes were visible in the fat body cells. The cytoplasm and membrane of these cells began to disintegrate and most of the nuclei were no longer visible (*Figure 4*). Although hyphae had ramified extensively in muscle tissues, only some of these tissues appeared to have disintegrated (*Figure 4*).

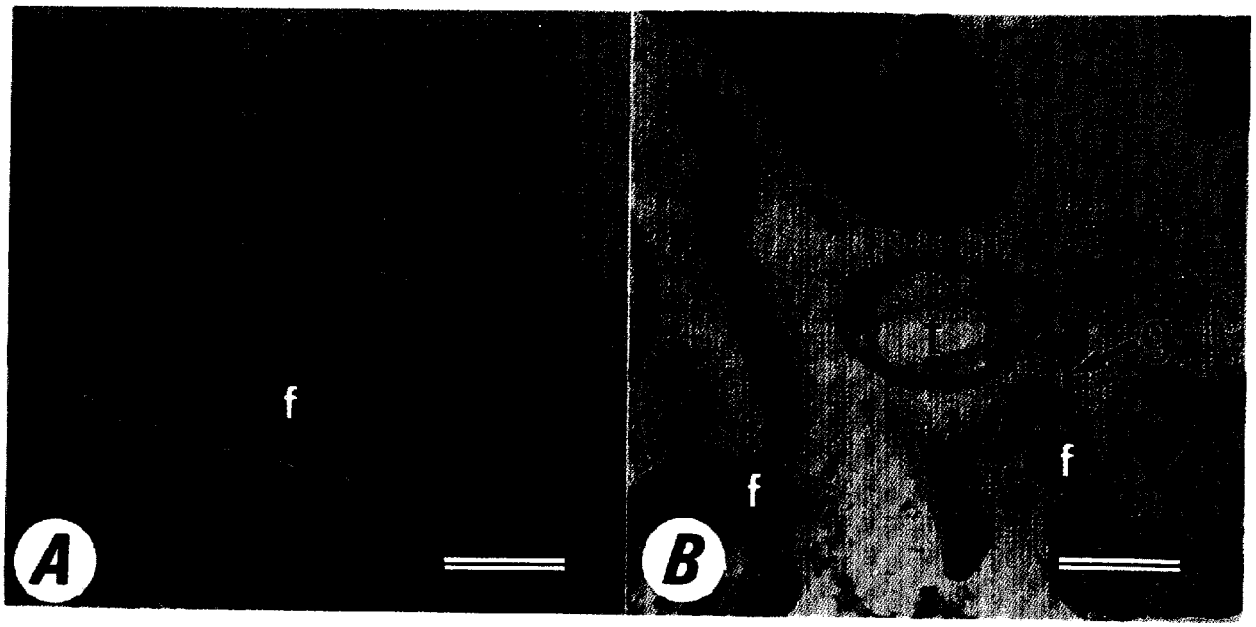

Figure 1. Transverse sections of ventral thoracic segment at 48 hours post inoculation. (A) Fungal hyphae (h) penetrating through the integument via a nucleus of an epidermal cell (arrow). (B) A germ tube (g) penetrating through the integument inside the trachea (t). Fat body tissues are labelled (f). Bar = 16 μm.

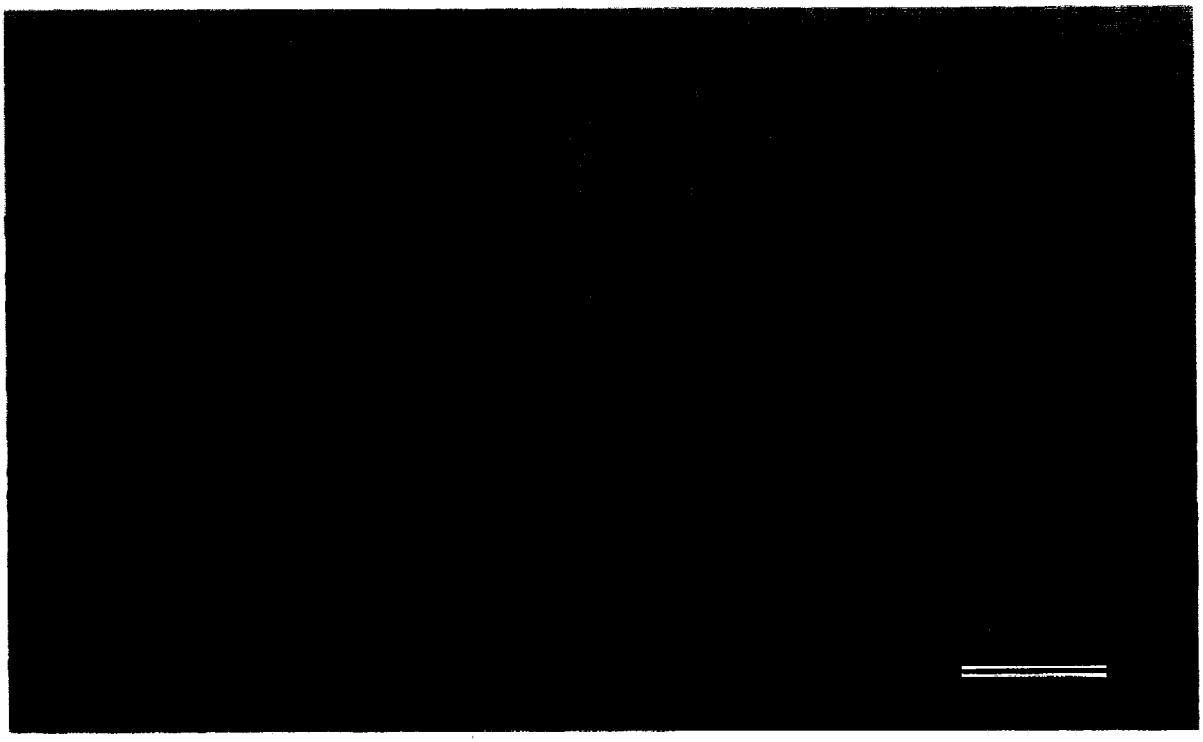

Figure 2. Longitudinal sections of abdominal region at 72 hours post inoculation. Fungal hyphae (h) colonizing the dorsal fat body tissues underlying the cuticle (c). Note the appearance of gut musculature (arrow) and gut lumen (l). Bar = 16 μm.

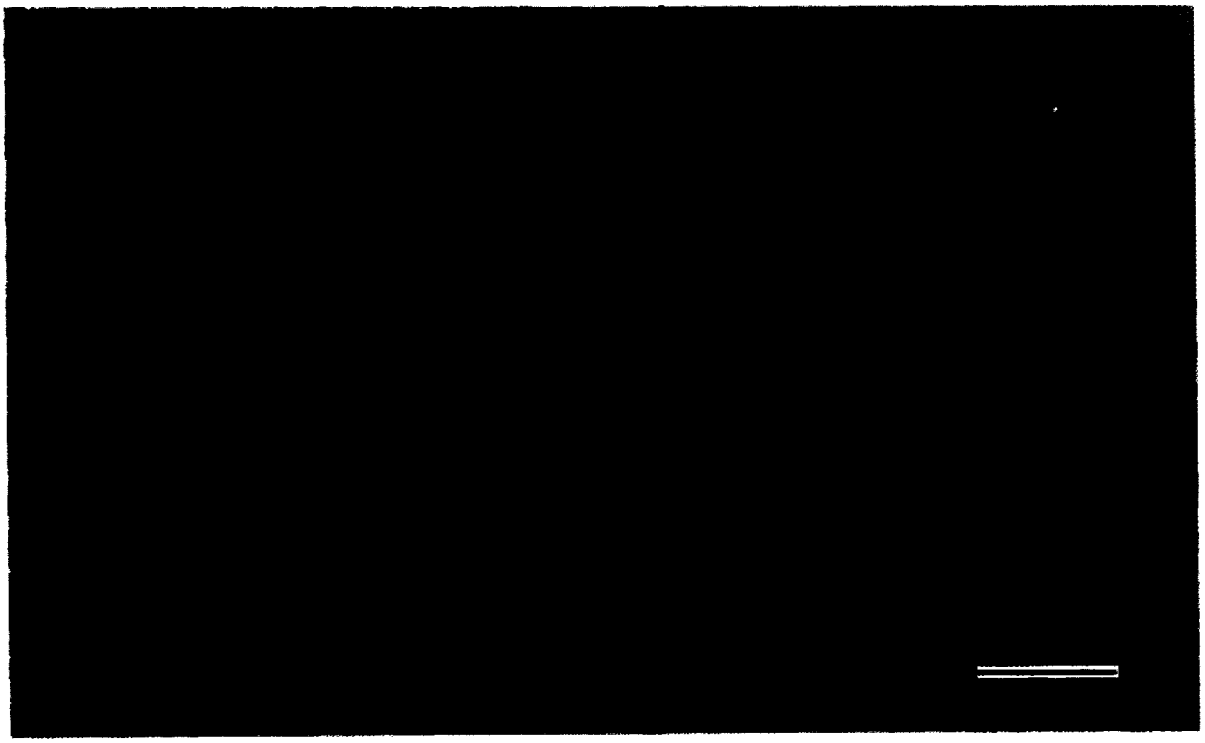

Figure 3. Longitudinal sections of abdominal region at 72 hours post inoculation. Fungal hyphae (h) attacking fat body tissues (f), and Malpighian tubules (mt). The ileum is labelled (i). Bar = 64 μm.

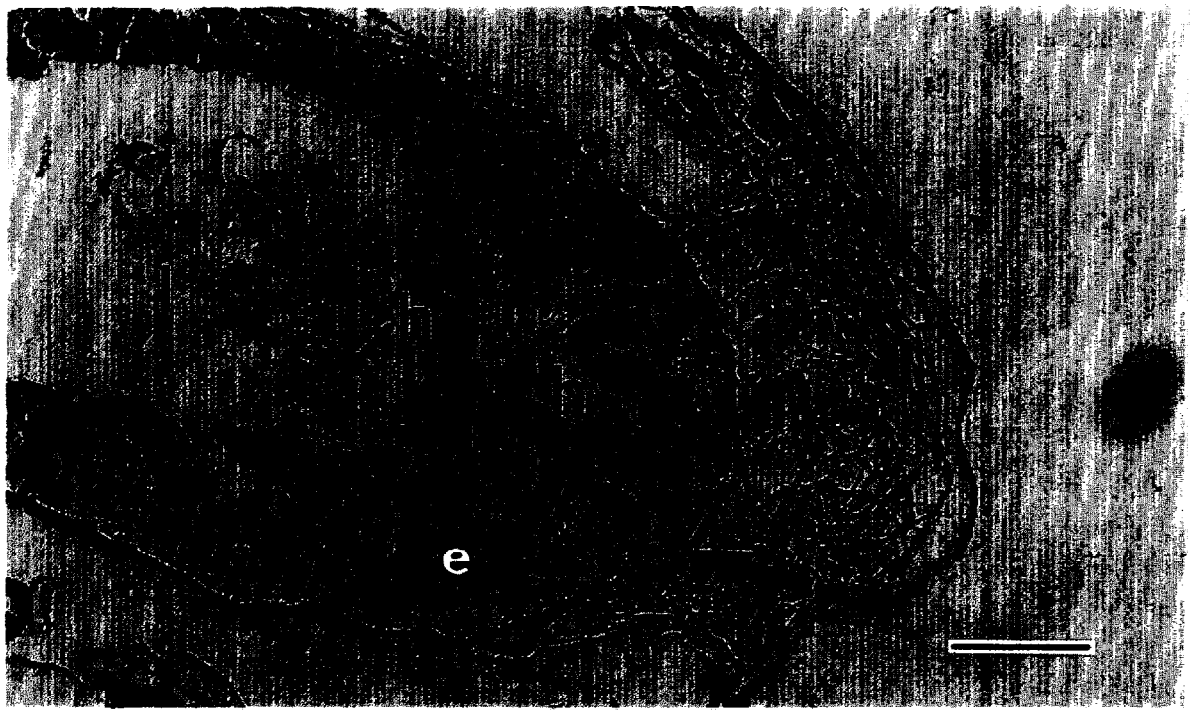

Figure 7. Transverse sections of abdominal region at 96 hours post inoculation. Fungal hyphae (h) penetrating through the gut musculature (arrow), epithelial cells (e), and ramified in the gut lumen (l). Bar = 64 μm.

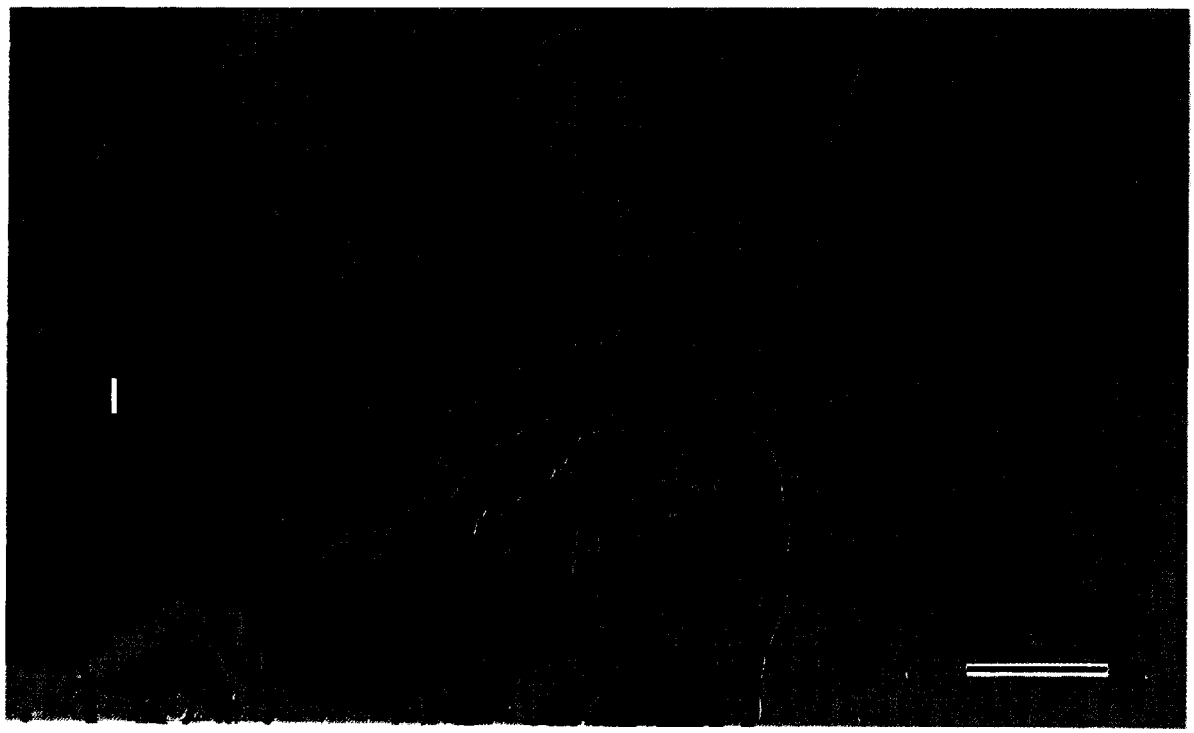

Figure 8. Transverse sections of abdominal region at 96 hours post inoculation. Fungal hyphae (h) protruding out through the intersegmental membrane of the abdomen. Note the appearance of trachea (t), muscle tissues (m), gut musculature (arrow) and gut lumen (l). Bar = 16 μm.

active cells contain mitochondria, dictysomes, ribosomes, and endoplasmic reticulum. Therefore, a high level of metabolic activity was occuring in these cells (Zacharuk, 1970a, 1970b). Germ tubes produced from these appressorial cells exhibited a strong orientation to penetrate the integument (Vey and Fargues, 1977). Additionally, elongations of germ tubes were supported by the presence of sufficient quantities of amino acids and glucosamine in the integument (Wood and Grula, 1984).

In bagworms, the germ tube penetrated the integument through the nuclei of epidermal cells (*Figure 1A*). In *H. zea*, penetration occurs near the horn-like nodule structures and at the bases of setae (Pekrul and Grula, 1979). It should be noted that penetration did not always occur at these structures, but in *H. zea*, a hole was formed by the enzymatic action of germ tubes at the integument (Pekrul and Grula, 1979). Zacharuk (1970a and b) concluded that penetration of the integument was primarily by an enzymatic mechanism, but was initiated by the mechanical activity.

Besides the direct penetration through the integument, the germ tubes also penetrated the integument inside the respiratory system (*Figure 1B*). This is possible because the conidia of *B. bassiana* are normally small enough (average diameter 2.5 μm) to be trapped inside a spiracle (diameter 5.3 μm or larger) and start to germinate. In *H. zea*, the germ tubes from the outside surface can enter the spiracle through the opening or by penetration through the side of a spiracle (Pekrul and Grula, 1979).

This study shows that no germinated conidia or hyphae appeared inside the gut lumen to cause infection at 48 hours post inoculation. The most critical condition influencing conidial germination inside the gut is the pH. However this study revealed that pH was not a factor influencing conidial germination inside the gut lumen of *M. plana*. The midgut pH for fourth instar of *M. plana* is 7.0 (Ramlah and Basri, 1994), which is suitable for the germination of *B. bassiana* conidia (Blazejewska and Wyrostkiewicz, 1976; Thomas *et al.*, 1987). Therefore, fungal infection in the gut lumen might be prevented by other factors such as the presence of digestive enzymes or microbial flora, insufficient contact time with the gut for conidial germination and penetration of the gut wall (Madelin, 1963) and, the rate of movement of food through the lumen (Pekrul and Grula, 1979; Leslie *et al.*, 1990).

In the insect body, large and varied amounts of free nutrients are available in the haemolymph and other vital tissues (Cheung and Grula, 1982). Once fungal penetration occurs, these free nutrients become available through the activities of extracellular hydrolytic enzymes such as proteinase, lipase and chitinase (Leopold and Samsinakova, 1970; Smith and Grula, 1983). These enzyme activities allow the mycelium to develop progressively inside the body and cause pathological changes in the haemolymph, mechanical blockages in internal systems and physical damage in vital organs (Bell, 1974). Malfunctioning of these systems and damage to vital organs will cause death of the insects.

This study shows that the presence of bags in the bagworm did not prevent fungal infection. We believe that the bags themselves can provide higher relative humidity inside than in the surrounding environment, and hence favourable condition for conidia to germinate and penetrate through the integument. Furthermore, the presence of the bag could inhibit the action of ultra violet radiation which could damage the conidia and consequently affect germination (Moore *et al.*, 1993).

CONCLUSION

A histopathological study has shown that *B. bassiana* infects *M. plana* through the integument and respiratory system. The presence of the bag in the bagworm did not seem to protect against infection by the fungus. Although the results from this study showed that *B. bassiana* can kill bagworms, its effectiveness as a pathogen in the field situation still needs to be investigated.

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