Histological Studies of *Cordyceps myrmecophila* (CES) 
Infection in the Ant *Palithothyreus tarsatus* Fab. 
(Formicidae: Ponerinae)

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(Received November 30, 1985)

Many moribund and dead *Palithothyreus tarsatus* Fab. and *Camponotus* spp. infected with *Cordyceps myrmecophila* (CES) were collected from Kibale and Zika forests (5 km outside of Entebbe in Uganda), and fixed for histological studies. These studies revealed that the fungus colonized in tissues of the head and thorax only. It invaded the tissue just behind the eye, attacked the brain, and the fat body was completely destroyed. The host cells in contact with the invading hyphae were quite completely destroyed and the surrounding tissues of the host were in a state of disorganization. The thoracic muscles and skeletal muscles were heavily invaded. The haemocoel between the cuticle and skeletal muscle was invaded by the vegetative hyphae. The mesentron was also attacked by the vegetative hyphae. There were major cellular losses due to a strong enzymatic activity. A variation in size of oblique perithecia was noted.

**INTRODUCTION**

Most of the species of *Cordyceps* are entomogenous. There are some that parasitize fungi: two have been described on sclerotia of *Claviceps*. A few species have been reported on hypogenous fungi of the genus *Elaphomyces*. Some occur on spiders. Most of the species develop on the larvae, pupae or adults of insects. In his summary of the species of the world, Kobayashi (see Mains, 1958) gives 5 on Arachnida, 39 on larvae of Coleoptera Scarabaeidae or on beetle larvae of the genus *Phyllophaga* spp., 36 on larvae of Lepidoptera and adult moths of the family Sphingidae, *Coccylius*, 20 on Hymenoptera Formicidae *Formica* spp., *Camponotus*, on hymenopteran wasp of the family Vespidae *Polistes*, *Tachytes*, and *Vespa* spp.; 12 on Hemiptera, 8 on Orthoptera cockroaches Blattidae, the mole cricket of the genus *Gryllotalpa hexadactyla*; 3 dipteran flies, on Isoperta and some have been reported on scale insects (Homoptera, Coccidae) and other Homoptera of family Cicadidae.

*Cordyceps* have been reported in Connecticut, New York, North Carolina, Kent Lake, Oakland, Michigan, Cuba, Jamaica, Panama, British Honduras, Tennessee, Brazil, British Guiana, Trinidad, Ohio, Europe and Asia, Ghana, and Uganda (Seaver, 1971; Jenkins, 1934; Mains, 1958; Evans, 1974; Samson and Evans, 1973, 1974, 1975, 1977; Samson et al., 1981, 1982; Kobayashi, 1941).

1 This investigation received financial support in part from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.
Over one hundred moribund and dead hymenopteran insects, Formicidae and Diptera flies, were collected from the Zika and Kibale forests 5 km away from Entebbe in Uganda. Some of these insects, *Paltothryeus tarsatus*, infected with *Cordyceps myrmecophilica* were fixed in formal saline for histological studies.

The purpose of the investigation was to obtain histological information on the extent of infection and also on the sclerotium formed in the dead species of *Paltothryeus tarsatus*.

**MATERIALS AND METHODS**

Moribund and dead specimens were fixed in formal saline (4% formalin containing 0.9% sodium chloride) for 24 hr. After fixation they were washed in water for 12 hr before dehydration. The fixed tissues were dehydrated, embedded in paraffin blocks and sectioned at 8 μm. An attempt was made to soften the embedded tissues using a microscopical reagent Mollifex (BDH). Tissues left in this chemical for 4 days were still difficult to section. The remaining tissues were decalcified using Gooding and Stewart's fluid: Formic acid (5.25 ml) and formalin (5 ml) in distilled water (100 ml). Sections were stained using haematoxylin-eosin. Some sections were stained with Gram's stain to differentiate the structure of asci walls, asci, and vegetative mycelium, and especially with a differential stain to show the relation of the mycelium to that of *Paltothryeus*. Heidenhain's iron alum haematoxylin was used to differentiate details of the nuclear structure. The sections were photographed.

**RESULTS AND OBSERVATIONS**

It was observed that dead and dying insects were clinging to wooden support at least a foot above the ground with their heads pointing either up or down in a vertical plane. The dead insects had mature and immature perithecia protruding through the thorax (see Fig. 1).

Before decalcification, these specimens proved difficult to section because of the large amount of calcium compound deposited in the alimentary canal. A few successful sections were made using decalcified material. Figure 2 depicts sections made from control insects through the head, thorax and fat body lobes. Figure 3 shows the colonization of tissues of the head by vegetative hyphae. The brain tissue was invaded by hyphae. Figure 4 shows endosclerotia or pseudosclerotia in thorax, skeletal and thoracic muscle. The endosclerotia and pseudosclerotia give rise to a dense vegetative mycelium which invades other tissues. Figure 5 shows the colonization of the skeletal muscle by the invading hyphae. Figure 6 shows the invasion of the fat body lobes in the moribund insects. This tissue was completely depleted at death. The haemocoel between the cuticle and skeletal muscle was colonized by vegetative hyphae. Figure 7 shows the colonization of the mesenteron by vegetative hyphae. Where a dense vegetative mycelium invaded a tissue, the cells in contact were completely destroyed, while the neighbouring tissues were in a state of disorganization.

Figures 8 and 9 depict a variation in the size of oblique perithecia prepared from different sclerotia of the same species and presented here for comparison. A section through a portion of a sterile stalk bearing a perithecial stroma was also made (see Fig. 2). Figure 10 shows cylindrical asci with septate filiform ascospores breaking into 1-celled fragments.
Fig. 1. Control longitudinal section through the head showing the eye capsule (E), cuticle (C) and the brain (B). ×180.

Fig. 2. Whole infected insects showing young stroma (y), mature sclerotium (s), sterile stalk (e).

Fig. 3. Longitudinal section through the head of an infected insect showing vesicle (V) of penetrant hyphae (p), cuticle (C), vegetative hyphae (N) and eye capsule (E). ×180.

Fig. 4. Longitudinal section through the thorax showing endosclerotia or pseudosclerotia (E). ×300.

Fig. 5. Heavy invasion of the skeletal muscle (S) by vegetative hyphae (V) Gram's stain—black dots are the nuclei. ×75.

Fig. 6. Longitudinal section through the thorax showing invasion of the fat body lobes (F) by vegetative hyphae (black strands) cuticle (C). ×180.
Fig. 7. Longitudinal section of the thorax showing the haemocoel between the cuticle and skeletal muscle invaded by vegetative hyphae which appear as black strands (Gram's stain). × 75.

Fig. 8. Longitudinal section through a perithecial stroma showing oblique perithecia (p), centrum (C).

Fig. 9. Longitudinal section through aperithecial stroma showing perithecia (p), centrum (C) with cylindrical asci. × 75.

Fig. 10. Part ascospores, i.e., segments of filiform ascospores. × 300.

**DISCUSSION**

**de Bary** (see **Jenkins**, 1934) accounted for the germination of endogenously produced conidia of *Cordyceps militaris* in the “Blood stream of larvae of *Gastropacha euphorbiae* to form sclerotia.”

Sclerotia were seen to give rise to hyphae that invaded neighbouring tissues. **Jenkins** (1934) noted that *Cordyceps agariciformia* destroyed groups of host cells (*Elephomyces*). In this study, it has been observed that the host cells in contact with the invading hyphae were rather completely destroyed, but the surrounding tissues of the host were in a state of disorganization. It was difficult to say whether the invading hyphae were the haustoria in a strict sense. But it was observed that the invading hyphae had a strong enzymatic action against the obstructive tissues. It is possible that the success of the invasion depends on both mechanical pressure as well as the secretion of enzymes. The fat bodies of the infected were completely destroyed. This destroyed the energy
source for the insect thus immobilizing it and leading to a moribund state. The attack on the brain tissue of the infected may lead to a loss of nervous control over the insect’s activity and orientation. The fungus destroyed the skeletal muscles, mesenteron and haemocoeel.

Sections through the sclerotia showed perithecial stromatic tissue containing oblique perithecia. The perithecia had a centrum made of cylindrical asci, with multiseptate filiform ascospores breaking into l-celled fragments. There was a variation in size of perithecia; of what significance this variation is could not be deduced from the data at hand. This collection is obviously a part of a wider C. australis epizootic, a common occurrence on this ant species in tropical forests. But unlike C. australis, C. myrmecophila has completely embedded oblique perithecia with cylindrical asci containing multiseptate filiform ascospores which break into l-celled fragments 5–10 × 1.5–2 (Figs. 8–10). The ascocarp is a cleistothecium-like type in C. myrmecophila. WASTI and HARTMANN (1982) reported that C. militaris killed 100% of gypsy moth larvae. ROBERTS (1980) reported culture filtrates of C. militaris toxic to Culex pipiens, Aedes aegypti and A. aegypti larvae.

Unlike mosquitoes, Palthothyreus tarsatus and Camponotus sp. are social insects with no harmful habits to man and his animals. Entomogenous fungi like C. myrmecophila that attack and kill harmless insects in any ecosystem would have no practical importance in a biological control sense.

ACKNOWLEDGEMENTS

My sincere thanks to Dr H. C. EVANS of the Commonwealth Mycological Institute of New England for identifying Palthothyreus tarsatus and confirming Camponotus sp., and for identification of Cordyceps australis and C. unilateralis. Thanks to Professor J. OKEDI, head of the Department of Zoology, Makerere University for his permission to use to facilities of the histology laboratory in his department. The supervision and close working relationship of Dr L. G. MUKWAYA, Head of Department of Entomology, Uganda Virus Research Institute is greatly appreciated.

This work was carried out with funds from the Ministry of Regional Co-operation and is submitted for publication with the permission of the Director of the Uganda Virus Research Institute. My regards to Miss Ruth NABAGISHERO for typing the manuscript.

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