Fungal and bacterial diseases of puriri moth, *Aenetus virescens* (Lepidoptera: Hepialidae), larvae

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Abstract

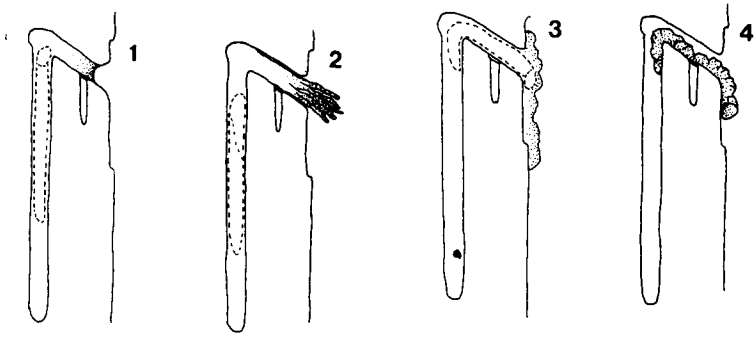
The general appearance of diseased larvae of *Aenetus virescens* is described for 1 bacterial and 3 fungal infections. Only one fungus has been identified—*Beauveria bassiana*. The morphology of the bacterium is illustrated by electron microscopy and is similar to that of "RM 17", a bacterium of doubtful taxonomic position causing a milky disease in scarab larvae.

Keywords: Lepidoptera; Hepialidae; *Aenetus virescens*; fungus; bacteria; pathology; *Beauveria bassiana*; New Zealand.

Larvae of *Aenetus virescens* (Doubleday) undergo 2 distinct phases of development. Initially they feed on fungi and dead wood on the forest floor (litter phase) before changing their habit to tunnel in and feed on live trees and shrubs (tree phase) for the main part of the life cycle (Grehan 1981a). Of the previously recorded diseases, *Cordyceps gunni* Berk has been recorded only from the tree phase while *Beauveria bassiana* (Balsomo) Vuillemin has been recorded from both the litter and tree phases (Grehan 1981b). This paper describes the appearance of diseased *A. virescens* tree phase larvae infected with *B. bassiana*, 2 unidentified fungi, and an unidentified bacterium.

Infected larvae were collected from the field and fungi were cultured on potato dextrose agar. This was successful only for *B. bassiana*. The morphology of the bacterium was studied by both light and electron microscopy. Dead larvae were smeared on to microscope slides and fixed in Carnoy's fluid before being triple-stained with Geimsa stain (Wigley 1980) for light microscopical examination. Tissues from infected larvae were fixed for electron microscopy in 5% gluteraldehyde in 0.05M phosphate buffer (pH 7.2) and then embedded in Spurr's resin. Sections were stained with uranyl acetate.

Four types of disease larvae could be distinguished in the field (Figs 1-4). One of the unidentified fungi (Fig. 1) is found on both larvae and pupae while the other (Fig. 2) has only been recorded from pupae. The hyphae of both fungi are white but they can be distinguished by the finger-like coremia of the fungus found only on pupae. The hyphae of *B. bassiana* are also white but extend out over the larval feeding area around the tunnel entrance (Fig. 3). Larvae attacked by the unidentified fungus (Fig. 1) are found in the inner part of the larval tunnel while larvae infected with *B. bassiana* are



Figs 1-4. Generalised diagrams illustrating relative position and form of diseased *Aenetus virescens* larvae (silk cover over tunnel entrance not shown). **1,** fungal pathogen of pupa and final instar larva; **2,** fungal pathogen of pupa with coremia protruding from tunnel; **3,** *Beauveria bassiana* infected larva with fungal hyphae extending over tree surface around tunnel entrance; **4,** larva infected by bacterium.

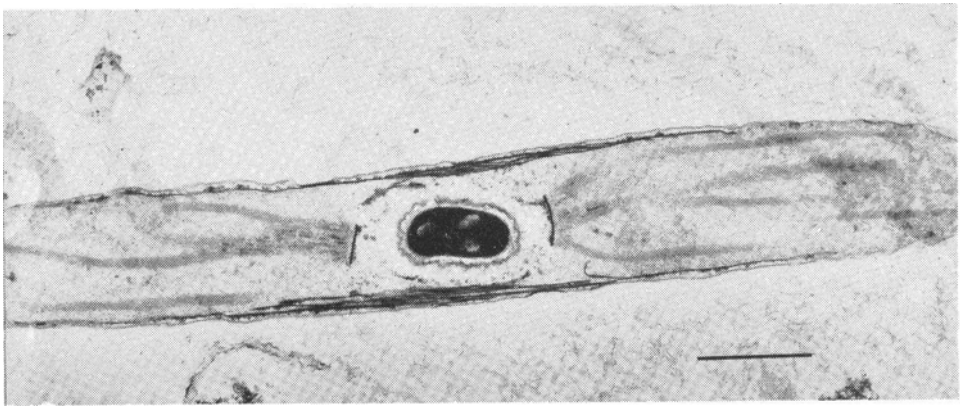


Fig. 5. Electron micrograph of longitudinal section through the bacterium infecting *A. virescens* showing the feather-like projections within the sporangium (bar = 1 μm).

found at or protruding from the tunnel entrance. It is possible that the 2 unidentified fungi are merely non-sporulating forms of *B. bassiana.*

Larvae killed by the bacterial disease are also found at the tunnel entrance (Fig. 4) and can be distinguished from the fungal-infected specimens by the lack of hyphae. Instead the larval body is darkly coloured, almost black and internally is packed with large numbers of the bacterium. Under the light microscope the bacterial sporangium is ephemeral and unusually elongated (8-10 μm) with a central highly refractile spore. Sections of the bacterium examined with the electron microscope (Fig. 5) show featherlike projections extending from the central spore to the distal ends of the sporangium. This structure is similar to that found in an unidentified bacterium ("RM 17") responsible for a milky disease in Australian scarabaeids (Milner 1981a; Milner & Beaton 1981). Two other infections with morphologically similar bacteria are known, one from a tipulid and the other from an isopod (Milner 1981b). As the bacterium forms spores and in Australian scarabs develops at the unusually low temperature of 12°C (Milner 1981a), it may have some potential for the biological control of porina *(Wiseana* spp.) and 'other soil inhabiting insects in New Zealand.

Records of larval mortality attributable to the diseases recorded in this paper are from Lake Pounui Reserve in the southern Wairarapa. All observations (except for *B. bassiana* attacking litter phase larvae, Grehan 1981 b) are for larvae in the host plant

Carpodetus serrata. The total observations for each disease are: fungus (Fig. 1), 3; fungus (Fig. 2), 1; *B. bassiana*, 7; bacterium, 4 (from a population of about 200-300 larvae between March 1978 to March 1983).

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REFERENCE

GREHAN, J. R. 1981a: Morphological changes in the three-phase development of *Aenetus virescens* larvae: (Lepidoptera: Hepialidae). *New Zealand journal of zoology 8*: 505-514.

———— 1981b: Infection of *Aenetus virescens* (Lepidoptera: Hepialidae) larvae by the fungus *Beauveria bassiana*. *New Zealand entomologist 7*: 327-329.

MILNER, R. J. 1981a: a novel milky disease organism from Australian scarabaeids: field occurrence, isolation, and infectivity. *Journal of invertebrate pathology 37*: 304-309.

———— 1981b: Identification of the *Bacillus popilliae* group of insect pathogens. Pp. 45-60 *In* BURGES, H. D. (Ed.) *Microbial control of pests and plant diseases 1970-1980*. London, Academic Press.

————; BEATON, C. D. 1981: A novel milky disease organism from Australian scarabaeids: ultrastructure. *Journal of invertebrate pathology 37*: 310-318.

WIGLEY, P. J. 1980: Diagnosis of virus infections: staining of inclusion body viruses. Pp. 35-39 *In* KALMAKOFF, J; LONGWORTH, J. F. (Eds.) *Microbial Control of Insect Pests. New Zealand DSIR bulletin 228*.