

a 66159

ENGLISH ONLY

(avec résumé en français)

INDEXED

A FUNGAL PATHOGEN FOR MOSQUITO LARVAE WITH POTENTIAL AS A
MICROBIAL INSECTICIDE

by

A. W. Sweeney,¹ D. J. Lee,² C. Panter³ and L. W. Burgess⁴

The sudden onset of a very high mortality in the larval culture trays of a laboratory colony of the mosquito Anopheles amictus hilli Woodhill and Lee 1944 during February 1972, led to the discovery of a new fungal pathogen of larvae of aquatic Diptera. Examination of dead larvae revealed the body cavity to be filled with a dense mycelium associated with a sporulating layer on the exterior surface. Hyphal branches were also observed in the coelom of apparently normal living larvae. The fungus was maintained in mosquito larvae by inoculating trays of healthy larvae with several dead infected specimens while attempts were made to isolate it in pure culture.

A successful isolation was achieved on 31 March 1972 using nutrient agar medium (0.5% peptone, 0.3% beef extract and 1.5% agar) containing 0.1% streptomycin and 0.02% neomycin. Mosquito larvae were infected with inoculum from this culture and re-isolation on nutrient agar medium was accomplished. After more than 40 subcultures the initial isolate is still pathogenic to mosquito larvae. Successful isolations from infected larvae have been made repeatedly and the fungus grows readily on a variety of common mycological media. Good growth has also been achieved in submerged culture in a broth consisting of peptone 0.5%, yeast extract 0.3%.

Dissections of living larvae have shown that the conidia germinate in the foregut and hindgut regions of the digestive tract. Hyphae have been observed arising from the alimentary tract, particularly the foregut, which appears to be the more important infection site. Infection through the midgut wall has not been seen. Penetration of the outer integument has not been detected although this is the common invasion site of insect pathogenic fungi.

Following penetration of the alimentary tract hyphae branch and proliferate in the coelom. Considerable hyphal growth may be present before the larvae show any behavioural symptoms, though sluggish twitching movements often occur in advanced stages of the infection. Death usually occurs from three to six days after conidia are introduced to culture trays maintained at 25°C. After death the body of the larva becomes completely filled with hyphae and assumes

¹ Malaria Research Unit, R.A.A.M.C., School of Public Health and Tropical Medicine, University of Sydney, N.S.W. 2006.

² Department of Entomology, School of Public Health and Tropical Medicine, University of Sydney, N.S.W. 2006.

³ C.S.R. Research Laboratories, Roseville, N.S.W. 2069.

⁴ Department of Plant Pathology and Agricultural Entomology, University of Sydney, N.S.W. 2006.

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation Mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

a bloated appearance. Within one or two days conidiophores emerge through the thoracic and abdominal cuticle and form a sporulating layer covering most of the body surface.

The taxonomic status of the organism is uncertain. It is a hyphomycete characterized by long-ovoid conidia borne singly at the apex of terminal and lateral flask shaped conidiogenous cells (see Fig. 1) and resembles the common terrestrial insect pathogenic fungus Metarrhizium anisopliae (Metsch.) Sorokin 1879. However, the present fungus may be distinguished from M. anisopliae by the production of the conidia which are borne singly rather than in basipetal chains compacted into columns (Barron, 1968).

Mosquito larvae of three genera (Anopheles, Culex and Aedes) have proved susceptible to infection in the laboratory but the host range is not restricted to the Culicidae. Larvae of the families Chironomidae and Ceratopogonidae have been infected but initial trials suggest that it may not be infectious outside a limited range of aquatic Dipteran hosts.

The fungus is being maintained in culture in three laboratories, the Malaria Research Unit of the Royal Australian Army Medical Corps, School of Public Health and Tropical Medicine, the Department of Plant Pathology and Agricultural Entomology, University of Sydney and the C.S.R. Research Laboratories, Roseville, Sydney.

At the present time the taxonomy of the fungus is being clarified and work on the determination of optimum culture conditions, standardization of inoculum and pathogenicity is in progress.

This organism is worthy of intensive investigation as a candidate microbial insecticide for mosquito control. It is rapid in action and can achieve very high levels of mortality under laboratory conditions. Infection by ingestion is particularly effective against mosquito larvae which feed non-selectively on particulate matter. Moreover, it appears that all mosquitos will be susceptible to infection. Mass production in simple media should be achieved without difficulty and prolonged subculturing does not lead to loss of pathogenicity. The possibility of control of insect vectors of disease by microbial techniques has been foreshadowed though no such agents have yet been developed to a stage where commercial availability is imminent (Laird, 1971). In our opinion the present pathogen shows great potential in the public health field and our current and proposed studies are directed towards this end.

SUMMARY

From a laboratory colony of Anopheles amictus hilli a fungal pathogen was isolated which had caused a very high larval mortality. The fungus, a hyphomycete which somewhat resembles Metarrhizium anisopliae grows readily on a variety of common mycological media and retains its high pathogenicity for aquatic stages of Diptera also after numerous passages on culture media. The fungus is being investigated for its potential use in mosquito control.

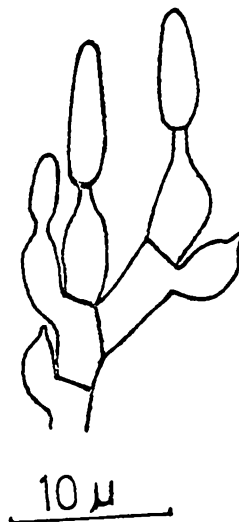
RESUME

Dans une colonie de laboratoire d'Anopheles amictus hilli, les auteurs ont trouvé un champignon pathogène pour les larves qui provoque une mortalité élevée dans les bacs de culture. Ce champignon a pu être isolé sur milieu nutritif gélosé. Il se développe bien sur plusieurs milieux mycologiques courants. Il s'agit d'un hyphomycete non encore classé qui ressemble un peu à Metarrhizium anisopliae. Même après 40 passages sur milieu nutritif, le champignon conserve une pathogénicité élevée pour les diptères aquatiques. Le principal siège de l'infection est, semble-t-il, l'intestin antérieur. Les hyphes se ramifient et prolifèrent dans le coelome. Les larves succombent en général trois à six jours après l'introduction de conidies dans les bacs de culture. On étudie la possibilité d'utiliser ce champignon pour la lutte contre les moustiques.

REFERENCES

- Barron, G. L. (1968) The genera of hyphomycetes from soil, Baltimore, The United States of America, Williams & Wilkins Co., pp. 223-225
- Laird, M. (1971) Microbial control of insects and mites, London, United Kingdom, Academic Press, p. 396

FIG. 1. A FUNGAL PATHOGEN FOR MOSQUITO LARVAE
WITH POTENTIAL AS A MICROBIAL INSECTICIDE



The purpose of the WHO/MAL series of documents is threefold:

- (a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;
- (b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;
- (c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

The mention of manufacturing companies or of their proprietary products does not imply that they are recommended or endorsed by the World Health Organization.