PATHOGENESIS OF AN *ASPERGILLUS FLAVUS* INFECTION
OF *GALLERIA MELLONELLA* EGGS

BY

C. N. BEHNKE & W. G. YENDOL (*)

Egg masses of the greater wax moth, *Galleria mellonella* L., experimentally infected with conidia of *Aspergillus flavus* LEX. became rapidly covered with the mycelium and were eventually destroyed. Germ tubes from conidia penetrated not only infertile, but also damaged eggs. Healthy eggs were often penetrated both by conidial germ tubes and mycelial elements. Once penetrated, eggs become filled with mycelium in as little as two days, after which conidiophores and mycelia protrude outwardly through the chorion.

It has been noted by STEINHAUS (1965) that information regarding the pathologies of infected insect eggs is almost nonexistent. Although some workers have mentioned eggs with an apparent mycosis, none of the reports include any detailed studies of the pathological condition. LOCKWOOD (1932) noted that eggs of the leafhopper, *Erythro- neura comes* (SAY), supporting growth of *Cladosporium* sp. and *Alternaria* sp. were dark brown to black and failed to hatch. Eggs of *Melolontha melolontha* (LINNAEUS) infected with *Metarrhizium anisopliae* (METCHNIKOFF) SOROKIN often turn brown and give a dried-up appearance (HURPIN & VAGO, 1958). CRIPS (1960) found tufts of zoosporangia protruding from the micropyle region of eggs of the water boat-man, *Corixa germania* FIEB, infected with *Saprolegnia* sp. When eggs of the differential grasshopper, *Melanoplus differentialis* (THOMAS), were infected with *Penicillium albicans* and a *Penicillium* sp. near *chrysogenum* they darken and collapse (BEHNKE, unpublished data). Other reports of apparent mycoses of insect eggs have also been mentioned in reviews by MADELIN (1963), MÜLLER-KÖGLER (1965) & STEINHAUS (1965).

The present investigation originated as an outgrowth of the need to explore the pathological relationship between entomogenous fungi

(*) Authorized for publication on November 7, 1968 as paper No. 3503 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Accepted for publication on December 30, 1968.
and their insect hosts. The fungus species *Aspergillus flavus* Link wax selected for experimental infection of eggs of the greater wax moth, *Galleria mellonella* (Linnaeus), and a subsequent study conducted of the resulting pathologies.

I. Materials and methods

A. Greater Wax Moth Rearing and Mycological Culture.

Wax moth eggs were obtained from insects reared in the laboratory through several generations. The stock culture was initiated from larvae and pupae collected from an infestation in the apiary of the Department of Entomology of The Pennsylvania State University. The larvae were reared in plastic containers on a medium modified from that described by Tanada & Tanabe (1965) by the addition of 50 ml. Carnation instant nonfat dry milk. Emerging adults were supplied absorbent cotton soaked with a saturated sugar water solution. Freshly-laid eggs were collected daily and stored in sterile petri dishes in a desiccator jar. Each desiccator contained distilled water to maintain a high relative humidity and was held at room temperature (22-27 °C).

The culture of *A. flavus* used to inoculate the wax moth eggs was originally isolated from eggs of the cabbage looper, *Trichoplusia ni* (Hubner) (Behnke & Paschke, 1966). The stock cultures were grown on slants of Sabouraud’s dextrose agar plus 0.2 % yeast extract and maintained at 6 °C.

B. Inoculation and Incubation of Eggs.

Experimental inoculation of wax moth eggs was accomplished by lightly dusting egg masses with conidia. Conidia for this purpose were harvested with an inoculating loop from 10-day-old plate cultures of the fungus grown on Sabouraud’s dextrose agar. Inoculated eggs were incubated at room temperature in sterile petri dishes in a desiccator jar containing distilled water to produce a high relative humidity. Inoculations were made at one, three, five, seven, and nine days after eggs were laid. Eggs representing control groups were treated in the same manner but were not inoculated.

C. Histological Technique.

At least one egg mass, containing approximately 10-50 eggs, from each inoculation group was fixed at six hours and one, two, three, four, and five days after inoculation. Eggs serving as the control group were fixed daily for nine days, at which time a portion of the group began to hatch.