Annual Report 1976

Almond Industry Research Projects

Correct Project Number 76-B3

TITLE : Project 75-B. Insect Pathology and Storage Control.

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I. OBJECTIVES: To investigate naturally occurring pathogens in NOW field populations and to test other microbial agents for NOW control.

II. INTERPRETIVE SUMMARY:

Viruses-- An extremely small nonoccluded (i.e., not enclosed in a protein matrix) virus which is a host-specific pathogen of NOW larvae was isolated from a mixed infection in larvae originally collected near Durham. The virus has been tentatively identified as belonging to a group known as the 'picornaviruses'. This chronic stunt virus (CSV) invades the cytoplasm of larval hemocytes (blood cells) and thus interferes with the larval hormone system, causing high mortality in very young infected larval. Older larvae (ca. 2 weeks old) that become infected cease growing and may survive in a stunted state for several weeks. Although the details of dosage/mortality relationships of CSV have not yet been studied, it is known that sublethally infected NOW may survive to emerge as dwarf moths, but usually they do not lay eggs. Larvae of other species of moths have been tested i.e., almond moth, raisin moth, Indian meal moth, and the tobacco moth, but none of these was susceptible to CSV.

Protozoa-- The life cycle of *Pleistophora* sp. was determined in an infected culture of NOW larvae. Although this pathogen is similar to at least 2 other known species, there is evidence to indicate that

it is distinct and not previously described. Larvae of several other moth species are also susceptible to infection.

Nematodes-- Adults and larvae of the following insect pests of almond orchards are susceptible to the nematode *Neoaplectana carpocapsae* (Mexican strain): navel orangeworm, peach twig borer, oriental fruit moth, tent caterpillar and the oblique banded leaf roller. This nematode was originally found in the codling moth. It is not harmful to plants but does parasitize a wide variety of insects especially moths (Lepidoptera). This nematode can seek-out and parasitize NOW larvae, reproduce itself, and remain viable for up to 10 days in the moist protected interior of a newly split almond.

III. EXPERIMENTAL PROCEDURE:

Viruses-- For histological studies, tissues from CSV infected larvae were fixed in Zenker's solution. Specimens were dehydrated in a graded series of alcohol and embedded in paraffin (MP=55^{o}C). Sections were cut at 6µm and stained with Mallory's triple connective tissue stain. Also, samples of infected tissue were prepared for examination in the electron microscope by fixing in 2.5% glutaraldehyde and postfixing in 1% osmium tetroxide. Specimens were embedded in Spurr's medium. Uncoated grids bearing sections stained with uranyl acetate and lead citrate were examined in a Siemens Elmiskop IA at 80 Kv.

Preliminary assays were conducted with homogenized tissues of infected larvae in an effort to establish a routine procedure for maintaining the infected culture of NOW in the laboratory. Known weights of infected larvae were homogenized in PO_4 buffered 0.65% saline solution

with a Polytron® homogenizer. The suspension was filtered and then mixed with known weights of bran diet to give the desired concentrations. Larvae of known age were placed on the virus-contaminated diets and subsequently (after ca. 30 days) evaluated for effectiveness of virus transmission. Twenty-five assays were conducted with 7, 10, and 15 day-old larvae. Concentrations of infected tissues tested ranged from 2.30 to 0.02 mg/g diet.

Host range studies were conducted with neonate larvae of the Indian meal moth, raisin moth, tobacco moth, and almond moth. These susceptibility tests were done on diets containing ca. 1.0 mg of tissue suspension/g diet.

Protozoa-- Histological and ultrastructure studies of *Pleistophora* were performed on tissues prepared as indicated above. Spores were measured with Vickers A.E.I. image splitting eyepiece at 1000X. LC_{50} and host susceptibility tests were conducted on diets containing fresh spore concentrations ranging from 1.6×10^4 to 1.2×10^7 spores/g diet.

Nematodes-- Cultures of *Neoaplectana carpocapsae* have been maintained by serial transfer in a laboratory culture of NOW.

IV. RESULTS:

Viruses-- Histological and ultrasturcture studies indicate that CSV is a nonoccluded virus that is limited to the cytoplasm of hemocytes. Tentatively it has been classified as belonging to the 'picornaviruses'. Infected hemocytes have been tentatively identified as plasmatocytes and granular hemocytes.

Assays indicate so far that only NOW is susceptible to CSV. Neonate larvae are highly susceptible and experience early mortality at all concentrations tested. However, for routine maintenance 15 day-old larvae are being reared on diets containing 0.02 mg of homogenized larval tissue/g diet.

Protozoa-- The *Pleistophora* originally isolated from NOW is also capable of invading larvae of the Indian meal moth, almond moth, raisin moth, tobacco moth, and the potato tuberworm. However, NOW is much more susceptible than any of the other larvae tested. The LC_{50} of NOW is ca. 1×10^6 spores/g, whereas the LC_{50} of the almond moth, for example, is 5x greater. Interestingly, the *Pleistophora* only invades the reproductive organs of male larvae of the Indian meal moth. Most tissues of NOW are invaded, including fat, muscle, nervous tissue, hypodermis, and reproductive organs.

V. DISCUSSION:

Viruses-- CSV probably blocks the hormonal function of the hemocytes and thus interferes with the production of the growth and molting hormone normally produced by the thoracic gland. This is a unique type of host-virus relationship which has not been previously reported from insects. Future studies will include the determination of the relative susceptibility of neonate and older larvae, and the influence of temperature on the stability of viral suspensions. (W. R. Kellen)

Protozoa-- The *Pleistophora* of NOW appears to be very similar to 2 known species of *Pleistophora* described from the potato tuberworm and the winter moth. However, the polar filament of the NOW species is only half as long as those of the described species.

A paper will be prepared describing the NOW *Pleistophora* as a new species. (W. R. Kellen)

Nematodes-- Future field tests with *Neoaplectana carpocapsae* will be directed toward spray applications at hullcrack to determine if the incidence of NOW damage can be reduced. (J. E. Lindegren)

VI. PUBLICATIONS:

W. R. Kellen and J. E. Lindegren. 1970. Previously unreported pathogens from the navel orangeworm, *Paramyelois transitella*, in California. J. Invert. Pathol. 16, 342-345.

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W. R. Kellen and J. E. Lindegren. 1974. Life cycle of *Helicosporidium parasiticum* in the navel orangeworm, *Paramyelois transitella*. J. Invert. Pathol. 23, 202-208.

J. E. Lindegren and D. F. Hoffmann. 1976. Ultrastructure of some developmental stages of *Helicosporidium* sp. in the navel orangeworm, *Paramyelois transitella*. J. Invert. Pathol. 27, 105-113.

J. E. Lindegren. 1976. Current prospects for microbial control of nitidulid beetles. Proc. Calif. Fig Inst. Res. pp. 15-18.

W. R. Kellen, D. F. Hoffmann, and S. S. Collier. Biology and ultrastructure of *Nosema transitellae* sp. n. in the navel orangeworm, *Paramyelois transitella*. J. Invert. Pathol. (in press).

W. R. Kellen, D. K. Hunter, J. E. Lindegren, D. F. Hoffmann, and S. S. Collier. Field evaluation of *Bacillus thuringiensis* for control of navel orangeworms on almonds. J. Econ. Entomol. (in press)