Project Number 77-B
Project Title: Insect Pathology and Storage Control-
Insect Parasitic Nematodes

Title: Project 77-B. Insect Pathology and Storage Control

Prepared By: W. R. Kellen **and Lindegren**

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

II. Interpretive Summary:

Chronic stunt virus (CSV) is a unique viral pathogen that invades the blood cells of NOW larvae. The virus has been isolated only once from field collected material, but it is now maintained in a laboratory culture for further study. CSV is restricted to certain NOW larval blood cells -- no other hosts or tissues are known to be susceptible to invasion.

In the laboratory the virus remains infectious for at least 12 months when frozen at -20°C. Additional studies of viral stability are in progress with infected larvae that have been frozen-dried and stored at -70°C. Under these conditions the virus may retain infectivity for many years.

In a preliminary study almonds were sprayed with a virus suspension at hull crack to test the stability and efficacy of the pathogen. After the spray treatment the nuts were removed from the trees and exposed to a high population of NOW larvae in the laboratory. When compared to controls, the treated nuts developed only about 1/5 as much damage. After one week, however, virus activity decreased about 50%.

III. Experimental Procedure:

Chronic Stunt Virus -- Storage tests

(1) Freeze-drying. Infected larvae were triturated in neutral phosphate buffered saline (0.8%) with a Polytron homogenizer. The crude suspension was centrifuged at 5000 rpm for 10 min. and at 10,000 rpm for 20 min. to remove tissue fragments. Sucrose (10%) and bovine serum (5%) were added to the supernatant containing virus particles. The material

was transferred to 10 ml drying bulbs, frozen at -70°C and dried under vacuum. The dried viral powders were stored at -70°C and tested periodically for infectivity with young NOW larvae. Whole infected larvae were also frozen-dried under vacuum and stored at -70°C. (2) In addition to freeze-drying, freshly infected whole larvae were also frozen and stored at -20°C. This material was tested at intervals of several weeks for virus activity.

For routine maintenance of virus infected NOW cultures, virus suspensions, were maintained in buffered saline at 4°C. These suspensions contained ca 0.20 - 0.60 mg tissue per ml of saline.

Electron microscope studies: An effort has been made to isolate and concentrate virus infected NOW hemocytes, so that the cells can be sectioned separately without the interference of other associated larval tissues. Infected larval hemolymph was collected in glass capillary tubes from puncture wounds, and immediately transferred to cold 2.5% glutaraldehyde (4°C). After post-fixation with osmium and washing, the hemocytes were pelleted in a clinical centrifuge. The pellet was embedded in warm agar, cut into blocks, dehydrated, and embedded in Spurr's medium in the usual manner.

Preliminary test of viral activity when applied to almonds at hullcrack:

Almonds were sprayed with a high concentration of virus in a water suspension. Immediately after the treatment, and again one week later, nuts were removed from the trees and exposed to a high population of NOW larvae in the laboratory. After about 4 weeks, nuts were examined for damage and compared to untreated controls.

IV. Results: Storage tests -- Although comparative LD_{50} studies have not been conducted, routine checks on the infectivity of frozen-dried virus suspensions and diseased whole larvae indicate no loss of virulence after 12 months storage at -70°C. Similarly, fresh infected larvae frozen at -20°C also retain infectivity after 12 months. Virus suspensions kept in saline at 4°C remained infective for at least 50 days (the longest period tested so far).

Electron microscope -- The technique of isolating and concentrating hemocytes for electron microscope studies is new to us and still needs further refinement before we can expect positive results. Preliminary specimens prepared in this manner were unsatisfactory because the pellet of hemocytes became dissociated and the cells were not concentrated in one area as desired. More experience with this technique should ultimately prove to be valuable, since the only alternative is to examine whole-body preparations which usually contain very few associated hemocytes.

Preliminary test with CSV on almonds -- The following data were obtained from nut samples collected immediately after sprayed with CSV and exposed to larvae in the laboratory:

	Treatment CSV			Control		
	1	2	3	1	2	3
Worm damaged nuts	1	1	2	6	8	6
Undamaged nuts	10	10	8	5	2	4
Adult moths emerged	0	0	0	9	13	19
Total Damaged		4			22	
Total Undamaged		28			11	
Total Moths		0			21	

The test indicates that CSV exerts a protecting influence on almonds against newly hatched NOW larvae immediately after the virus is applied. After one week, however, additional samples showed a reduction in virus activity, possibly due to the effects of sunlight or unfavorable pH on the nut surface. This was not determined.

V. Discussion: Storage tests -- Results of our low temperature tests show that we probably should have no difficulty in maintaining highly virulent preparations of CSV over long periods of time. In fact, we expect that the frozen-dried preparations should remain stable for many years when maintained under vacuum at -70°C. Virus stability under storage temperatures in the range of 15 to 35°C need to be investigated. Because of mechanical problems with our constant-temperature cabinets, we were unable to start these temperature dependent studies last year.

Electron microscope -- The technique of concentrating hemocytes by low rpm centrifugation is somewhat dependent upon collecting adequate numbers of cells initially. The technique, however, is good and its value has been demonstrated by several researchers working with insect hemocytes for electron microscopy. Our main problem is that infected NOW larvae have reduced numbers of hemocytes and it is difficult to obtain sufficient quantities of blood. The technique, however, is essential to our study of the progression of CSV disease in vivo. As a follow-up to this study, we are also attempting to establish a tissue culture cell line from NOW hemocytes to support our cytopathology studies.

Preliminary field test -- The small field test was encouraging because the virus treatment showed positive influence on the reduction of worm damage to nuts and the survival of worms to the adult moth. A

larger scale test should be conducted. As mentioned in earlier reports, the entire question of the action and possibly synergistic effects of chemical adjuvants on CSV needs study, especially as to enhancement of longevity and virulence under field conditions. This is an area which we have not yet explored.

Publications: None

Title: Project 77-B. Insect Parasitic Nematodes

Prepared By: James E. Lindegren

I. Objectives: To determine the biological control potential of the insect parasitic nematode *Neoaplectana carpocapsae* for the control of navel orangeworm.

II. Interpretive Summary:

A test was conducted in 1977 to determine the host-parasite response to a spray application of an insect parasitic nematode *Neoaplectana carpocapsae*. Test results indicate that nematode spray applications at hull split can reduce navel orangeworm adult and larval populations by approximately 60% and nut damage caused by navel orangeworm larvae by 34%.

III & IV. Experimental Procedure and Results:

Field Application --

Initial studies were made in 1976 by treating individual almonds in the field to determine the biological control potential of *N. carpocapsae* (Mexican strain) invasive stage nematodes. Results indicated that total suppression of a NOW larval population was possible if the quantity of nematodes applied was sufficient (Table 1).

A larger test was conducted in 1977 to determine the host-parasite response to a spray application of this nematode (Table 2). Spray applications were made in the evening when the relative humidity was at least 60%. This test application resulted in average adult and larval mortalities of approximately 60%. Reduction in nut damage caused by NOW larvae was about half that figure.

Monitoring Methods --

Larvacidal action and nematode distribution were measured by baiting 10 almonds per tree with 2 colony-reared NOW larvae per almond,

8 hours before the nematode spray application. Baited almonds were collected the following morning, held at ca. 27°C 6 days and dead NOW larvae were then examined microscopically (20X) for nematode parasitic stages.

Adult mortality was monitored by the following method: Ten colony-reared NOW adults were placed on a 9-cm dia. cardboard disc coated on one side with Stikem^{R} Special[1/]. For transporting to the field, the disc was placed in a plastic petri dish containing moist filter paper. prior to nematode application, three discs were placed in the canopy of each target tree, 2 at about 8 feet and 1 at 16 feet above the ground.

V. Discussion:

Results from the 1977 spray application at hull split indicate that spray applications of this nematode can reduce navel orangeworm populations and the resultant nut meat damage.

Tests will be conducted to determine the most effective time and method for the application of this insect parasitic nematode. An additional test is planned to confirm the 1977 field applications.

Efforts are continuing towards obtaining an exemption from tolerance for the use of this nematode for the control of navel orangeworm on almonds. Should data from the 1978 tests appear promising, safety tests would be contracted with applied Biological Science Lab for partial fulfillment EPA requirements.

1/ Michel & Pelton Co., Emeryville, CA.

Margins 1" 1 1/2"

Margins 1 1/2" 1"

Table 1.--Control of the Navel Orangeworm in Newly Split Almonds [1/] in the Field by the Spray Application of *Neoaplectana carpocapsae* (Mexican strain), 1976.

No. nematodes/almond	% larval mortalities
1,500	100.0
483	100.0
218	97.7
150	95.0
108	91.6
46	23.5

1/ Two, 14 day old colony reared NOW larvae/almond
10 almonds/tree, 3 trees each/test and control

2.-- CONTROL OF NAVEL ORANGEWORMS IN FIELD ALMONDS BY THE SPRAY APPLICATION OF NEOAPLECTANA CARPOCAPSAE, 1977

53 ROWS

A test was conducted to evaluate the biological control potential of an insect parasitic nematode. Test results indicate (see table below) that nematode spray application at hull split can reduce navel orangeworm populations and the resultant nutmeat damage.

Future tests will be concerned with determining the most effective time and method for the application of this parasite.

Plot Size - 4 Nonpareil Almond trees

3	4
2	1

34
33

Applications - Beginning at ∿ 20 % hull split

1) 8/2/77 ∿ 10:00 pm
2) 8/9/77 ∿ 9:00 pm
3) 8/16/77 ∿ 8:30 pm

3	4
2	1

28
27

Application Rate

1) Spray (*N. carpocapsae* (M)) - 3 x 10^6/tree (∿ 225 x 10^6/Acre) in 6 gal H_2O.

Equipment Used - Solo Port. 423 Backpack Sprayer

Harvest - 8/30/77

Sample Size - 2 of ∿ 285 nuts/tree (equiv. to 1 lb inshell)

1) one sample for worm count - 55% Reduction
2) second sample sent to CAGE for determination of % Rejects - 34% Reduction

Spray Application Monitored W/ Colony NOW

Larvae - 2/nut - 10 nuts/tree at each application

1) 65% mortality
2) 60% nematode distribution (as measured by nuts containing parasitized NOW larvae in the treatment).

Adults - 10/sticky disc, 3 discs/tree

1) 60% mortality

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Studies of a Chronic Stunt Virus

Chronic stunt virus (CSV) is a unique viral pathogen that invades the blood cells of NOW larvae. The virus has been isolated only once from field collected material, but it is now maintained in a laboratory culture for further study. CSV is restricted to certain NOW larval blood cells -- no other hosts or tissues are known to be susceptible to invasion. Infections that are acquired by very young NOW larvae are quickly fatal, however, the details of this dosage - "time of mortality" relationship have not been clarified. When exposed to certain low concentrations of virus, larvae frequently develop long-lasting infections that interfere with normal growth, hence the name "chronic stunt virus." Chronically infected larvae may survive in a stunted state for several weeks (up to 8 weeks in laboratory tests); however, larvae do not feed in this diseased condition and they are usually moribund.

CSV is a small nonoccluded virus (25 mm diameter). Invasion is limited to the cytoplasm of the host cell. In the laboratory the virus remains infectious for at least 12 months when frozen at -20°C. Additional studies of viral stability are in porgress with infected larvae that have been frozen-dried and stored at -70°C. Under these conditions the virus may retain infectivity for many years.

In a preliminary study almonds were sprayed with a virus suspension at hull crack to test the stability and efficacy of the pathogen. After the spray treatment the nuts were removed from the trees and exposed to a high population of NOW larvae in the laboratory. When compared to controls, the treated nuts developed only about 1/5 as much damage. After one week, however, virus activity decreased about 50%.

The test indicates that CSV might have value as a protectant on almonds against newly hatched NOW larvae. However, the stability of the virus under field conditions could be a limiting factor and the addition of chemical screens against delitarious ultra violet rays might be necessary.

CONTROL OF NAVEL ORANGEWORMS IN FIELD ALMONDS BY THE SPRAY APPLICATION OF *NEOAPLECTANA CARPOCAPSAE*

53 ROWS

A test was conducted to evaluate the biological control potential of an insect parasitic nematode. Test results indicate (see table below) that nematode spray application at hull split can reduce navel orangeworm populations and the resultant nutmeats damaged by them.

Future tests will be concerned with determining the most effective time and method for the application of this parasite.

W

Plot Size - 4 Nonpareil Almond trees

3	4
2	1

34
33

Applications - Beginning at ∿ 20 % hull split

1) 8/2/77 ∿ 10:00 pm
2) 8/9/77 ∿ 9:00 pm
3) 8/16/77 ∿ 8:30 pm

3	4
2	1

28
27

Application Rate

1) Spray (*N. carpocapsae* (M)) - 3 mil/tree
(∿ 225 x 10^6/Acre in 6 gal H_2O)

Equipment Used - Solo Port. 423 Backpack Sprayer

Harvest - 8/30/77

Sample Size - 2 of ∿ 285 nuts/tree (equiv. to 1 lb inshell)

1) one sample for worm count - 55% Reduction
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Spray Application Monitored W/ Colony NOW

Larvae - 2/nut - 10 nuts/tree at each application

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2) 60% nematode distribution (as measured by nuts containing parasitized NOW larvae in the treatment).

Adults - 10/sticky disc, 3 discs/tree

1) 60% mortality

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Tree No. 26 6 5 1