78-B5B

Title: Project 78-B5B - Insect Parasitic Nematodes

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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 1978 to determine if the invasive stages of N. carpocapsae (Mexican strain) could protect windrowed almonds caught in a summer rain from navel orangeworm (NOW) larvae. Test results indicate that existing NOW larvae and pupal stages could be parasistized by the nematode and that the nematode could persist in moist almonds up to 19 days after the initial application. There was, however, no reinfestation by NOW in the moist windrowed almonds and therefore no NOW larvae to protect against.

A commercial sprayer, Air-O-Fan 375 proved compatible as a delivery system for the application of insect parasitic nematode invasive stages.

NOW adults were found to be very susceptible to the Mexican strain of N. carpocapsae and a testing procedure has been established utilizing adult NOW for determining the viability and comparative virulence of this insect parasitic nematode.

A technique has been developed utilizing adult NOW for the simple economical rearing of N. carpocapsae and other insect parasitic nematodes. The resultant invasive stage nematodes can then be stored in a concentrated condition without the need for mechanical aeration.

III. & IV. Experimental Procedure and Results:

Field Application.--N. carpocapsae (Mexican strain) invasive stage nematodes were applied to windrowed Nonpareil almond test plots approximately 2 meters long X 1 meter wide by 15 centimeters high. Each test plot and control was replicated 5 times. Almonds were premoistened with approximately 40 liters of water at 12 hr intervals with a total of 160 liters applied before and 40 liters after the nematode application. Nematodes were applied August 11, 1978, at 7-9 pm with a Sears 20 gallon centrifugal type pump sprayer. Test applications were 20 liter suspensions of 3 or 6 million nematodes per treatments with water applied to the controls. Marked almonds baited with one 14 day old colony reared NOW larvae, ten almonds per treatment, were placed on the surface of each test plot and examined 3 days after the initial treatment. All of the baited almonds recovered contained parasitized NOW larvae. Test plots were examined at 1 and 2 week intervals to determine nut damage and host-parasite response in naturally occurring NOW field infestations (Table 1).

A commercial sprayer, Air-O-Fan 375 with double sided centrifugal fan and positive mechanical agitation, was evaluated as a delivery system for N. carpocapsae.

To determine if high water temperatures, which can be detrimental to the nematode, might be encountered during the agitation process; return water temperatures, pumped through a centrifugal pump, were measured at the recirculating line at 1 minute intervals for 30 mintes (Table 2).

Nematode damage resulting from agitation of the nematode suspension was measured by sampling the suspension approximately 140 liters at 158.4 nematodes per ml, at the recirculating line. Five 15 ml samples were taken after 10 minutes of continuous agitation. Four 1/5 ml alloquates from each of the 5 samples was then examined under a dissecting microscope (20X) for live or dead nematodes (Table 3).

Nematode samples during spray application were then collect in plastic bags, 13x12x24x.002, held 3 feet from the nozzles. Nematode mortality was assessed as previously described for Table III.

The experimental susceptibility of NOW adults to the Mexican strain of N. carpocapsae was determined by placing 5, 10, 20, 40, 80 or 160 nematodes per 20 x 100 mm glass petri dish. Four (9 cm) filter papers moistened with 4 mls deionized water were used as a moist substrate for the nematodes. Ten adult NOW were added per petri dish (3 replications) and incubated at 27°C for 72 hr. Host mortality was recorded at the end of the 72 hr period with a calculated LC_{50} of 13.4 nematodes per petri dish Table V.

Techniques for rearing insect parasitic nematodes are described in the enclosed manuscript "A modified technique for the propagation and storage of Neoaplectana carpocapsae Weiser."

V. Discussion:--Data from the windrowed almond- N. carpocapsae treatment (Table I) indicates that: a high natural mortality of NOW larvae and pupae occurred after the almonds were knocked; no reinfestation of NOW larvae occurred in the moist windrowed almonds; no significant difference in nutmeat damage was observed between the treatment and control; and a proportionate increase in nutmeat damage occurred from week I to week II in both the treatments and the control.

It would appear that high soil surface temperatures in the Fresno area during the first part of August were responsible for the natural occurring NOW mortality. The early knocking, August 10, 1978, limited the NOW infestation to the first invading generation of the new almond crop. NOW stages at this time were predominantly late instars, pupae and emerging adults. Once the almonds were on the orchard floor, the recorded damage in both treatments and control increased from week 1 to week 2 samples even though the almonds were not reinfested by NOW. This increase in nutmeat damage may be the result of accelerated fungal growth causing light insect damaged nutmeats to become more obvious.

The lack of NOW reinfestation in knocked almonds limits the usefulness of this nematode as protectant to wet windrowed almonds.

A commercial sprayer, Air-O-Fan 375^{R}, was found to be a compatible delivery system for N. carpocapsae (Mexican strain) invasive stages (Tables II, III & IV). Parameters of temperature increase and nematode damage will be monitored in future applications with other commercial delivery systems.

A bioassay technique, utilizing NOW adults as a host, has been developed for determining the viability of N. carpocapsae invasive stages. This technique is currently being used to determine the effects of spray adjuvants and fumigants on nematode invasive stages. Its future use should include nematode compatibility studies with insecticides and fungicides as well as the effects of environmental factors such as temperature, moisture, sunlight and storage on nematode viability.

Effective techniques for the rearing and storage of N. carpocapsae invasive stages (manuscript #1) have been developed. These techniques have and will be used to supply invasive stage nematodes for laboratory and field NOW control studies.

VI. Publications

James E. Lindegren, Charles E. Curtis and George O. Poinar 1978. Parasitic nematode seeks out navel orangeworm in almond orchards. California Agriculture 32, (6) pp 10-11.

James E. Lindegren, Darlene F. Hoffmann, Susan S. Collier and Rodney D. Fries. 1979. A modified technique for the propagation and storage of Neoaplectana carpocapsae Weiser. In press.

Table 1.--Navel orangeworm - N. carpocapsae, host parasite response and resultant nut damage in wet windrowed almonds.

Time of sample	% NOW mortality			% NOW parasitized			% nutmeat damage		
	C[1/]	T1[2/]	T2[3/]	C	T1	T2	C	T1	T2
1 week	23.7	66.7	74.3	0.0	46.7	62.9	12.8	10.6	11.8
2 weeks	[4/]	-	-	-	-	-	16.8	14.4	17.4

1/ Control-water application

2/ Three million nematodes per test plot

3/ Six million nematodes per test plot

4/ No live NOW present and dead NOW obliterated by fungi

Table 2.--Water temperature increase[1/] during 30 minutes of agitation by a chemical sprayer[2/]

Minutes	Water Temperatures	Minutes	Water Temperatures
0	19.8	-	-
1	21.0	16	22.0
2	21.0	17	22.0
3	21.0	18	22.0
4	21.0	17	22.1
5	21.0	20	22.1
6	21.0	21	22.2
7	21.2	22	22.3
8	21.2	23	22.5
9	21.2	24	22.6
10	21.5	25	22.6
11	21.6	26	22.7
12	21.7	27	22.8
13	21.8	28	23.0
14	21.9	29	23.0
15	22.0	30	23.0

1/ Water temperatures taken at the recirculating line. Total water volume tested approximately 200 liters.

2/ Air-O-Fan 375

Table III.--Nematode[1/] mortality after 10 minutes of continuous agitation in a commercial sprayer[2/]

	Sample aliquots				
Vial No.	1	2	3	4	Total
I	26/0[3/]	33/0	31/0	30/1	120/1
II	27/1	26/0	33/0	29/0	115/1
III	34/0	35/0	32/0	31/0	132/0
IV	29/0	30/0	30/0	29/0	118/0
V	29/1	29/0	37/0	31/0	126/1
				TOTAL -	608/3
				TOTAL MORTALITY -	0.5%

1/ Neoaplectana carpocapsae (Mexican strain)

2/ Air-O-Fan 375

3/ Sample size/mortality

Table IV.--Nematode[1/] mortality after spray application with a commercial sprayer[2/]

	Sample aliquots				
Bag No.	1	2	3	4	Total
I	26/3[3/]	30/2	33/3	33/2	122/10
II	35/3	31/2	32/3	33/3	131/11
III	32/1	33/1	29/2	32/0	126/4
IV	30/1	34/2	32/1	29/0	125/4
V	30/3	33/1	35/2	33/2	131/8
				TOTAL	635/37
				TOTAL MORTALITY	5.8%

1/ Neoaplectana carpocapsae (Mexican strain)

2/ Air-O-Fan 375

3/ Sample size/mortality

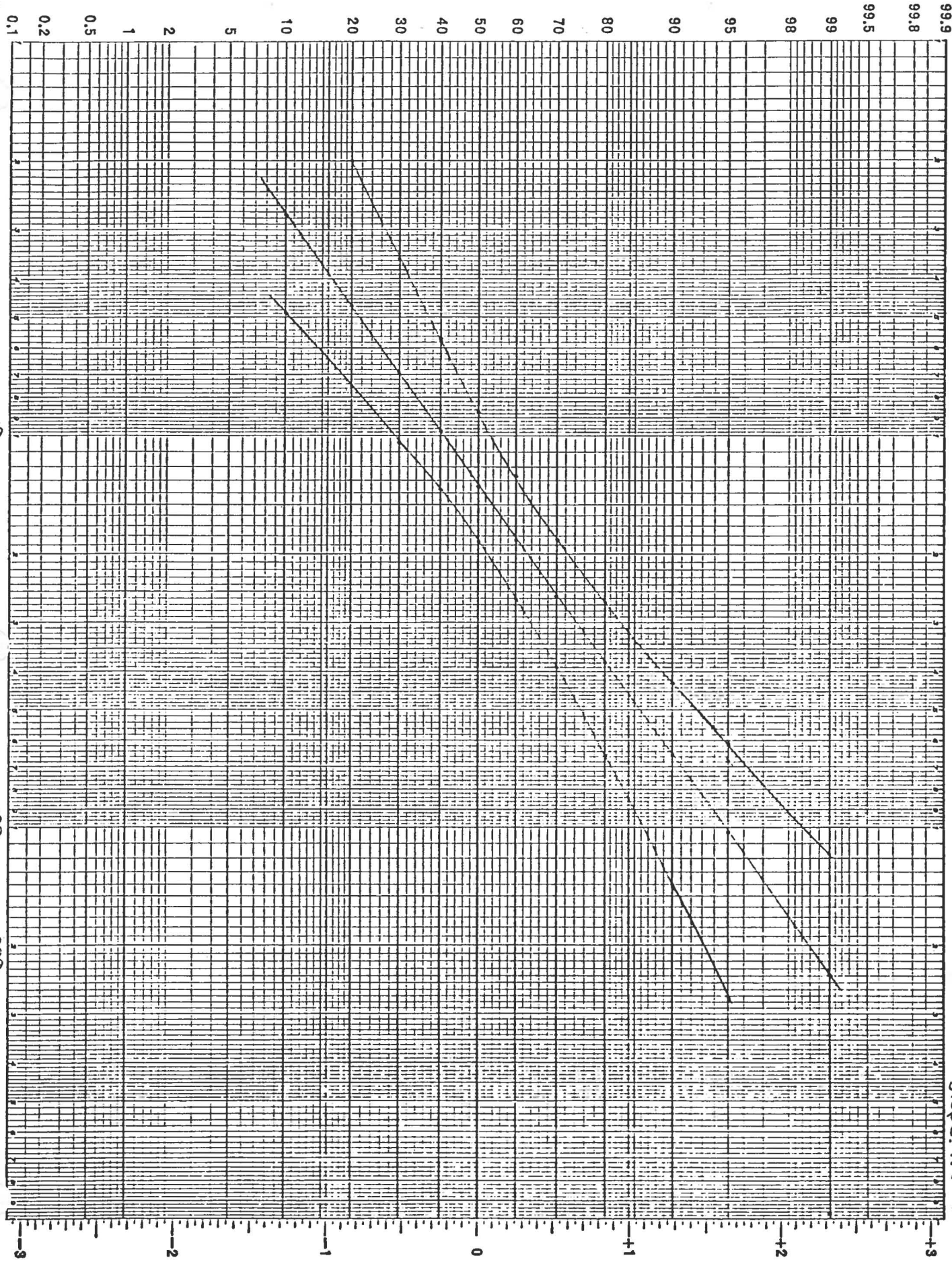

Table V Neoplectana carpocapsae on NOW 72 hr

Slope: 1.8580

Normalized Probit Axis

ABSTRACT

A technique is described for the use of adult Lepidoptera in the simple economical rearing of *Neoaplectana carpocapsae* and other entomogenous nematodes. The resultant invasive stage nematodes are stored without mechanical aeration at 6°C in compact stackable plastic petri dishes.

Margins 1" 1 1/2" Margins 1 1/2" 1"

A simple technique for the propagation and storage of *Neoaplectana carpocapsae* Weiser using *Amyelois transitella* (Walker) adults

by Lindegren, James E., Hoffmann, Darlene F., Collier, Susan S. and Fries, Rodney D.

Introduction

The insect parasitic nematode *Neoaplectana carpocapsae* Weiser has been reared on artifical diets (Glaser et al. 1942, Dutky 1964, House et al. 1965, Poinar and Thomas 1966, Hansen et al. 1968, Bedding 1967) as well as in lepidopteran larvae (Dutky et al. 1964, Poinar 1975). The low rearing cost of $.02 per million nematodes reported by Bedding (1967), compared to $1.00 per million reported by Poinar (1971), favors the use of artifical rearing of this nematode.

However, in vivo rearing methods described by Dutky et al. (1964) and Poinar (1975) are useful for the isolation and maintenance of new and possibly obligate insect parasitic nematodes, as well as for the rearing of limited numbers of nematodes for laboratory or small scale field tests. Work in this area has prompted the following modifications of the simplified rearing method described by Poinar (1975).

Nematode Rearing

Navel orangeworm, *Amyelois transitella* (Walker), adults reared on a modified bran diet* described by Finney and Brinkman (1967) are substituted for last instar wax moth larvae as an insect host for *N. carpocapsae*. The adults inactivated by storage at 4°C for 1 hr are then added to 100 x 15 mm plastic petri dishes containing 4 filter

papers** premoistened with 4 ml 0.1% formalin solution, prepared with 37.8% formaldehyde and deionized water, and 1 drop of concentrated (ca. 15,000) invasive stage nematodes (Fig. 1). The adults are then incubated at 27°C for 48 h (Fig. 2). The bottom of the exposure petri dish, containing the dead parasitized *A. transitella* adults, is then placed inside a 150 x 25 mm plastic petri dish containing 25 ml of 0.1% formalin solution. Invasive stage nematodes are then trapped in the formalin solution as they move out of the exposure petri dish (Fig. 3). The trapped nematodes are harvested (Fig. 4) 1 wk after the initial host exposure and bi-weekly thereafter for 4-6 wk.

Two ml of concentrated nematodes (ca. 3 x 10^6 per ml) are then transferred with a disposable pipette to a 150 x 15 mm plastic petri dish containing 25 ml of 0.1% formalin solution. The nematodes are then stored at 6°C (Fig. 5) until needed.

Formalin solution must be added periodically to replace evaporated moisture. The solution level should be 2-3 cm deep to allow for oxygen exchange. Concentrations of *N. carpocapsae* invasive stages, up to 4 million per petri dish, have to date remained viable, ca. 94%, for 9 months under these storage conditions.

An overall view of this procedure is presented in Fig. 6.

Fig. #6

(A) Exposure dishes with ca. 15,000 nematodes and 4 ml 0.1% formalin solution can be stored at 6°C until needed.

(b) Exposure dishes 48 h after the addition of NOW adults.

(C) Bottom half of exposure dish in 150 x 25 mm trapping dish with invasive stage nematodes in 0.1% formalin solution.

(D) Settling flask with nematodes starting to collect at the bottom.

(E) Disposable pipette for transferring concentrated nematodes.

(F) Formalin (0.1%) solution dispenser.

*The raw bran used in this diet was autoclaved to prevent insect pathogen contamination and a solution of Nutritional Biochemical Corporation Vitamin Diet Fortification Mixture was added to help replace heat labile vitamins lost during the sterilization process.

**#1 qualitative 7 cm dia. Whatman.

Results and Discussion

The average nematode production per adult from the 1,325 navel orangeworm adults sampled bi-weekly for 6 wk is 7,000 with 91% of the total nematode production occurring within the first 3 wk of the sampling period.

As a result of a multidisciplinary approach to control the navel orangeworm, a multimillion dollar pest of almonds in California, ca. 6,000 adults are produced per week at this laboratory. The nematode production capability is therefore ca. 42 million nematodes per week.

Navel orangeworm adults and other adult Lepidoptera either salvaged as by products from insect rearing or possibly collected from light traps represent a low cost food source for rearing entomogenous nematodes. At this facility entomogenous nematodes have been reared on navel orangeworm adults since 1976. Nematodes successfully reared with this system include the Mexican and DD136 strain of *Neoaplectana carpocapsae, N. glasseri* Steiner, and *Heterorhabditis heliothidis* (Khan, Brooks and Hirschmann).

Summary

Adult Lepidoptera provide a previously unutilized food source for the rearing of *N. carpocapsae* and other entomogenous nematodes.

The advantages of this rearing and storage system are: 1) it is economical if cost free adult Lepidoptera are utilized, 2) it provides a clean culture of invasive stages, 3) it is adaptable to the isolation and maintenance of other entomogenous nematodes, and 4) it provides for the concentrated simplified storage of the harvested invasive stage nematodes.

economical if cost free adult Lepidoptera are utilized, 2) it provides a clean culture of invasive stages, 3) it is adaptable to the isolation and maintenance of other entomogenous nematodes, and 4) it provides for the concentrated simplified storage of the harvested invasive stage nematodes.

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Legends

Figure 1 -- Adding *N. carpocapsae* invasive stages to exposure petri dish.

Figure 2 -- Dead parasitized navel orangeworm adults 48 h after exposure to nematodes.

Figure 3 -- Invasive stage nematodes migrating from parasitized navel orangeworm adults into formalin solution.

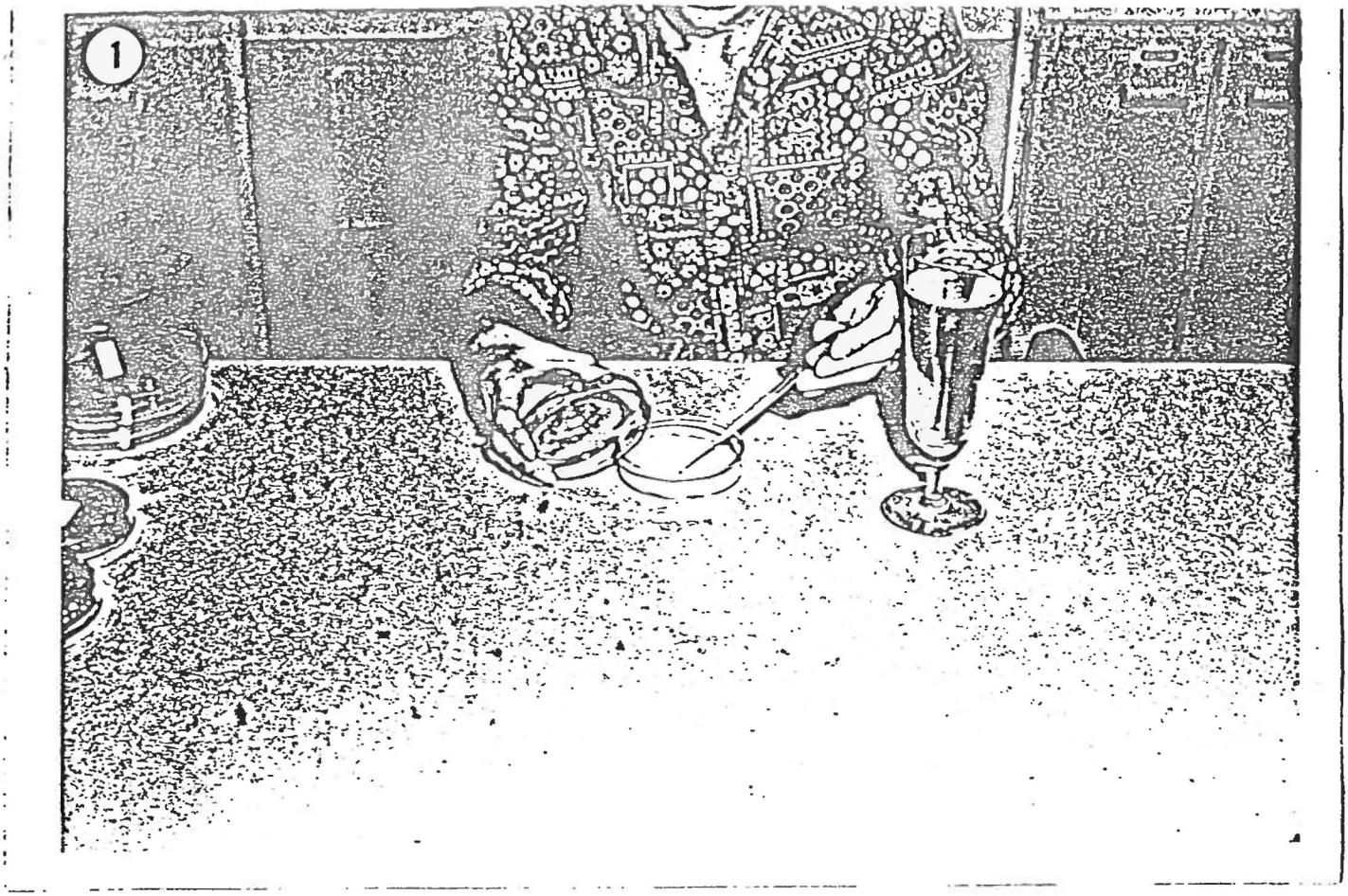
Figure 4 -- Invasive stage nematodes trapped in formalin solution are settled for storage.

Figure 5 -- Concentrated invasive stage nematodes ca. 6 million per petri dish, stored at $6^{o}C$.

Figure 6 -- Overview of nematode rearing procedure.

Key words:

Entomogenous nematodes, *Neoaplectana carpocapsae;* propagation and storage; Lepidoptera adult hosts, navel orangeworm adults.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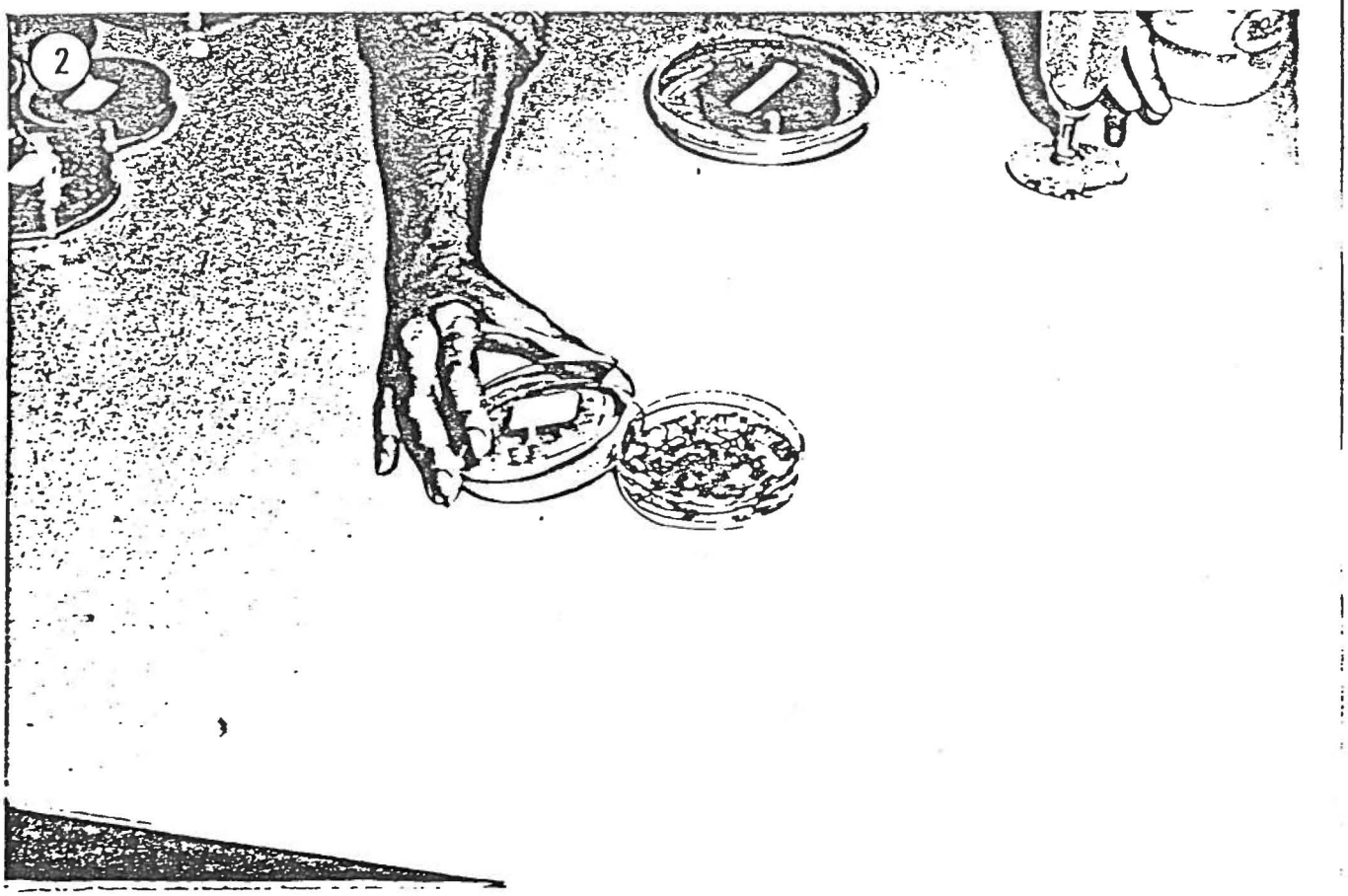
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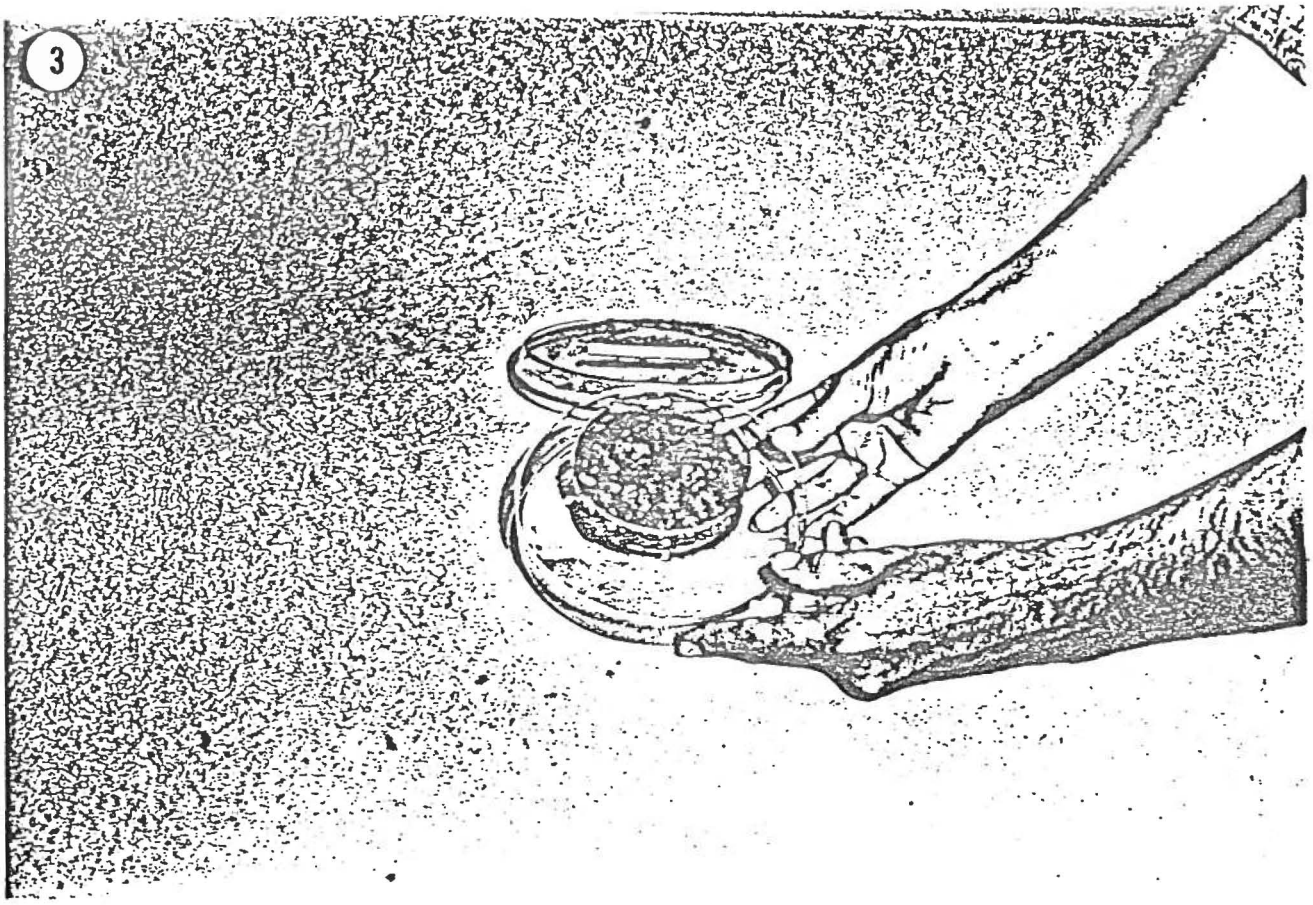
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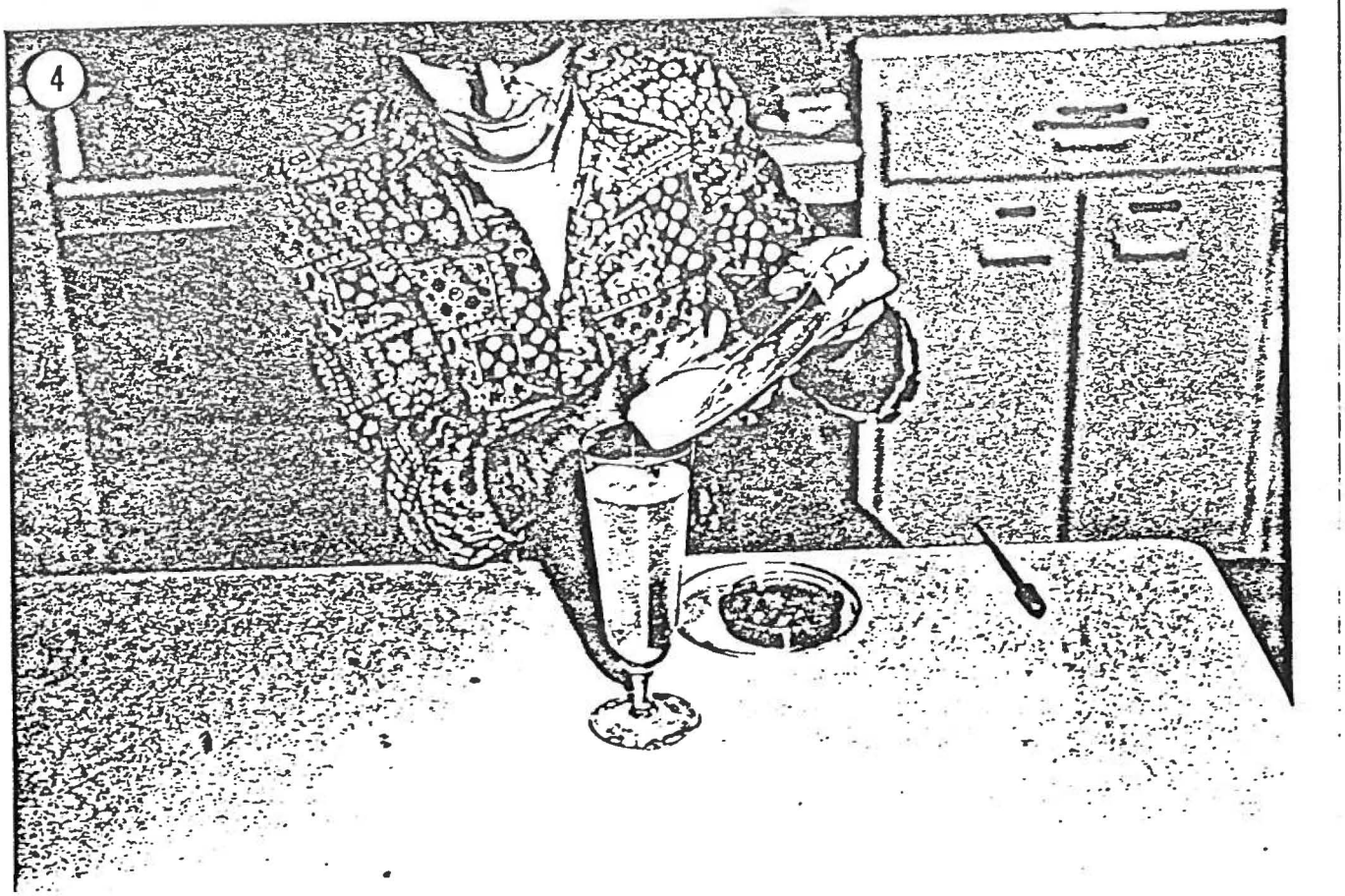
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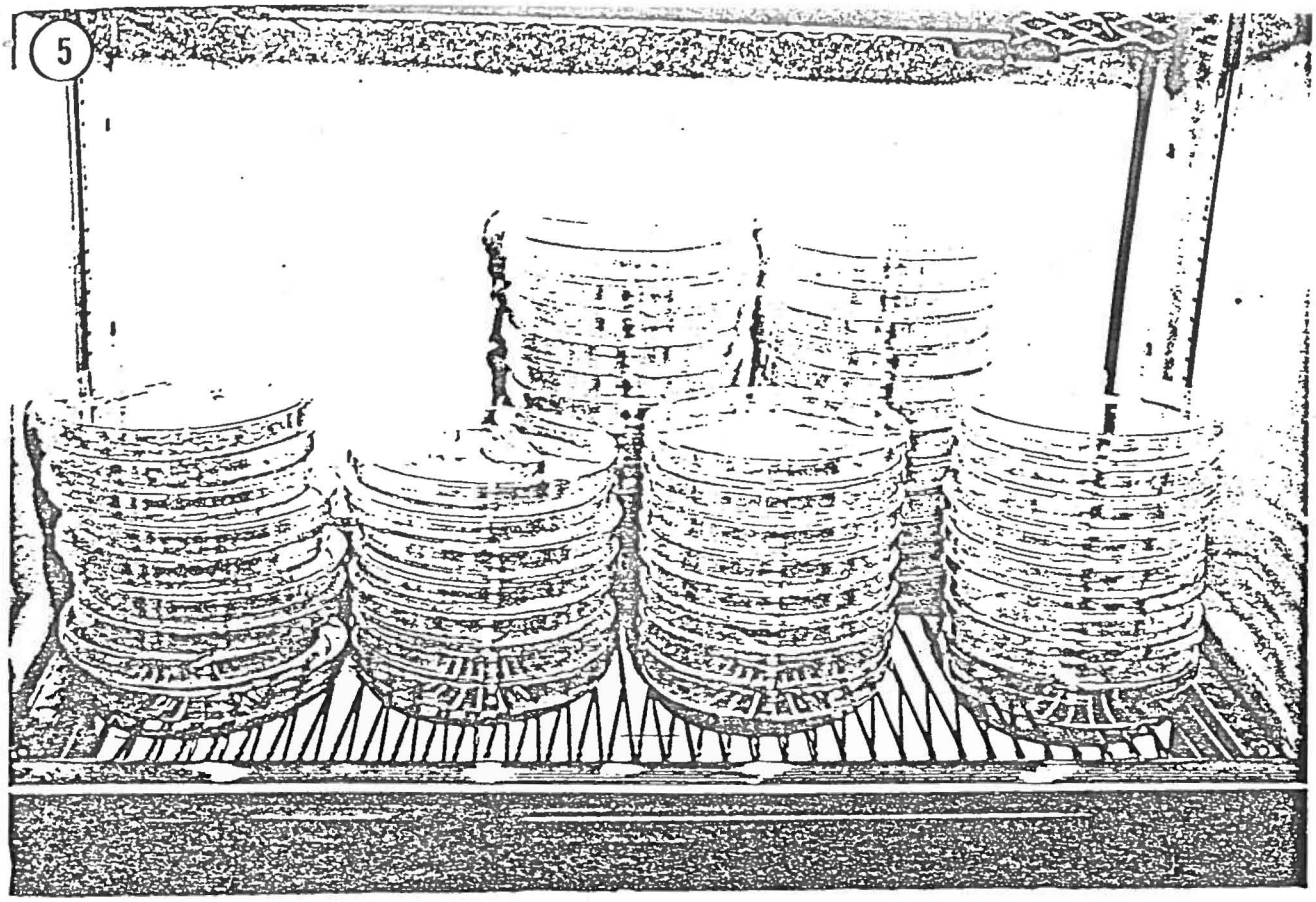
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