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Efficacy of Heterorhabditis HL-81 strain (Nematoda: Heterorhabditidae) against the black vine weevil Otiorhynchus sulcatus (Coleoptera: Curculionidae)

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Abstract

Efficacy of Heterorhabditis HL-81 strain
(Nematoda: Heterorhabditidae) against the Black Vine Weevil
Otiorhynchus sulcatus (Coleoptera: Curculionidae)

by Arash Agah

The entomopathogenic nematode Heterorhabditis HL-81 strain was studied for its efficacy to control the black vine weevil (BVW) Otiorhynchus sulcatus an important pest in agriculture and horticulture fields. The overall results showed that the nematode is an ideal biological control agent against the BVW. The nematode was capable of parasatizing and killing insect larvae located at 5 cm and 9 cm depth. Early instar larvae were less susceptible to HL-81 when compared to mature larvae, demonstrating the importance of timing of application. Most importantly, the efficacy of HL-81 was comparable to the standard insecticide Orthene. The future prospect of utilizing HL-81 against BVW is discussed.

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I. Literature Review

A. Entomopathogenic Nematodes

Records of nematode diseases in insects extend back for more than 350 years. The first reference to nematodes parasitizing insects was made by Aldrovandi in 1623 (Poinar, 1975). There are over 3000 natural associations between insects and nematodes with eight orders of nematodes containing representatives capable of parasitizing healthy insects (Poinar, 1975). One of the three major lines of insect-parasitic nematodes are rhabditoids like Steinernema (Steinernematidae) and Heterorhabditis (Heterorhabditidae). They evolved from bacterial-feeding microscopic rhabditoids (referred to as entomopathogenic nematodes). Major research efforts have been focused on these nematodes because of their ability to kill an insect host within a relatively short period of time (Kaya, 1985).

B. Entomopathogenic Nematodes Steinernema and Heterorhabditis

These two genera are interesting as biological control agents because they are dependent on bacteria as a source of food and have evolved methods of carrying and introducing bacteria of the genus Xenorhabdus into living insects. These bacteria occurring in

Steinernema species have been classified as X. nematophilus, and those associated with Heterorhabditis nematodes are X. luminescens (Akhurst, 1983). Because of their associated bacteria, these nematodes are able to kill and develop in most insects. As a result, they can be used as biological control agents and are commercially available today.

1. Mode of action

Steinernematid and heterorhabditid nematodes are mutualistically associated with Xenorhabdus spp. The free-living infective-stage nematode, which does not feed, carries its bacterial symbiont monoxenically within its intestine. The infective stage nematode is the invasive form which locates insects, initiates infection and is the only stage in the nematode's life cycle that survives outside the insect in the soil (Figure 1). The infective-stage nematode enters the insects via natural openings (mouth, anus or spiracles) and penetrates mechanically into the hemocoel where it releases the bacterium. In addition, heterorhabditids have the ability to enter the hemocoel of certain insects by penetrating through the intersegment. The bacteria proliferate, cause a septicemic death of the insect within 24-72 hours, and establish favorable conditions for nematode reproduction by providing nutrients and inhibiting the growth of many foreign microorganisms. The nematodes feed on multiplying bacteria and dead host tissue, passing through several generations . Eventually infective stage nematodes, carrying the mutualistic bacteria

in their gut, emerge from the depleted insect cadaver. Depending on the species, , it takes 8-20 days for the nematodes at 18-28°C to complete their life cycle in most insects.

The relationship between the nematode and the bacterium is considered mutualistic because the bacterium cannot enter into an insect's hemocoel without the nematode and the nematode cannot proliferate without the presence of the bacterium. Neither can survive in nature without the other.

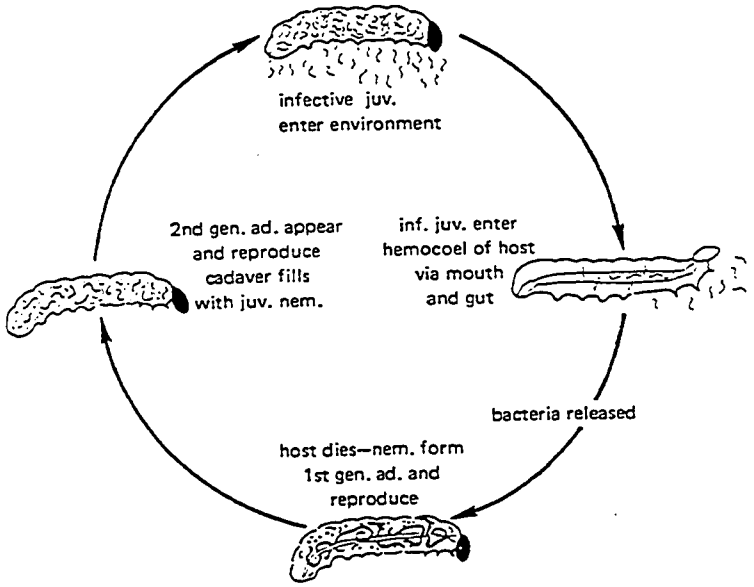


Figure. 1 Life cycle of steinernematids and heterorhabditids nematodes. (Annual Review of Entomology, Vol. 17 1972 by Annual Reviews Inc.)

2 Environmental safety and host range

The effects of entomopathogenic nematodes on nontarget arthropods in the laboratory, field soils, and a stream were assessed. According to Georgis, et al , 1991 predatory adults in laboratory tests were less susceptible to the nematodes than immature stages. Entomopathogenic nematodes that had significantly suppressed pest population in field tests did not adversely affect the numbers of nontarget soil arthropods in comparison with the untreated control. In contrast, broad-spectrum chemical insecticides used as chemical standards significantly reduced or showed a tendency to reduce nontarget arthropod populations (Georgis, et al , 1991). Under laboratory conditions, nearly 300 insect species from 10 orders are reported to serve as hosts for these nematodes (Poinar, 1979). However, the effective host range in the field is limited by the nematode's moisture requirement to insects found in soil and cryptic habitats. On the other hand, insects in such habitats are difficult to control with chemical pesticides, and these nematodes may provide an effective means of insect control (Kaya, 1985). There is no evidence that these nematodes or their associated bacteria can develop in vertebrates as shown with tests conducted in rats (Gaugler and Boush, 1979), mice and chicks (Poinar, et al , 1982) and guinea pigs (Oberndorf, et al , 1983). Furthermore, these nematodes are host specific and would not harm other arthopods such as earthworms and spiders. The US Environmental Protection Agency stated in November 1982, that pest control organisms such as insect predators, nematodes, and microscopic parasites are considered biorational pesticides and are exempt from the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as authorized by section 25(b) 7 of FIFRA and specific in the exemption from regulation of certain biological control agents published in the federal register of June 2, 1982 (Betz, et al , 1982).

3. Dispersal and Persistence

Steinernematids and heterorhabditids are widely distributed and have been isolated on every inhabited continent and in many islands. Few epizootics have been documented (Poinar, 1990). Nematode persistence as indicated by the insect mortality with time, decline significantly 3-6 weeks after their releases as shown in most field studies. However, few reports showed the ability of the nematodes to achieve longer term control. Kaya (1990) reported long-term (nearly one year) persistence of Heterorhabditis when alternate hosts were available in the soil. Apparently the nematodes were able to recycle and infect new hosts in the soil. Many abiotic and biotic factors affect the persistence and dispersal of nematodes in the soil. Abiotic factors includes soil type, water, aeration, temperature, and chemistry of the soil solution. Biotic factors includes nematophagous fungi, predacious nematodes, mites, and collembollan species (Poinar, 1986, 1988; Ishibashi, et al , 1987).

4. Field application and efficacy

Species and strains of Steinernema and Heterorhabditis differ in virulence, tolerance to adverse environmental conditions, ability to seek out hosts, and behavior in soil. Selecting a proper species/strain against a particular insect or environment is important in maximizing the potential against the insect host. Georgis and Gaugler (1991) demonstrated that biological control attempts using nematodes without careful consideration to optimal strain and soil parameters risk a high probability of failure.

C. Black Vine Weevil (Otiorhynchus sulcatus) (Fabricius)

1. Economic importance

Otiorhynchs sulcatus was recognized as an economic pest of greenhouse plants in Germany in 1834 (Simons, 1981). Riley (1871) recorded the first economic damage caused by these weevils in North America. In 1875 Bolton recorded severe injury to grapes, and in 1881 Ormerod found that these weevils damaged strawberries and raspberries. Unlike many agricultural and horticultural insect pests, the Black Vine Weevil is a destructive insect in both larval and adult stages. Extensive damage is usually made by larvae feeding on roots` and destroying the smaller rootlets, finally causing death of plants. Because nursery infestations become widespread through the movement of infested plant material, shipping of infected plants is prohibited.

2. Distribution

Otiorhynchus sulcatus has been recorded from the following countries: Australia, Austria, Belgium, Canada, England, France, Germany, Ireland, Italy, Netherlands, New Zealand, Norway, Russia, Scotland, Sweden, and Switzerland. In the United States the insect has been found in the following states: Alaska, Arizona, California, Connecticut, District of Columbia, Illinois, Maine, Maryland, Massachusetts, Michigan, Montana, Nevada, New Hampshire, New Jersey, New Mexico, New York, North Carolina, Ohio, Oregon, Rhode Island, Texas, Utah, Vermont, Virginia, Washington, and Wisconsin.

3. Life Cycle

Adult weevils are flightless. They hide during the day in the soil or under litter of plants, becoming active only at night. They feign death when disturbed. The eggs are usually dropped indiscriminately by the female or placed in the soil or plant crevices. The grubs hibernate in winter, feed again on the roots in spring, then pupate and emerge as adults in early summer. The larva molts five or six times during its development in the soil. The normal period of development ranges from 72 to 113 days. The mature larva forms a cell in the soil where it enters the prepupal stage which lasts from three weeks to eight and a half months, depending upon the temperature. The pupal stage ranges from 15 to 22 days with an average of 18 days. The adult remains in the soil on an average of 8 days, with extremes of 4 and 17 days, before emerging. There is only one generation a year. (Figure. 2)

Fall

Larvae, pupae, or adults prepare to overwinter in soil.

Winter

Adults, pupae, or larvae over winter in soil among roots of host plants.

Spring

Adults become active. Pupae ready to emerge as adults. Larvae become active again.

Summer

Adults emerge. Two to four weeks later adults deposit eggs around the crown of plants. Emergence occurs in about ten days to two weeks and tiny larvae burrow in soil and feed on roots. Pupae develop.

Figure 2. Life cycle of Otiorhynchus sulcatus (Fabricius)

4. Host plants

There are more than 77 host plants recorded for the Black Vine Weevil. The list of a few of the most important hosts is shown in Table 1.

Table 1. **BVW Host Plants**

Type of plant	Site	Major plant hosts
1. Perenials	Nurseries / Greenhouses	Primrose
2. "	" / "	Cyclamen
3. Flowering shrubs	" / "	Azalea
4. " "	" / "	Rhododendron
5. Shrubs	Nurseries / Landscape	Photinia
6. "	" / "	Escallonia
7. Trees	" / "	Liquidamber
8. "	Landscape	Maple
9. Crops	Agriculture	Strawberry
10. "	"	Raspberry

II. Efficacy of Heterorhabditis HL-81 against Otiorhynchus sulcatus.

A. Introduction

Although chemical insecticides have been the primary means of controlling soil insects for many years, concerns about public safety, environmental contamination, and reduced efficacy due to possible microbial deterioration or insect tolerance and resistance have created a need for alternative control strategies (Klein, 1990).

The use of microbial agents for the control of soil inhabiting insects has serious drawbacks. These include narrow host range, slow build-up in the soil, poor growth of in vitro cultures, and registration requirements. Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae lack these limitations and possess many qualities that make them excellent biolgical control agents. HL-81, as well as strains of many species of Heterorhabditis, can be easily mass produced. They have ability to seek out their hosts, kill them rapidly, and are environmentally safe. This combination of attributes has generated an intense interest in the development of these nematodes for use against soil insect pests (Gaugler, 1988).

B. Material and Methods

All experiments were conducted at room temperature of 26-27°C and 40-60% relative humidity. Soil temperature ranged between 22-23°C.

1. Heterorhabditis HL-81

Heterorhabditis HL-81 was isolated from soil in the Netherlands by Simons (1981). The original inoculum of Heterorhabditis HL-81 strain was obtained from Biosys company in Palo Alto, California. In this study HL-81 strain was produced in larvae of the Greater Wax Moth Galleria mellonella according to a method described by Duky, et al , (1964).

2 Storage

Storage of up to 4 months was achieved by placing one million infective stages of HL-81 on moist sponge (15 by 15 Cm) enclosed in a zip lock bag and maintained at 10-12°C.

3. Black Vine Weevil / Greater Wax Moth

Greater Wax Moth larvae were used in experiments wherever Black Vine Weevil larvae were not available. Wax Moths were obtained from Biosys company in Palo Alto, California, and Black Vine Weevils were collected from a commercial plant nursery in Watsonville, California.

4. Experimental materials

a. Soil :

The soil consisted of 50% organic matter (peat moss, redwood compost), and a 50% mixture of sand, loam, and perlite. The soil was obtained from the Department of Botany at the San Jose State University.

b. Plants :

Four to six weeks old potted (4" sq. pots) primroses and liquidambers were used as hosts for black vine weevils. Plants were obtained from commercial nurseries in Watsonville and Sunnyvale, California.

c. Cages :

To prevent movement in the soil or outside the pot, the larvae were caged in small aluminum screens.

d. Chemicals :

Orthene (Acephate) from Cheveron chemical company was used in one experiment at a rate of 1 1/2 fl.oz per 1 gallon of water to determine the efficacy gap between the HL-81 and the insecticide.

C. Experimental results

1. Test #1

Objective: To determine the infectivity of HL-81 against wax moth larvae placed in soil at different depths (no plant host).

Method: Two larvae per pot were placed at 5cm or 9cm deep in soil. Infectives of HL-81 were applied by pipet at a rate of 20,000 per pot.

Result: The experiment suggested that HL-81 can move downward and infect insects located 9 cm in soil. However, the level of control was higher against insects placed 5 cm below the soil surface. These results are in agreement with various reports related to heterorhabditids movement (Klein, 1990) (Table. 1 and Figure. 1).

Table 1. Mean survival of Galleria mellonella exposed to Heterorhabditis HL-81 strain at different time and depth.

	Mean number of live larvae ± SE [a]			
	9 Cm deep		5 Cm deep	
	48 Hrs	72 Hrs	48 Hrs	72 Hrs
HI-81	0.40±0.55 [c]	0.20±0.45 [c]	0.00±0.00 [a]	0.00±0.00 [a]
CONTROL	-	-	1.80±0.45 [b]	2.00±0.00 [b]

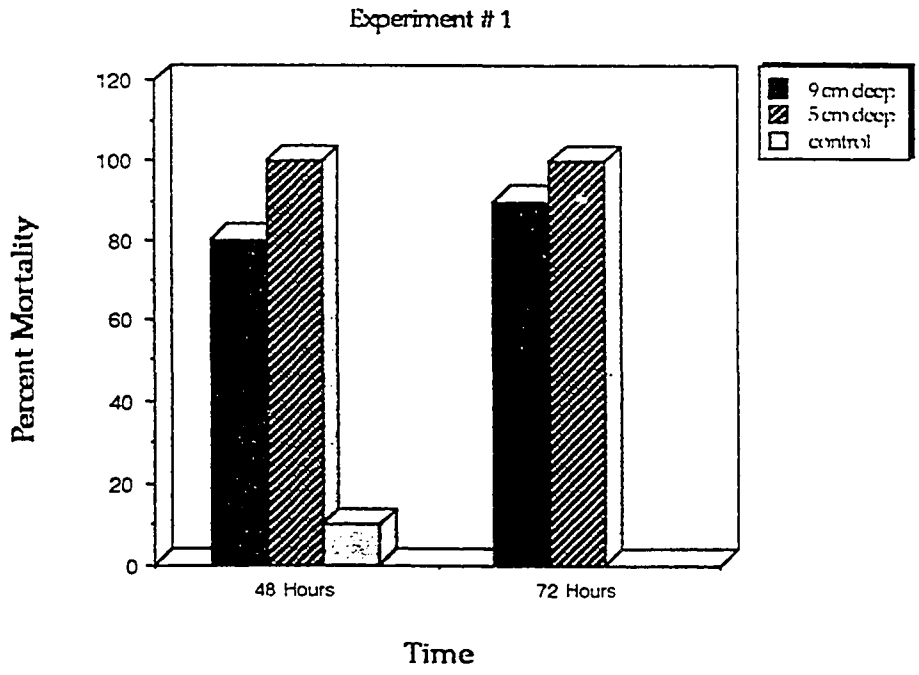
a. Mean of 3 trials each of 5 replicates
b. Means followed by the same letter are not significantly different using analysis of variance ($p<0.05$).
c. F value is equal to 36.02

Test #1 continued

ANOVA Table

source of variation	degree of freedom	sum of squares	mean squares
among groups	5	21.07	4.214
within groups	24	2.80	0.117
total variation	29	23.87	0.823

Figure 1.



2. Test #2

Objective: To determine the infectivity of HL-81 in potted primroses aganist wax moth larvae placed in soil at different depths.

Method: Two larvae and 20,000 nematodes were applied to each pot (similar to test #1).

Result: In contrast to experiment 1, at 48 hours after treatment the level of control was low at both depths. However, high control was recorded at 72 hours. This delay may be attributed to the presence of plant roots (Table. 2 and Figure. 2).

Table 2. Mean survival of Galleria mellonella in potted primroses exposed to Heterorhabditis Sp. HL-81 strain at different time and depths.

	Mean number of live larvae ± SE [a]			
	9 Cm deep		5 Cm deep	
	48 Hrs	72 Hrs	48 Hrs	72 Hrs
HI-81	1.80±0.45 [a]	0.40±0.55 [b]	1.80±0.45 [a]	0.00±0.00 [c]
CONTROL	-	-	2.00±0.00 [a]	1.80±0.45 [a]

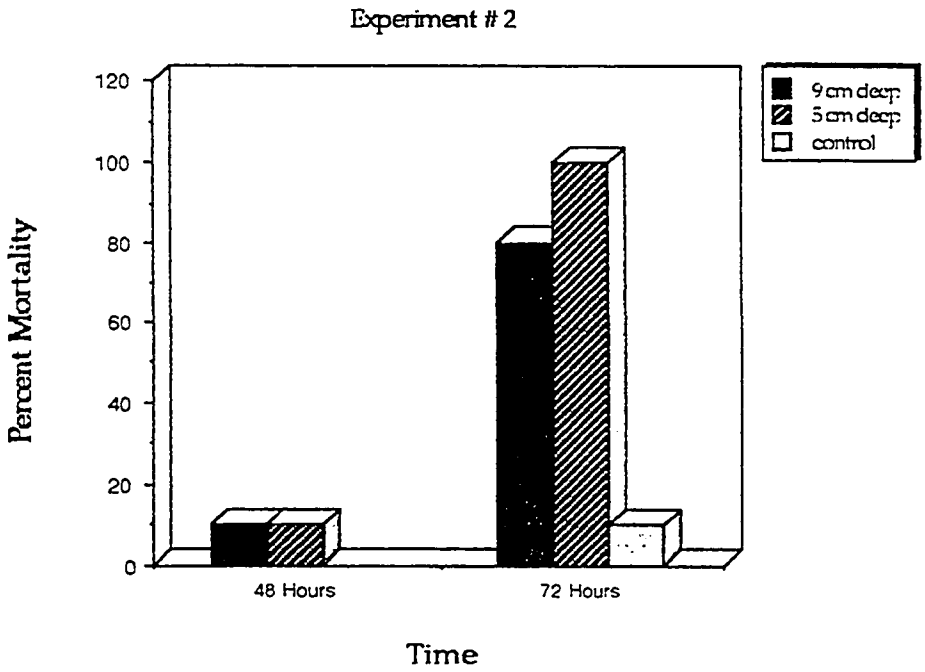
a. Mean of 3 trials each of 5 replicates
b. Means followed by the same letter are not significantly different using analysis of variance ($p<0.05$).
c. F value is equal to 24.7

Test #2 Continued

ANOVA Table

source of variation	degree of freedom	sum of squares	mean squares
among groups	5	18.70	3.70
within groups	24	3.60	0.15
total variation	29	22.30	0.77

Figure 2.



3. Test #3

Objective: To determine the infectivity of HL-81 at different depths and different number of larvae per pot.

Method: Primrose plants were used in this experiment. Results were examined after 72 hours. The application rates were as follow.

HL-81 dosage rate	Number of larvae per pot
2,000	2
5,000	5
10,000	10
15,000	15
20,000	20

Result: At 2,000 nematodes per pot, an average of 99 % control was obtained against larvae placed 9 cm deep, whereas the mortality was 86% against larvae located 5 cm deep. In contrast, the results of test #1 showed a better control at 5 cm when compared to 9 cm depth. These results could be attributed to the larval population, i.e. nematode movement was enhanced as the insect population increase (Table. 3 and Figure. 3).

Exp.#3 Continued

Table 3. Mean survival of Galleria mellonella exposed to Heterorhabditis HL-81 strain at different depth and differet dosages of nematodes per pot.

	Mean number of live larvae ± SE [a]		
HI-81	9 Cm deep	5 Cm deep	control
2,000	1.00±0.00 [a]	0.00±0.00 [b]	2.00±0.00 [d]
5,000	0.00±0.00 [b]	0.50±0.00 [a]	5.00±0.00 [e]
10,000	0.50±0.00 [a]	0.00±0.00 [b]	10.00±0.00 [f]
15,000	1.50±0.00 [c]	2.25±0.00 [d]	15.00±0.00 [g]
20,000	3.00±0.00 [d]	1.00±0.00 [a]	20.00±0.00 [h]

a. Mean of 4 trial each of 5 replicates

b. Number of Galleria were increased as in each replicate.

c. Means are significantly different using analysis of variances ($p<0.05$)

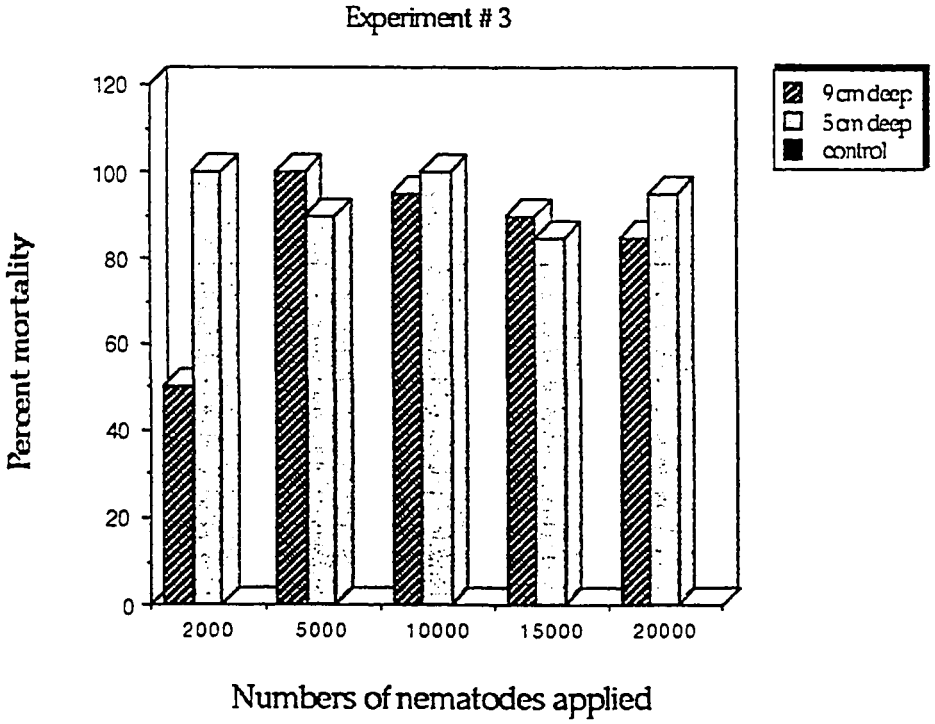
d. F value is equal to 5.18

Exp.#3 Continued

ANOVA Table

source of variation	degree of freedom	sum of squares	mean squares
among groups	2	293.27	146.640
within groups	12	339.85	28.320
total variation	14	633.12	45.220

Figure 3.



4. Test #4

Objective: To determine the infectivity of HL-81 at different dosages against insect host (no host plants).

Method: Mortality rates were checked 72 hours after application. Five dosages were tested as shown below.

HL-81 dosage	# of larvae per pot
100	5
500	5
1,000	5
2,000	5
5,000	5

Result: The level of insect control was low at dosages of 100 and 500 infective nematodes (HL-81) per pot. A dosage of 1,000 infective nematodes gave a moderate control. Best control was achieved at 2,000 and 5000 infectives, demonstrating that dosages of 2,000 infectives and higher per pot are needed to provide satisfactory control (Table. 4 and Figure. 4).

Exp.#4 Continued

Table 4. Mean survival of Galleria mellonela exposed to Heterorhabditis HL-81 strain at different dosages per pot.

	Mean number of live larvae ±SE[a]	
Hl-81	9 Cm deep	control
100	4.00±0.00 [a]	5.00±0.00 [e]
500	4.00±0.00 [a]	5.00±0.00 [e]
1,000	2.00±0.00 [b]	5.00±0.00 [e]
2,000	1.00±0.00 [c]	5.00±0.00 [e]
5,000	0.00±0.00 [d]	5.00±0.00 [e]

a. Means are significantly different using analysis of variances ($p<0.05$)

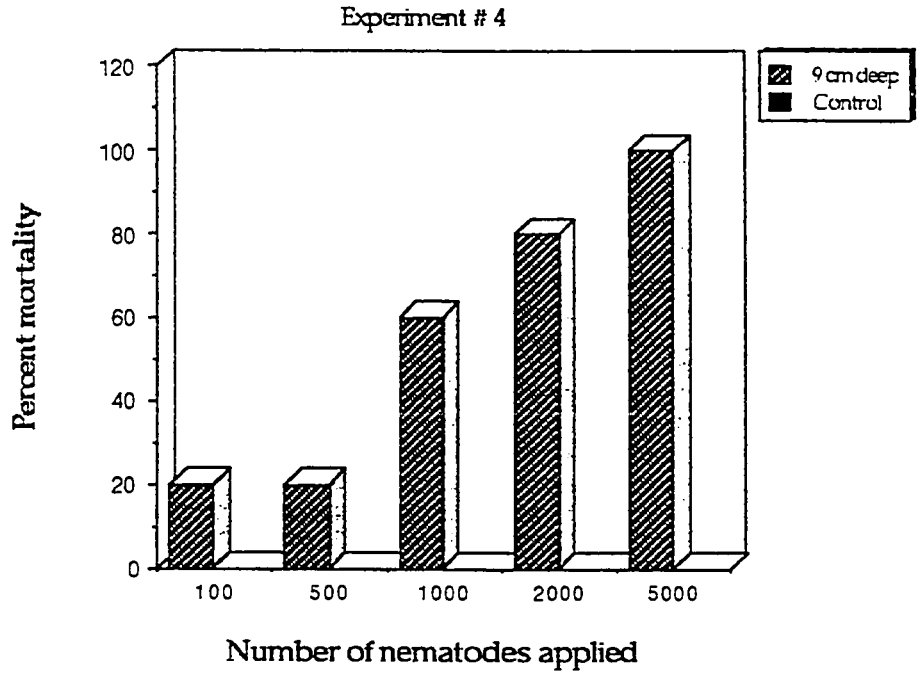
b. F value is equal to 12.25

ANOVA Table

source of variation	degree of freedom	sum of squares	mean squares
among groups	1	19.6	19.60
within groups	8	12.8	1.60
total variation	9	32.4	3.56

Exp.#4 Continued

Figure 4.



5. Test #5

Objective : To determine the infectivity of HL-81 against the larval instars of Otiorynchus sulcatus .

Method : Potted primrose plants were used. Each pot was inoculated with 5, 17-day old larvae collected from laboratory culture. Nematodes were applied at the rate of 6,800 infectives per pot. Results were recorded 7 and 31 days, after treatment.

Result : The results after 7 days were satisfactory because no larvae (dead or alive) were found, possibly due to disintegration of infected insects. Good results were obtained from the second test where larvae were examined after 31 days. Data indicates that older larvae were more susceptible to Heterorhabditis HL-81 strain nematodes than the younger stages (Table. 5 and Figure. 5).

Table 5. Mean survival of Otiorhynchus sulcatus exposed to Heterorhabditis Sp. HI-81 strain at two larval stages (24 and 48 days old).

	Mean number of live larvae ±SE [a]	
	24 days old larvae	48 days old larvae
HI-81	5.00±0.00 [a]	0.50±0.84 [b]
CONTROL		4.67±0.52 [a]

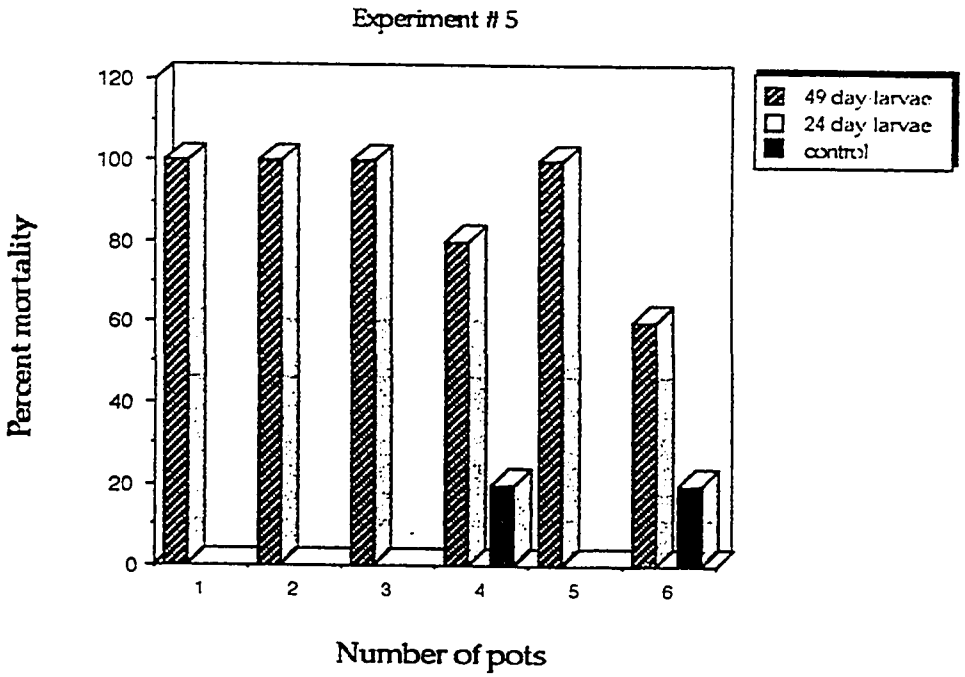
a. Mean of each trial with 6 replicates
b. F value is equal to 117.9

Exp.#5 Continued

ANOVA Table

source of variation	degree of freedom	sum of squares	mean squares
among groups	2	75.45	37.73
within groups	15	4.83	0.32
total variation	17	80.28	4.72

Figure 5.



6. Test # 6

Objective : Comparable efficacy of HL-81 with S. carpocapsae (entomopathogenic nematode) and Orthene (insecticide).

Method : Infected pots were randomly selected from greenhouse nursery in Watsonville, California. Pots were infested naturally with Otiorynchus sulcatus. Plants grown in the infested pots were approximately 14 months old Liquidamber styraciflua seedlings. The insecticide Orthene (Acephate) was used as a standard. Mortality rate was recorded 21 days after inoculation.

Result : Because of the high variability between the replicates, significant differences were not detected. However, there was a trend showing that both nematode species and orthene provided a better insect control compare to untreated control (Table. 6 and Figure. 6).

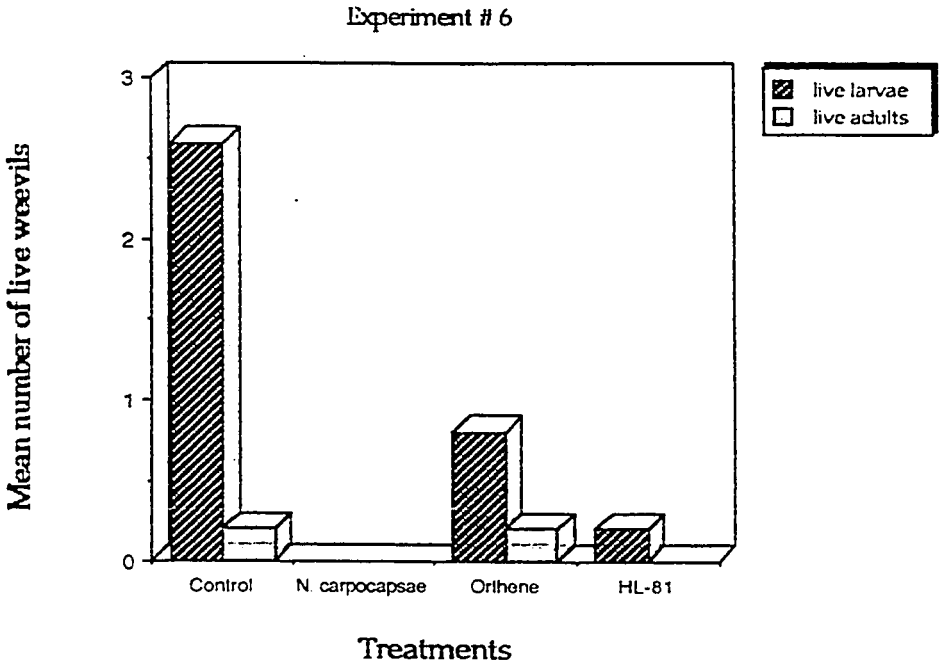
Exp.#6 Continued

Table 6. Mean survival of Otiorhynchus sulcatus exposed to two nematode species and an insecticide.

	Mean number of live larvae ±SE[a]	
	number of live larvae	number of live adult
HI-81 strain	0.200±0.447	0.000±0.000
Orthene (insecticide)	0.800±0.837	0.200±0.447
S. carpocapsae	0.000±0.000	0.000±0.000
Control	2.600±3.782	0.200±0.447

a. Mean of 4 trials each of 5 replicates
b. Comparison of means would not be possible since no variance among some of the groups was found ($p<0.05$)

figure 6.


Experiment # 6
Mean number of live weevils
3
2
1
0
live larvae
live adults
Control
N. carpocapsae
Orthene
HL-81
Treatments

IV. Discussion and Conclusion

For many years chemical pesticides were the only solutions for controlling insect pests. Environmental hazards, residue persistence, pest resistance, and adverse effects on beneficial organisms have encouraged scientists both in chemical industries and biological firms to work together to find an alternative solution for controlling insect pests.

The entomopathogenic nematode Heterorhabditis HL-81 strain has not previously been tested to determine its insecticidal activity. Simons and Schaaf (1986) were the only researchers who studied the infectivity of Heterorhabditis HL-81 strain on the black vine weevil. However, to have a better understanding of the potential of HL-81, the current research was carried out under laboratory and greenhouse conditions.

The results showed that HL-81 strain has promise for controlling Black Vine Weevil larvae. HL-81 has the capability to move downwards and infect hosts located at 5 cm and 9 cm depth. This demonstrates, as it is the case with other heterorhabditid species, the ability of HL-81 to seek out the insect host (Gaugler, 1988). Furthermore, the data indicated that mature larvae of Black Vine Weevil are more susceptible than early instar, thus demonstrating the importance of timing of application to maximize the effectiveness of this strain. HL-81 was comparable to the standard insecticide Orthene and the nematode Steinernema carpocapsae in reducing the larval population of Black Vine Weevil larvae. Dosages of 2,000 and 5,000 infectives per pot were sufficient to cause significant insect control. The dosage range is comparable to other commercially available Steinernema and Heterorhabditis species.

The commercial development of HL-81 is promising considering the recent development in vitro mass production method and formulation.

The results generated in this study are important data towards recommending an application strategy for HL-81 strain against Black Vine Weevil larvae. However, further studies as outlined below are needed to reach a full understanding on the factors affecting its efficacy. Predictable insect control can be obtained once these studies are completed.

1. Genetic manipulation: Recent studies by Gaugler (1991) have suggested that host-seeking enhancement can be achieved through 10-13 breeding cycles of nematodes in insect hosts. Improvement in persistence, tolerance to UV light and biotic and abiotic factors have also been reported (Poinar, 1990).

2. Soil ecology: Because the nematodes are applied as a soil insecticide, it is important to understand the effect of biotic and abiotic factors on the survival, movement and pathogenicity of HL-81.

3. Quality assurance: In-vitro commercial production methods have been developed for heterorhabditids and steinernematids.

Formulation stability was achieved by immobilizing the nematodes in gel polymer materials, thus achieving up to 6 months shelf life. Therefore, there is good indication that shelf life up to 6 months can be implemented with HL-81 strain. Research is needed to find a standardized quality control method that could be used to measure the pathogenicity and viability of HL-81 throughout all stages of its development (i.e production, formulation, storage, and application). Such research is needed to prevent the production of low quality nematodes.

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