

PROGRESS REPORT
EFFECTS OF ENTOMOPATHOGENIC NEMATODES ON
INSECT PESTS OF AVOCADO

Nancy E. Beckage
Department of Entomology
University of California
Riverside, CA 92521

The withdrawal of several chemical pesticides from the market for pest management has diverted research attention from reliance on chemical control towards developing alternative biological control strategies. Growing concern about environmental contamination with pesticides, and insecticide resistance of the pests themselves, have also caused us to seek new biological control agents. For example, several strains of the bacterium *Bacillus thuringiensis* have been successfully used in insect pest management programs.

The growing number of instances where nematodes are being successfully used in controlling insect pests prompted us to assess the viability of these nematodes in controlling key pests of avocado. The mutualistic bacterium *Xenorhabdus* sp uses the nematodes from two families (*Steinernematidae* and *Heterorhabditidae*) as vectors to enter the insect hosts' hemocoel and cause tissue degradation and subsequent septicemia. Bacterial gene products are thought to confer the pathogenicity of the nematodes to the insects through this tripartite association.

A long term goal of our research program is to develop the bacteria or bacterial gene products as control agents alone or in combination with other pathogens such as viruses. In the short-term, we are testing efficacy of the nematodes themselves. This report describes the effects of entomopathogenic nematodes on the lepidopteran pest, amorbina (*Amorbia cuneana*). Experiments were conducted by Ph.D student Ashok Pullikuth with two widely used nematode species, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, to assay pathogenic effects on the larval and pupal stages of *A. cuneana*.

MATERIALS AND METHODS

STEINERNEMA CARPOCAPSAE CULTURES:

Nematode cultures of *S. carpocapsae* All were obtained from Biosys as the commercial product -Biosafe. Nematodes were processed according to the manufacturer's instruction and used to infect fifth instar larvae of *Manduca sexta* (tobacco hornworm) and *Galleria mellonella* (greater wax moth). Infective stage juvenile nematodes were harvested from cadavers and used within 3 to 5 days following exit from the cadavers in pathogenicity studies.

HETERORHABDITIS BACTERIOPHORA CULTURES:

Nematodes were obtained from Dr. Harry Kaya, UC Davis. Extracted nematodes were multiplied as above for use in our studies.

BIOASSAY

Caterpillars of *Amorbia* were raised on a semi-synthetic pinto bean based diet at 25 ° C. A modified petri dish bioassay procedure (Woodring and Kaya, 1988) was followed to ensure constant contact of the caterpillars with the nematode suspension on filter papers. The percent mortality of larvae was recorded 24, 36, and 48 hrs after infection.

Death due to parasitization by nematodes was recorded. Eight caterpillars from each treatment were randomly chosen and incubated in moist chambers for 8 days. Each caterpillar was dissected to observe the developing nematodes inside the carcass to confirm that mortality was due to nematode infection. Data were arc sine transformed and subjected to analysis of variance.

RESULTS

Both nematode species caused considerable mortality in all three larval stages of *Amorbia cuneana* tested. *Steinernema carpocapsae* All caused 54.8% mortality in pupae while *Heterorhabditis bacteriophora* NC caused 57.4% mortality in the pupal stage (Table 1). Thus, pupae as well as larvae were susceptible to nematode infection.

The highest mortality (88.12%) caused by *S. carpocapsae* was observed in the fourth stage while *H. bacteriophora* caused highest mortality (89.01%) in the fifth stage. Percent mortality ranged from 83.19 to 88.12% with *S. carpocapsae* and 81.3 to 89.01% with *H. bacteriophora*. Table 2 and 3 show that nearly all hosts were dead by 48 hrs post-infection.

DISCUSSION

Both the nematode species tested were effective in causing significant death to larval and pupal stages of *Amorbia cuneana*. Even though 81-89% mortality was seen after exposure of larval stages of *Amorbia* to the nematodes, the pupal stages were less susceptible and only 55-57% mortality occurred. The reduced kill in the pupal stage could be attributed to the reduced number of entry portals accessed by the nematodes, their thick cuticle and also due to the lack of mobility of pupae. Since each population of nematodes is a mixture of 'ambushers' and 'hunters' (Gaugler and Campbell, 1991) mobility of the insect greatly compensates for the proportion of the nematode population lost to ambushing individuals which do not actively seek out hosts. Our present findings suggest that both *S. carpocapsae* and *H. bacteriophora* are potent biocontrol agents of *Amorbia cuneana*. We will next be extending our studies to identifying the toxic gene products of the bacteria involved in causing the mortality.

As an initial assessment we had used a relatively high dosage of nematodes (350 nematodes/caterpillar). We are now carrying out LD50 tests to find out the optimum number of nematodes required to cause mortality. These findings were reported by Ashok Pullikuth at the 1991

Annual meeting of the Entomological Society of America at Reno, Nevada and further findings will be presented at the 1992 Society of Nematologists meeting in August

REFERENCES

Gaugler, R. and J. F. Campbell, 1991. Behavioural response to the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* to oxamyl. *Annals of Applied Biology* 119: 131-138.

Woodring, J. L. and H. K. Kaya, 1988. Steinernematid and Heterorhabditid Nematodes: A Handbook of Techniques. Southern Cooperative Series Bulletin 331.

Table 1. Percent mortality of *Amorbia cuneana* caused by entomopathogenic nematodes

Nematode	Stages of Amorbia			
	3	4	5	Pupa
Steinernema	83.19**	88.12**	83.67**	54.83**
Heterorhabditis	83.19**	81.30**	89.01**	57.41**
Control	0.99	0.99	0.99	0.99

Figures were angular transformed for analysis; Mortality recorded after 48 hrs post-infection; nematode inoculum level 5000N/replication.**, significantly different at 1% level.

Table 2. Percent mortality of *Amorbia cuneana* caused by *Steinernema carpocapsae* at different time intervals post infection

Stages	Hours post infection		
	24 hrs	36 hrs	48 hrs
Larva 3	43.08**	63.44**	83.19**
Larva 4	46.59**	63.92**	88.72**
Larva 5	48.19**	68.34**	83.67**
Pupa	0.99ns	35.00**	54.83**
Control	0.99	0.99	0.99

** , significant at 1% level.

Table 3. Percent mortality of *Amorbia cuneana* caused by *Heterorhabditis bacteriophora* at different time intervals post infection

Stages	Hours post infection		
	24 hrs	36 hrs	48 hrs
Larva 3	37.22**	83.19**	83.19**
Larva 4	40.22**	60.21**	81.30**
Larva 5	45.00**	70.80**	89.01**
Pupa	0.99ns	47.54**	57.41**
Control	0.99	0.99	0.99

** , significant at 1% level.