Workgroup/Department: Almond

University of California
Division of Agricultural Sciences

PROJECT FINAL REPORT

Project Year 1996

Project Leader H. Kaya[1]

Cooperating Personnel Paul Verdegaal[2], Joe Grant[2], and R. A. Van Steenwyk[3]

Project Title Control of Tenlined June Beetle with insect-parasitic nematodes

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[1] Dept. Nematology, UC Davis; [2] UC Farm Advisors, San Joaquin county; [3] Dept. Entomology, UC Berkeley

Project Objectives:

1. Identify prospective bio-control agents
2. Evaluate natural parasitization
3. Develop feasible control measures using entomopathogenic nematodes

I. Identify prospective bio-control agents (1995) - We have developed procedures for keeping field collected tenlined June beetle (TJB) grubs for several months and have reared them on a limited scale. A bioassay was developed to screen entomopathogenic nematodes (EN) against TJB. Individual grubs were kept in sandy soil and fed on almond roots. EN were added and grub mortality assessed after 8 days. Screening of 7 nematode species/strains against 2nd instar TJB larvae showed that TJB had a low susceptibility to EN but one species, Steinernema kushidai, provided very high TJB mortality (Table 1). Further bioassays showed that 3rd and 1st instar larvae were similarly if not more susceptible to S. kushidai even at relatively low concentrations (Table 2). Laboratory experiments in citrus containers showed S. kushidai infected TJB larvae at depths greater than 15 cm in soil; mortality at greater depths, however, was limited. This indicated that in field applications EN will have to be irrigated into deeper soil layers to infect TJB grubs throughout the soil profile.

II. Evaluate natural parasitization (1995 and 1996) - No field-collected grubs were observed with obvious symptoms of parasitization. A strain of Heterorhabditis bacteriophora designated TJB strain was isolated from soil samples taken in an almonds orchard in Manteca but it had a low pathogenicity against TJB grubs. An entomopathogenic fungus, Metarhizium anisopliae, isolated from the same soil samples is being kept in culture; it has not been tested against TJB.

III. Greenhouse testing (1995 and 1996) - EN have been tested against TJB grubs in greenhouse experiments. Almonds seedlings planted in citrus containers (45 cm deep x 20 cm diam) in sandy non sterile orchard soil were infested with TJB larvae. After 4 days to allow for natural dispersal of the larvae, the containers were treated with S. kushidai and post-irrigated with 2.5 cm of water to wash the EN deeper into the soil. After 2 weeks, the number of surviving larvae was counted. This system proved to establish conditions very close to the field. In trial 1 with 12 1st instar TJB and 25,000 EN/pot ($5x10^9$/ha), we observed a significant 40.5% reduction of TJB larvae (Table 3). In trial 2, 50,000 EN/pot caused only 18% reduction of 1st instar grubs. In trial 3, three 2nd and three 3rd instar grubs were added per pots and treated with 50,000 EN/pot. There was a 33% reduction in 2nd instar grubs but no reduction on 3rd instars (Table 3).

IV. Field testing - Because of the limited control of 1st and 2nd instar grubs and lack of control of 3rd instars even under favorable greenhouse conditions, no field tests were conducted.

Conclusions - TJB grubs generally have a low susceptibility to EN and only one among the species tested showed any promise as a control agent for this pest in the laboratory. Although we did not conduct field experiments, we suspect that 2nd and especially 1st instar grubs because they tend to stay closer to the soil surface could be reduced to some extend with S. kushidai. High nematode application rates and repeated applications required, however, would make EN applications too expensive. In addition, the 3rd instar grub which is probably the stage that causes the most serious damage can not be reduced with EN alone. Based on other research with white grubs of turf, stressing TJB grubs with another agent (e.g., more environmentally acceptable insecticides such as imidacloprid or Bacillus thuringiensis subspecies japonensis) to increase nematode susceptibility may be useful approach in the future.

Table 1. Mortality of tenlined June beetle 2nd instar larvae after 8 days exposure to infective juveniles (IJs) of different species/strains of entomopathogenic nematodes in soil at 20°C.

Nematode species/strain	200 IJs	500 IJs
Control	0	0
Steinernema anomali	3 ± 3	24 ± 3 b
S. glaseri NC	0	33 ± 12 b
S. kushidai	--	91 ± 3 a
Heterorhabditis bacteriophora TJB	0	3 ± 3 b
H. bacteriophora NC1	6 ± 6	12 ± 12 b
H. megidis	3 ± 3	18 ± 5 b
H. hepialus	3 ± 3	36 ± 5 b

Means followed by same letter within column are not significantly different (Tukey, $P < 0.05$).

Table 2. Mortality of the three larval instar (L_{1-3}) stages of the tenlined June beetle after 8 days exposure to different concentrations of Steinernema kushidai in soil at 20°C.

# IJs	L_1	L_2	L_3
Control	0	0	0
50	--	--	52 ± 11 b
100	--	83 ± 7	66 ± 6 ab
200	82 ± 5	93 ± 3	82 ± 8 ab
500	--	--	94 ± 2 a

Means followed by same letter within column are not significantly different (Tukey, $P < 0.05$).

Table 3. Reduction of tenlined June beetle (TJB) larvae in 15 L pots with almond seedlings in the greenhouse three weeks after treatment with 25,000 or 50,000 infective juveniles (IJs) per pot of the entomopathogenic nematode, *Steinernema kushidai*.

Trial	I	II	III
# IJs / pot	25,000	50,000	50,000
TJB Stage	L_1	L_1)	L_2 / L_3 / L_2+L_3
% Reduction	40.5%	18%	33% / 3% / 14%
P <	0.0001	0.001	0.02 / 0.4 / 0.03

Originator's Signature Date 3/27/97

Department Chair Date 3/27/97

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COLLEGE OF AGRICULTURAL AND
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AGRICULTURAL EXPERIMENT STATION
COOPERATIVE EXTENSION
(916) 752-6905
FAX: (916) 752-5809

DEPARTMENT OF NEMATOLOGY
UNIVERSITY OF CALIFORNIA, DAVIS
DAVIS, CALIFORNIA 95616-8668

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Dr. Harry K. Kaya

Davis 3/27/1997

Kandi Cruz
Almond Board of California
1104 12th street
Modesto
CA 95354

Final report Project No. 96-HK1

Dear Ms. Cruz:

Please find enclosed the Final report for Project No. 96-HK1.

Sincerely,

Harry Kaya, Professor