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University of California
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**PROJECT INTERIM REPORT**

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

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**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** - 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 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 some EN species had a useful level of pathogenicity and one EN species provided very high TJB mortality (Table 1). Further bioassays showed that 3rd and 1st instar larvae were similarly if not more susceptible to EN even at relatively low dosages (Table 2). Laboratory experiments in citrus containers showed that the best EN species 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 with water to infect TJB grubs throughout the soil profile.

**II. Evaluate natural parasitization** - Neither in the field nor in the laboratory, any grubs were observed with obvious symptoms of parasitization. Using wax moth larvae as baits, a strain of Heterorhabditis bacteriophora designated TJB strain was isolated from soil samples taken in almonds orchards in Manteca. This nematode, however, had a low pathogenicity against TJB grubs and was not further investigated. An entomopathogenic fungus, Metarhizium anisopliae, isolated from the same soil samples is being kept in culture. Further examination of field samples for natural enemies will be conducted in 1996.

**III. Field testing** - EN have been tested against TJB first instar larvae in greenhouse experiments. Almonds seedlings were planted in 45 cm high citrus containers in sandy orchard soil. After time for establishment, the seedlings were infested with TJB larvae. After 4 days to allow for natural dispersal of the larvae, the containers were treated with EN 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. In a first trial, this system proved to establish conditions very close to the field and a significant 40% reduction of TJB larvae was observed at a relatively low nematode concentration. Further trials are being conducted including higher nematode concentrations.

Table 1. Mortality of tenlined June beetle 2nd instar larvae after 8 days exposure to 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 stages of the tenlined June beetle after 8 days exposure to different dosages of Steinernema kushidai in soil at 20°C.

| # IJs | 1st | 2nd | 3rd |
|---|---|---|---|
| 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, $P < 0.05$).

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