Turfgrass Insect Biocontrol Project

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Fungal pathogen isolates of soil insects were tested against Japanese beetle grubs. There were considerable differences in performance under various common environmental conditions.

1992 studies suggest that under specific environmental conditions, commercially available entomopathic nematodes may outperform standard turfgrass insecticides for controlling grubs. Research in 1993 will focus on field studies to verify these laboratory and greenhouse results.

Studies during 1992-93 in our long-term research and development program to introduce biologically based control agents for scarab grub control in turfgrass has focused on evaluating a number of entomopathogenic agents (entomopathic nematodes and fungi) against Japanese beetle grubs.

Fourteen *Metarhizium anisopliae* isolates, a fungal pathogen of soil insects, were tested against Japanese beetle grubs. Two isolates, MADA and 1020 are currently being considered for commercialization. The other twelve isolates were chosen because they were isolated from scarab grubs from around the world. MADA and 1020 performed well at both treatment rates, but there were clearly isolates that performed consistently better over the study. There was considerable differences in how well several fungal isolates performed under a variety of environmental conditions that are commonly found in Metropolitan area golf courses. Studies were also initiated to determine if combinations of fungal pathogens and traditional insecticides might increase grub mortality and reduce the lag time common for many insecticides in soil. These preliminary studies suggest that this may occur. Further work in this area will be undertaken in 1993. The addition of fungal pathogens to composts for top dressings was also studied in 1992. Although this work is preliminary we have encouraging results and will continue this study during the rest of the project.

A second 1992 research focus was in evaluating entomogenous nematodes under a variety of environmental conditions to determine those conditions that promoted maximal grub mortality. These studies suggest that under specific environmental conditions, commercially available entomopathic nematodes may outperform standard turfgrass insecticides for controlling grubs. Research in 1993 will focus on field studies to verify these laboratory and greenhouse results. We have also studied several nematodes that have been collected by Cornell University entomologists that appear to compare favorably to commercially produced nematodes against Japanese beetle grubs. Large plot studies are planned for 1993.

Summary of Fungus Tests

All isolates

Twelve *Metarhizium anisopliae* isolates were obtained from the USDA-ARS collection of entomopathogenic fungal cultures (Plant Protection Research Unit, U.S. Plant Soil and Nutrition Laboratory, Tower Road, Ithaca, NY): 1046, 1298, 2547, 2548, 2136, 2139, 3329, 3330, 3334, 3337, 3340, 1914. Additionally two isolates of *M. anisopliae* were obtained as formulated mycelia (MADA and Bio 1020). All isolates were cultured on plates of one-half strength Potato Dextrose Agar (PDA). Conidia were harvested from these plates, dried and stored at 4℃. Conidia from each isolate mixed into soil at a rate of 10^7 CFU/g of soil. Grass seed was added. Five grams of the soil/seed/conidia mixture was added to a scintillation vial and a third instar Japanese Beetle grub was placed on the top. Fifteen insects were treated for each insect. The amount of condia permitting, this procedure was replicated three times at 10^7 CFU/g of soil and one time at 10^6 CFU/g of soil.

MADA and 1020 performed well at both treatment rates but there were clearly isolates that performed consistently better over the study (see figures 1 and 2). These isolates are being formulated and will be tested against third instar Japanese beetle grubs this fall. If there is adequate funding we would like to test these isolates against other common scarab grubs including Oriental beetles, Asiatic Garden beetles and European chafers. There was some inconsistency in the viability of the conidia so there may have been some underestimation of the rates applied.

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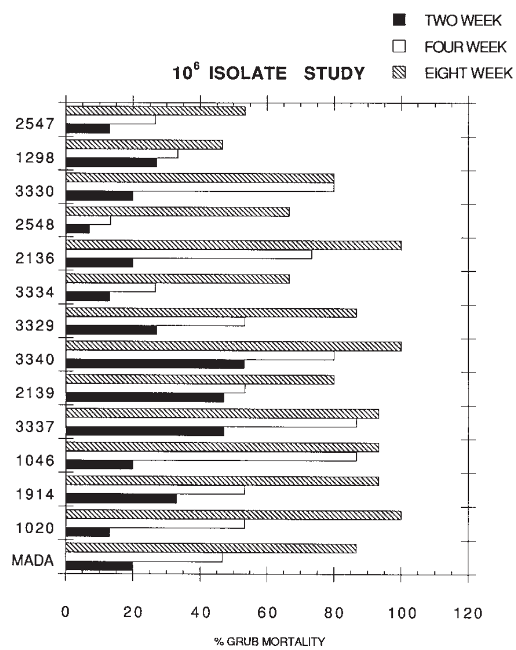
Figure 1

Effects of fungal isolates on grub mortality



Entomopathogenic fungal culture isolates (1046, 1298, 2547, 2548, 2136, 2139, 3329, 3330, 3334, 3337, 3340, 1914) of Metarhizium anisopliae*. MADA & 1020—two isolates of* Metarhizium anisopliae *as formulated mycelia isolates.*

Figure 2

Comparison of effects of fungal isolates on grub mortality

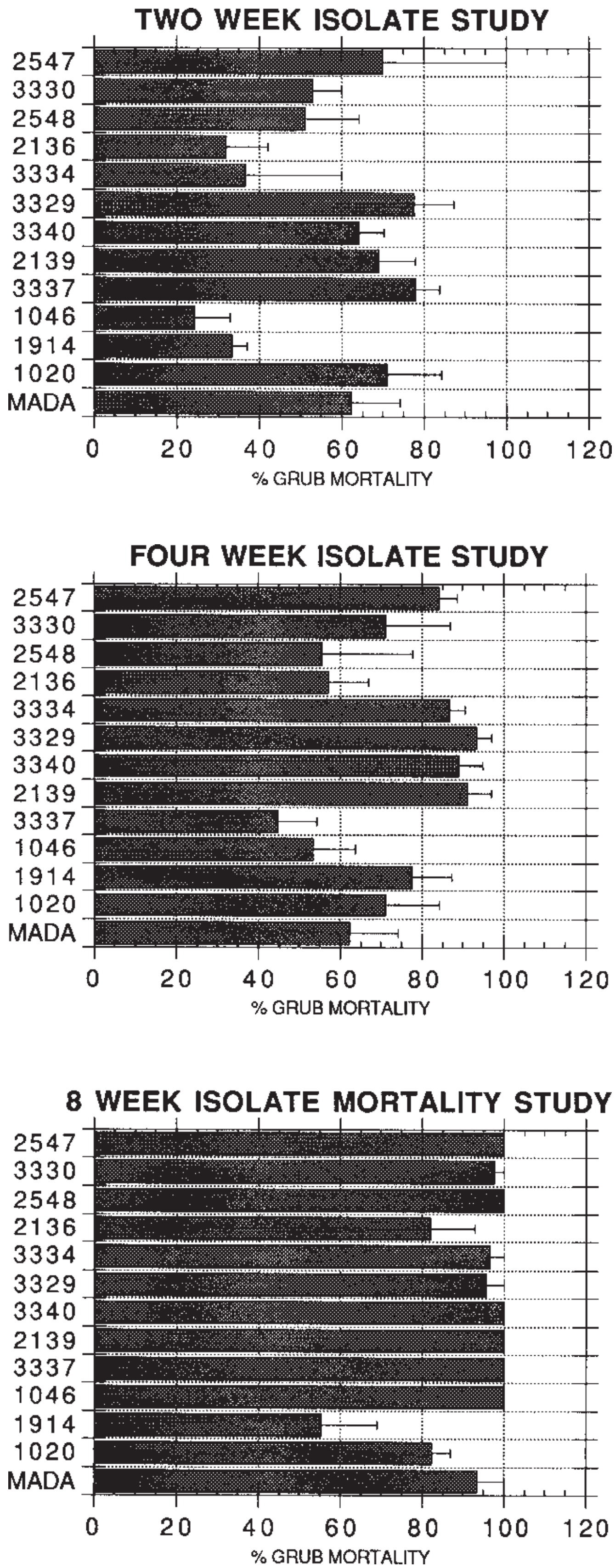
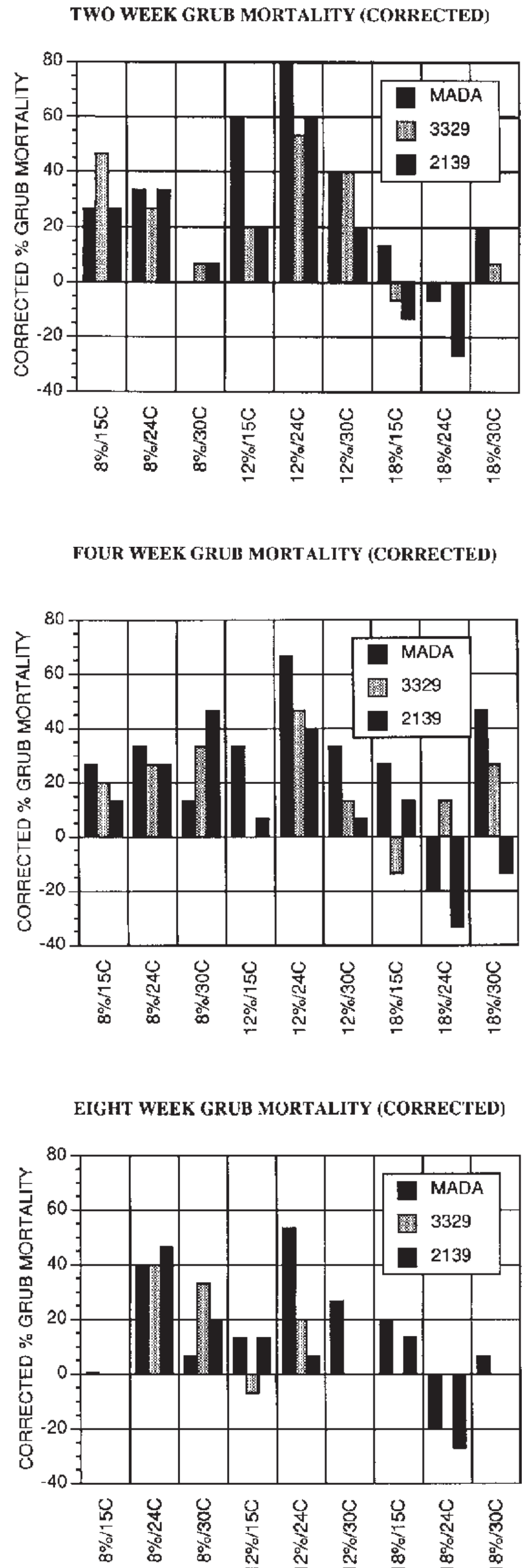


Figure 3

Influence of temperature and moisture on effectiveness of 3 fungal isolates



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All isolates appeared to perform best under intermediate soil moisture and temperature conditions.

Clearly under these experimental conditions both MADA and 1020 performed extremely well. We would propose that a similar study be undertaken this coming year with the newly formulated isolates.

Fungus Isolates at 3 Temperatures and 3 Moistures

The effect of three fungus isolates, MADA, 3329, 2139, at three temperatures (15℃, 24℃, 30℃) and three soil moistures (8%, 12%, 30%) on Japanese Beetle mortality was investigated. Fungal conidia were mixed into soil at a rate of 10^6 CFU/g of soil. Each tube contained 4.7 g of soil. Third instar JB larvae were added to the top of each scintillation vial. Fifteen insects were set up in each treatment. Readings were done at 2, 4 and 8 weeks. Graphs were corrected for check mortality (see figure 3). An additional trial at 24℃ and 12% soil moistures was set up with Cranberry root grubs (*Lichnanthz vulpina* Hentz) [not included in summary].

All isolates appeared to perform best under intermediate soil moisture and temperature conditions. The two additional isolates did not appear to provide significantly higher mortality at higher soil moisture. If this appears to be a limiting factor for use then we might screen additional isolates at higher or lower temperature and/or moisture conditions to identify these isolates. We will retest all formulated isolates at multiple temperature and moisture conditions against Japanese beetle grubs this fall.

Earth Gro and Bio 1020

Six treatments were set up:

1) Check with Earth Gro (20% by volume)—incorporated,

2) Check with Earth Gro (20% by volume)—top dressing (TD),

3) Bio 1020 (.48g/30g soil) with Earth Gro (20% by volume)—incorporated,

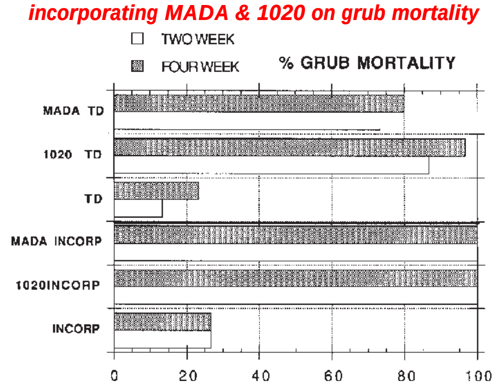
4) Bio 1020 (.48g/30g soil) with Earth Gro (20% by volume)—top dressing (TD),

5) MADA (.48g/30g soil) with Earth Gro (20% by volume)—incorporated,

6) MADA (.48g/30g soil) with Earth Gro (20% by volume)—top dressing (TD).

Figure 4

Comparison of effectiveness of top dressing (TD) or incorporating MADA & 1020 on grub mortality

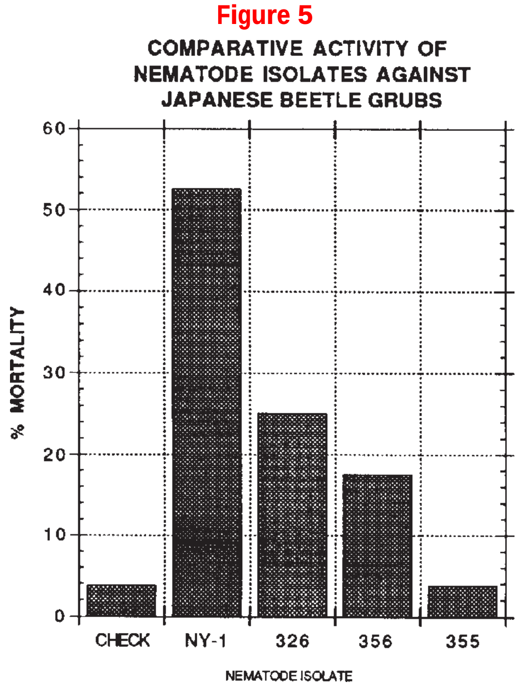


These rates were used to insure that grubs could be infected by a compost-based fungal product. If these studies are to continue then rate-response under the different application regimens should be studied. Thirty third-instar Japanese beetles (*Popilia japonica*) were used in each treatment. The appropriate formulated material was added into the Earth Gro compost and mixed in with a glass rod. For incorporated treatments, the Earth Gro/Formulated material was mixed into the soil. One-ounce cups were filled with this mixture. For top-dressed treatments, one-ounce cups were 4/5 filled with soil; the remaining 1/5 on the surface was filled with the Earth Gro/Formulated mixture. The insects were placed on the top of each cup and allowed to crawl into the soil. Readings were done at 2 and 4 weeks. Clearly under these conditions both MADA and 1020 performed extremely well (see figure 4). We would propose that a similar study be undertaken this coming year with the newly formulated isolates. Also, the shelf-life of formulated material in compost under a variety of moisture-temperature conditions should be studied.

Comparison of Entomogenous Nematode Strains for Japanese Beetle Grub Control

Four isolates of entomopathic nematodes were evaluated in the laboratory for efficacy against third instar Japanese beetle grubs. Our standard nematode was *Steinernema glaseri* (326) that is being commercialized by Biosys (Palo Alto, CA) for use against soil insects. We have studied this isolate in the laboratory and in the field for the past

Figure 5



several years. Also tested in this study were two additional *Steinernema* isolates (Venezuela and Rio Bravo) and a *Heterorhabdtis* nematode (NY-1), isolated and cultured by P. Schroeder and C. Ferguson (in alfalfa, Oswego NY), was also evaluated. Grub mortality caused by the various nematodes can be seen in Figure 5.

Our studies indicate that Biosys 326 was the most effective *Steinernema* isolate evaluated but that *Heterorhabdtis* nematode (NY-1) clearly outperformed all other nematodes and should be considered in further studies. These results parallel similar comparisons between these nematode groups. *Steinernema* currently is of greater interest to commercial enterprises because as a group they tend to be more easily formulated and shipped than the more fragile *Heterorhabdtis*. For this reason considerable effort has been spent to improve the performance of this group. However, there is increasing interest from small commercial enterprises to produce *Heterorhabdtis* nematodes for use on high value horticultural crops (including lawns and golf courses). Since nematodes are not considered pesticides there are no regulatory roadblocks to their commercialization. The only roadblocks currently is the lack of comprehensive research to determine the best nematode isolates and the environmental conditions that will maximize their performance. Studies proposed for 1993 include the large scale testing of Biosys 326 and P. Schroeder and C. Ferguson NY-1 in high maintenance and low maintenance turfgrass.

Environmental Factors Influencing the Performance of *Steinernema glaseri* (326) Against Japanese Beetle Grubs in Turfgrass

Effective long-term control of the scarab grub complex was achieved with organochlorine and cyclodiene insecticides until their use was discontinued due to insect resistance and government intervention; less consistent control has been achieved with organophosphate and carbamate insecticides and with a variety of biological agents (nematodes, fungi, bacteria and viruses). The entomogenous nematode *Steinernema glaseri* (326) [Biosys, Palo Alto, CA] has shown promise for controlling grubs in turfgrass but the results have been inconsistent.

One reason for this inconsistency may be the lack of basic understanding in the interaction of the nematode, the grub population, and the soil physical and biotic environment (soil moisture, temperature, texture, compaction, and microbial flora, etc.). While each of these factors has been independently studied to varying degrees by basic and applied researchers, their interdependence has largely been ignored. The nature of the specific interactions between target insect population(s),

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Figure 6
Physical properties of 6 soil samples

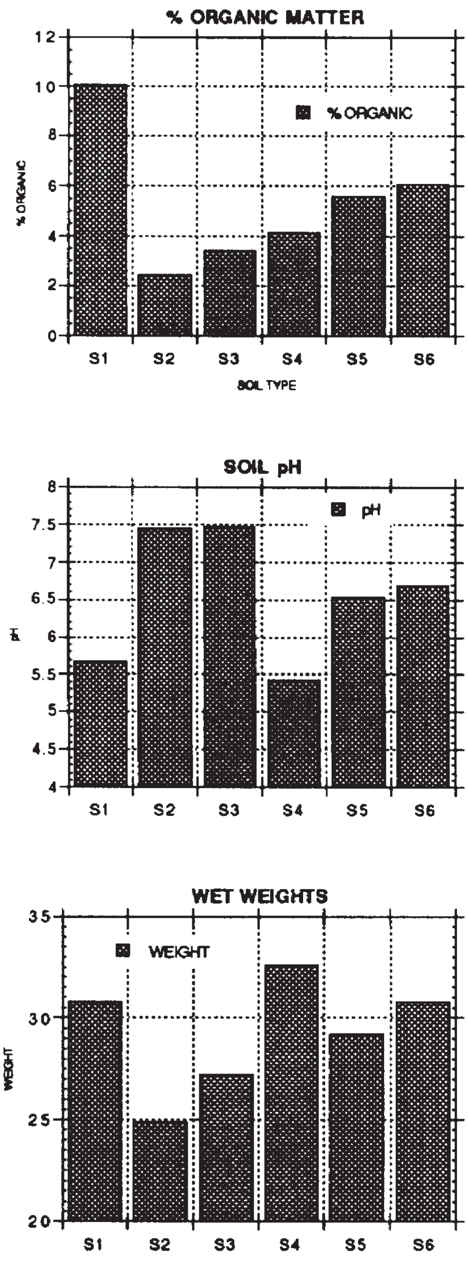
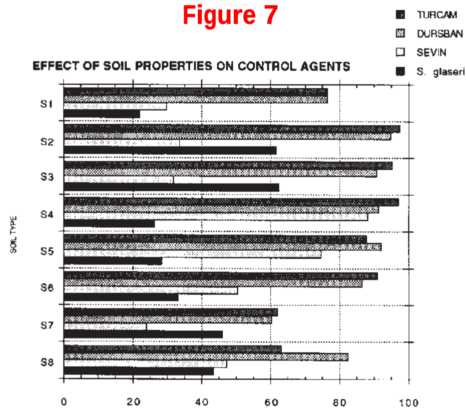


Figure 7



***Heterorhabdtis* nematode (NY-1) clearly outperformed all other nematodes and should be considered in further studies.**

***Steinernema* currently is of greater interest to commercial enterprises because as a group they tend to be more easily formulated and shipped than the more fragile *Heterorhabdtis*. For this reason considerable effort has been spent to improve the performance of this group.**

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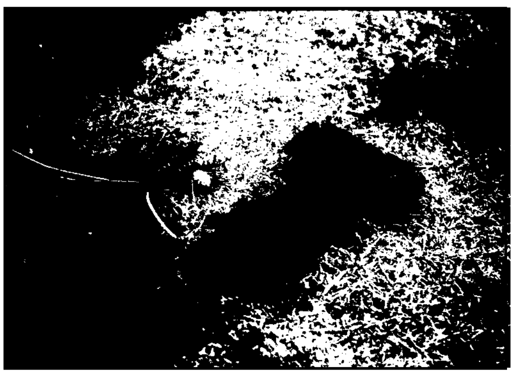
The nature of the specific interactions between target insect population(s), control agent(s) and the soil environment were examined in this study. While each of these factors has been independently studied to varying degrees by basic and applied researchers, their interdependence has largely been ignored.

control agent(s) and the soil environment were examined in this study. In the first stage of our study we hoped to determine the influence of soil physical properties (% organic matter, soil pH, soil bulk density) on the performance of *Steinernema glaseri* (326) against third instar Japanese beetle grubs.

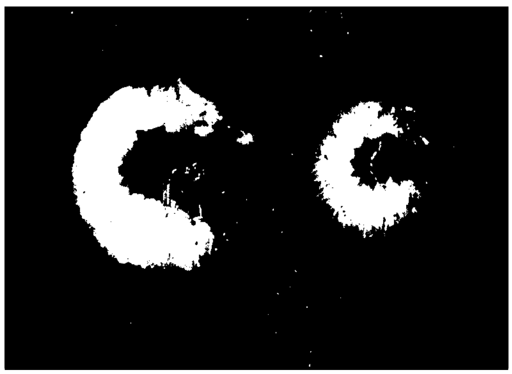
We determined the efficacy of this nematode in eight different soils with varying combinations of a number of potentially important soil characteristics. Six of these soils have been characterized (see figure 6) [2 pending at Cornell soil analysis lab in Ithaca]. The performance of *Steinernema glaseri* (326) was compared with the performance of three common grub insecticides (see figure 7). Our results indicated grub mortality of 22% to 64 % from our *Steinernema glaseri* (326) treatments in the various soil types.

Three soils (S1, S3, S4) were then selected for more comprehensive studies. These soils encompassed the range of soil factors (% organic matter, soil pH, soil bulk density (see figure 8)) and the range of grub control (22%, 66%, 30%) due to *Steinernema glaseri* (326). These studies were conducted in test chambers that allowed late third instar Japanese beetle grubs to escape through the soil profile during the four week experiment in response to internal physiological changes and /or changes to their external environment. Control agents were applied at the field rates and twice the field rate as a drench and then irrigated with 1.00 cm and .25 cm of distilled water. Results are indicated in figure 8.

Our results indicate that in our more complex bioassay arenas *Steinernema glaseri* (326) performed as well as or significantly better than either soil insecticide for grub control. There may be several reasons for our results: 1) Large third instar Japanese beetles are very tolerant of insecticides but show no increase tolerance to nematodes when compared to earlier stages. 2) Late instar grubs may be moving down into the soil profile to find pupation sites. Nematodes can and will move down through the profile and can reach grubs whereas soil insecticides are bound to the thatch soil interface. 3) Environmental factors that may move grubs down into the soil profile may also move nematodes down thereby increasing the opportunity for overlap and infection. Increases in irrigation and application rate also improved control agent effectiveness. The impact of increases in irrigation was most evident in high organic soil (S1) where free water was limiting at the lower irrigation rate.



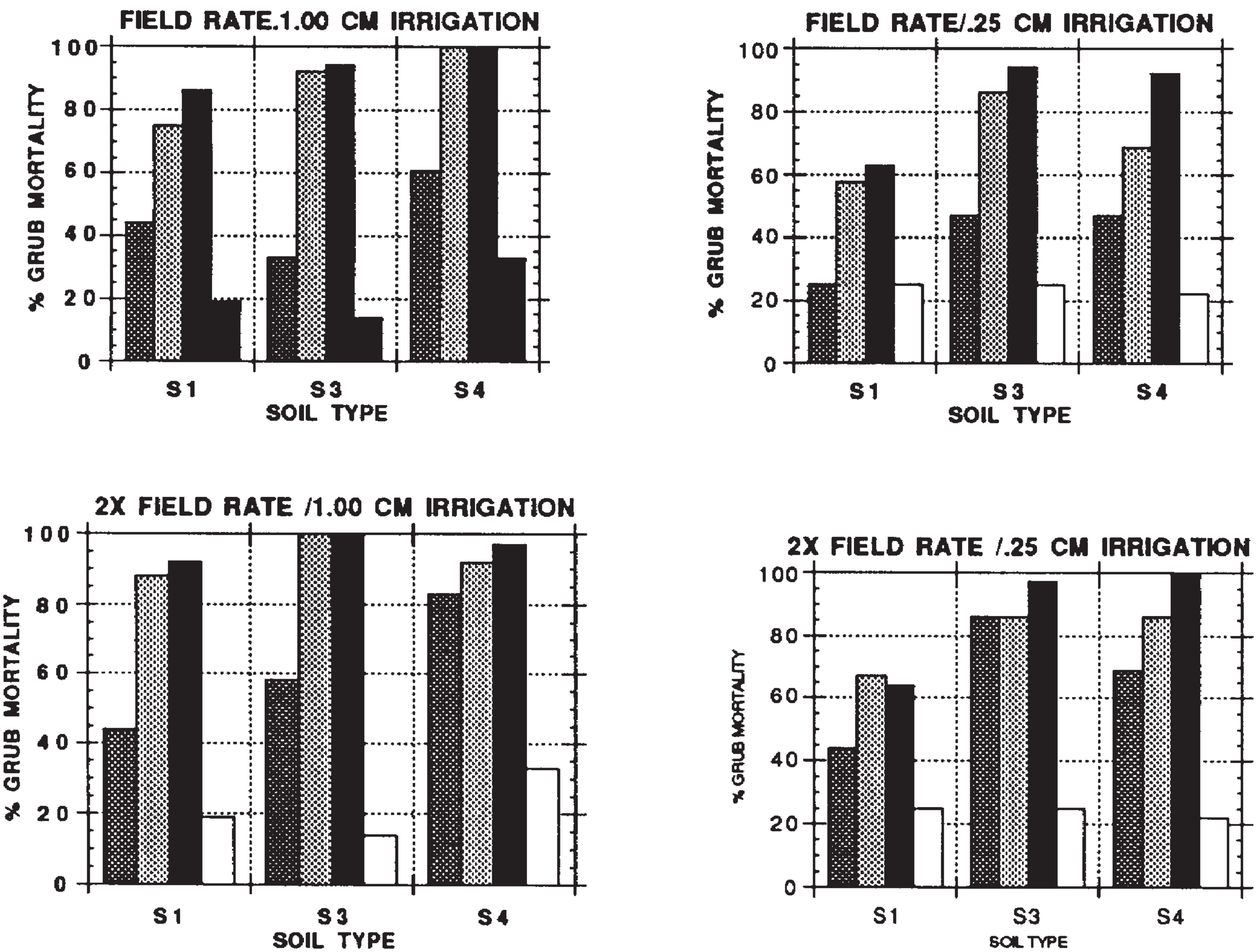
White grub damage



Grubs at different instars can be found in the same area.

Figure 8

Comparison of effects of insecticides and nematode treatments depending on soil type and irrigation



In each set, stippled bars indicate insecticides, solid black bar indicates nematode treatment and empty bar indicates check.