Set-up for white grub suppression greenhouse experiments.

Use of a Biological Stressor to Enhance the Efficacy of Entomopathogenic Nematodes for White Grub Suppression in Turfgrass

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Entomopathogenic nematodes can provide good control of white grubs, but the results are not consistent. *Bacillus thuringiensis* subspecies *japonensis* Buibui strain (Btj) is effective against the early instar of scarabs but not against 3rd instar grubs. Our laboratory studies in the first year of this project have shown that combinations of Btj and nematodes result in additive or synergistic mortality of 3rd instar grubs. To achieve this enhancement, the grubs have to be stressed for at least one week by exposure to Btj before nematodes are applied.

Progress of Research

Several greenhouse pot experiments were conducted with 3rd-instar *C. hirta* or *C. pasadenae* grubs. Treatments in the various experiments consisted of different applications rates of Btj and the entomopathogenic nematodes *Heterorhabditis bacteriophora* or *Steinernema kushidai* and the combination of nematodes species with Btj. The pots (14 cm diam x 11 cm height) were filled with loamy sand to a height of 9 cm and seeded with a ryegrass mixture. After 3-5 weeks, the pots were seeded with 9 grubs/pot and the grubs were allowed to acclimatize for 3 days. Experiments started with a drench application of Btj suspension or water followed 10 days later by a drench application of nematode suspension or water. The pots were destructively sampled 14 days after nematode application and the number of surviving grubs counted.

We also conducted two field ring tests. Rings (25 cm diam x 6 cm height) were driven in the ground in turf areas, 12 3rd-instar grubs/ring were released and given 5 days for acclimatization. Then Btj or water was applied as a drench followed by the nematodes 10 days later. The plots were destructively sampled 3 weeks after nematode application. The first field test was conducted in a turf area on the UC Davis campus area with 3rd-instar *C. hirta*. The second field test was conducted on the Balboa Park golf course in San Diego with 3rd-instar *C. pasadenae*.

The data showed the same trend as last years laboratory data. *C. pasadenae* was more resistant to the nematodes alone than *C. hirta*. Both species, *C. hirta* more so than *C. pasadenae*, were relatively resistant to Btj alone. In the combination treatments, the results with *C. hirta* (Btj concentrations between 0.5 and 10 kg AI/ha) were variable but the general trend was an additive interaction between Btj and both nematodes species. The field trial with *C. hirta* using the nematode *H. bacteriophora* failed because a resident *H. bacteriophora* population that had not been detected in soil samples taken when selecting the experimental site, infected grubs in many replicates throughout the treatments.

The results with *C. pasadenae* (Btj concentrations between 0.1 and 2.5 kg AI/ha) were more promising. In a first greenhouse trial we observed a synergistic interaction. In the field test, we observed additive and weak synergistic interactions between *H. bacteriophora* and various Btj concentrations (0.1-2.5 kg AI/ha). In a second greenhouse trial, the grubs had been collected at a later time and their size was very heterogeneous. We therefore blocked the experiment over grub size, grubs without externally visible fat body (weight 160-430 mg) and grubs with externally visible fat body (weight 570-910 mg). This trial indicated that the younger 3rd-instar grubs are considerably more susceptible to Btj. The trends for the older grubs were similar to the ones observed in the experiments using *C. hirta*; the interactions were mostly additive, although in one combination it was synergistic. For the younger grubs, however, we observed a strong synergistic interaction between the nematodes and Btj at 0.25 kg/ha.

Conclusions

Our greenhouse and field ring test confirm our laboratory observations from 1995/1996. Combinations of entomopathogenic nematodes and Btj provide a higher grub mortality compared to these control agents alone with interaction being mostly additive and sometimes synergistic. The interactions are variable and this may be explained by different factors. First, as one of the greenhouse experiments showed, the already high Btj resistance of 3rd instar *Cyclocephala* grubs further increases as they grow and age. Since we were not aware of this effect before this experiments was evaluated, the average age and size of grubs used in all other experiments might have varied and influenced the results. Second, to sufficiently stress the mostly larger 3rd instar grubs in our experiments, we had to use relatively high Btj rates that are not only uneconomic but may also have had a negative effect on grub feeding and negative effects on the nematodes. We think that the interaction between Btj and nematodes may be stronger and more stable when lower Btj rates can be used against more susceptible grubs, i.e., younger grubs and/or grubs of more susceptible species such as the Japanese beetle.

Whitegrubs, *C. hirta*. Left is healthy, right was killed by entomopathic nematode, *Heterorhabditis bacteriophora.*

Research for 1997/1998

As described in the project proposal, we will conduct a field trial with nematode-Btj combinations in natural *Cyclocephala* populations. Because the high Btj concentrations needed to sufficiently stress older 3rd instar *C. hirta* grubs are not economic and may also have a negative effect on the nematodes, we are planning to conduct this experiment when predominantly younger 3rd instars will be present at the field site. This will allow us to use lower, more economic Btj rates.

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