

Suppression of Landscape Insect Pests Using a Combination of Biological Control Agents

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Objective:

To determine whether combining insect pathogens against two different insect species which are spatially separated in a landscape situation is an effective approach for insect suppression.

Summary of Results:

A number of insect pathogens have been or are being developed as biological control agents of various insect pests in agriculture, forestry, and landscape. Two of the most commercially successful biological control agents are the bacterium, Bacillus thuringiensis subspecies kurstaki (Bt), and entomopathogenic nematodes. Bt is effective against most foliage-feeding caterpillars, but some such as armyworms and cutworms are less susceptible to this bacterium. Many of these armyworms and cutworms feed at or near the soil surface. Entomopathogenic nematodes, such as Steinernema carpocapsae (Sc), S.glaseri (Sg), and Heterorhabditis bacteriophora (Hb) are effective against many soil insect pests, especially weevil and scarab larvae and cutworms and armyworms, and are not effective against foliage-feeding pests.

Recently, it has been demonstrated that these nematodes have different search or attack behaviors. Some entomopathogenic nematode species (e.g., Sc) have a “sit and wait” strategy whereas others have an “active foraging” strategy (e.g., Sg and Hb). In the first case, the nematodes will sit and wait for a mobile host (e.g., caterpillars) to come to them whereas in the second case, the nematodes will actively seek out a sedentary host (e.g., weevil and scarab larvae). Our objectives in this study were to determine (1) whether entomopathogenic nematodes with different search strategies can effectively control two insect pest species occupying different parts of

the soil habitat and (2) whether an entomopathogenic nematode species can be combined with Bt to control a soil insect pest and a foliage-feeding pest.

In our greenhouse study, the combination of two entomopathogenic nematode species with different attack strategies is a feasible control tactic when two pest species occur in different parts of the soil habitat. Sc with its sit and wait strategy was effective against the black cutworm, *Agrotis ipsilon* which feeds at the soil-grass interface, whereas Hb with its foraging strategy was effective against larvae of the black vine weevil, *Otiornchus sulcatus*. Soil temperature influenced the results. Sc was effective against the black cutworms at 16 and 22° C, but Hb was effective against the black vine weevil only at 22° C. (In these tests, 1.1×10^9 nematodes/hectare was used to keep the percentage host mortality below 100%. This rate is equivalent to about 0.5×10^9 nematodes/acre. Recommended rates are $1-2 \times 10^9$ nematodes/acre.) Unfortunately, we encountered great difficulties in conducting the field aspect of this study because the Argentine ant, a voracious predator, consumed the black cutworms that were placed in our testing arena. Several attempts on the UC Davis soccer field and in a private lawn in Davis failed because of the ants. However, we were able to publish the greenhouse part of our study.

In further greenhouse studies, the combination of Bt (1 lb/acre) and Hb (1×10^9 /acre) proved to be an effective means to control a foliage-feeding caterpillar and a root-feeding pest. In the first test, ornamental kale was used as a food source for the larvae of the cabbage looper, *Trichoplusia ni*, and grass roots was used as a food source for the white grub, *Cyclocephala hirta*. (White grubs cannot be reared in the laboratory and must be field collected each year.) Ten cabbage looper larvae and 10 white grubs were included in each test. Four days after treatment, the kale plant was assessed for foliage damage rating (1 = no damage, 2=10-25% damage, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100% damage) independently by two individuals and averaged. Dead cabbage looper larvae were collected and examined for Bt or Hb infection. White grubs were collected from the soil, washed in water, and held in clean soil for 48 hours. Figure 1A shows the results of this test

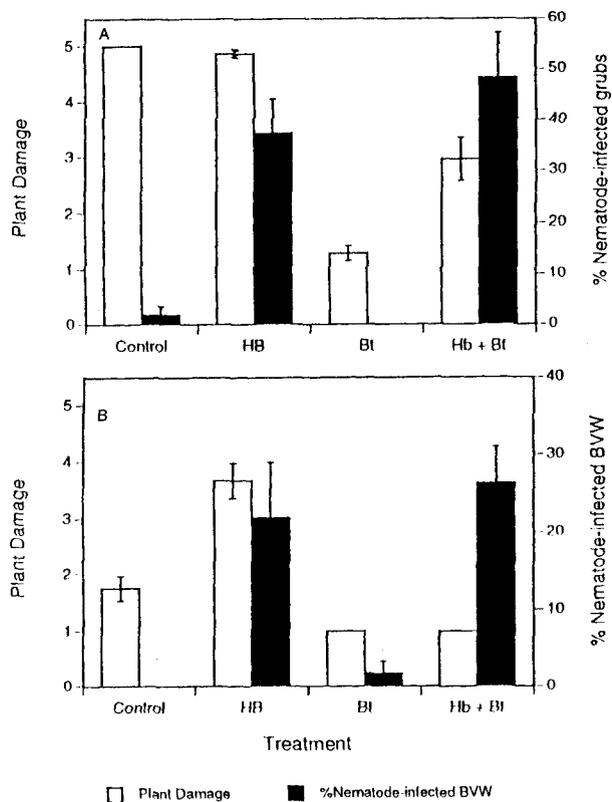


Figure 1. Suppression of the foliage-feeding pest on ornamental kale and a soil-inhabiting pest with the combination of the bacterium, *Bacillus thuringiensis* (Bt), and the entomopathogenic nematode, *Heterorhabditis bacteriophora* (Hb). A. The control of the cabbage looper on foliage and the white grub. B. The control of the cabbage looper and the black vine weevil (BVW).

where the combination of Bt and Hb provided foliage protection and white grub suppression. Bt alone provided foliage protection but no grub control and Hb alone provided grub control but no foliage protection. The control treatment provided neither grub control nor foliage protection. One grub was infected with an unknown *Heterorhabditis* species, but this grub was probably infected in the field. In the Bt treatments, all cabbage looper larvae recovered were killed by Bt and no live larvae were recovered. None of the cabbage looper larvae were killed by Hb. In the Hb treatments, all dead white grubs were killed by Hb and none by Bt. The mortality of white grubs was low, but only 4 days were allowed to elapse before the treatment was assessed.

In the second test, larvae of the black vine weevil were used as the root-feeding pest. The results were similar as the first test (Figure 1B) except that the degree of defoliation in the control was not as

extensive and the Hb treatments had a lower percentage of infection. In the Bt alone treatment, one black vine weevil larva was found infected with a heterorhabditid species. The black vine weevils were collected from the field and may have been infected by the heterorhabditid.

The third test was the same as the first test except that Bt and Sg were used. The Bt was effective against the cabbage looper larvae, but Sg was ineffective against *C. hirta*. The reason(s) for this failure is unknown because the Sg was active and infected the laboratory host larva. We speculate that some antagonist of Sg was present in the soil that adversely affected the nematode.

The compatibility of entomopathogenic nematodes with commercial formulations of Bt varied depending on how the nematodes were treated. Sc proved to be a hardy nematode and survived in two different commercial Bt formulations (a wettable granule and a liquid) for up to 4 days whether the holding container was placed on a shaker (to provide aeration) or left standing on the laboratory bench. However, Hb and Sg were adversely affected by the commercial Bt formulations and high nematode mortality were observed after 3 days when the combinations were left standing on a laboratory bench. In contrast, when the Bt-nematode suspension was provided aeration on a shaker, Hb and Sg survived very well for 4 days. Figure 2 shows the data for Hb that were shaken (aerated) and not shaken (not aerated). These nematodes maintained their infectivity against a laboratory hosts. Nematodes in unformulated Bt or without Bt survived very well with aeration on a shaker or on the laboratory bench.

In summary, we have demonstrated that the combination of two nematode species with different attack strategies against two pest species occupying separate parts of the soil habitat can be effectively controlled. Greenhouse studies showed that low soil temperature can influence the results, but the use of the recommended nematode rate and a longer evaluation time should provide effective control.

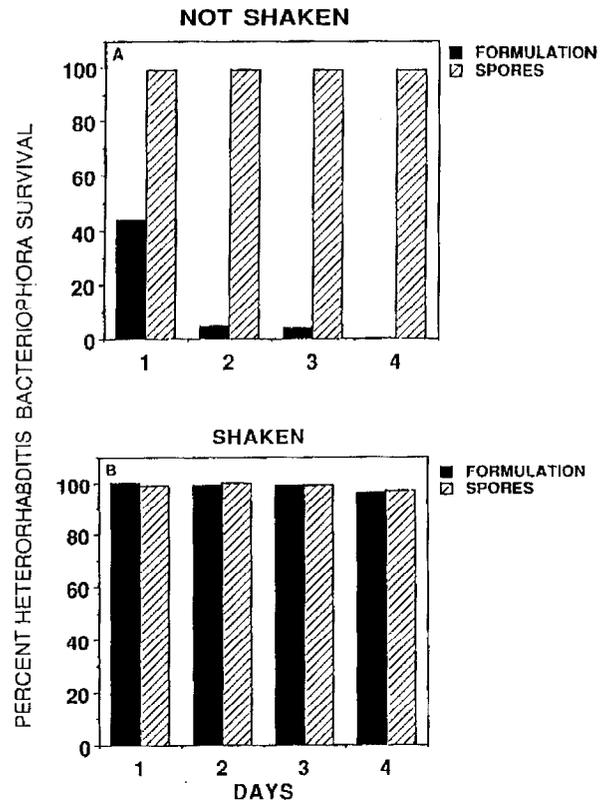


Figure 2. The effect of storage of the entomopathogenic nematode, *Heterorhabditis bacteriophora*, in a wettable granule formulation of *Bacillus thuringiensis* compared with storage in a suspension of *B. thuringiensis* spores. A. *H. bacteriophora* was not shaken (not aerated). Note the poor survival of the nematode in the formulated suspension versus the spores only suspension. B. *H. bacteriophora* was shaken (aerated). Note the survival of the nematode in both formulated and spores only suspensions.

Although we were unable to conduct the test in the field because of the Argentine ant, this two nematode species combination should be effective. We will pursue this aspect to demonstrate field effectiveness. The Bt-Hb combination also proved to be effective against a foliage-feeding caterpillar and against a soil-inhabiting pest. A higher rate of Hb should provide more efficacious results against the soil pest. However, at this time, the use of Sg in combination with Bt is discouraged. The combination of Hb or Sg and commercial formulations of Bt should be used immediately and prolonged storage of this combination is not recommended.

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