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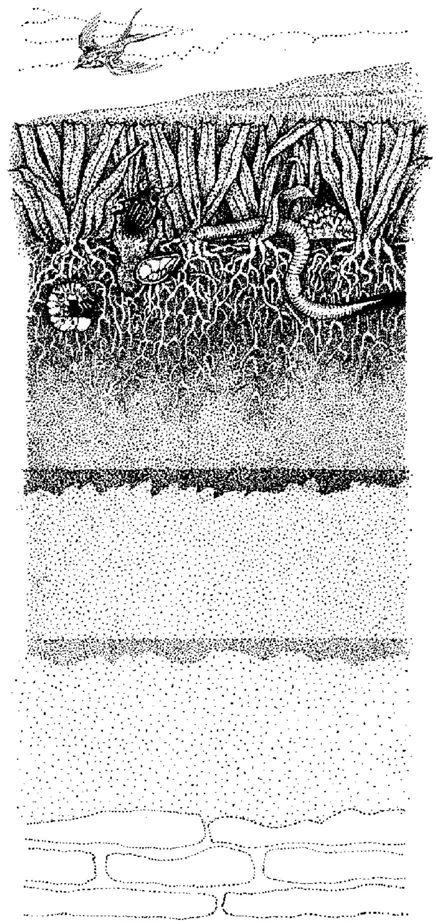

This Times

Biorational Control Agents for Japanese Beetle Management

The development of specialty pest management products often takes a back seat to products that targeted pests of crops such as field corn, soybeans and cotton. Products that were not effective against one or more insect pests on these major commodities had little chance to be tested against insects particular to turfgrass. Although the crop protection industry has focused greater attention on so-called specialty markets such as turfgrass, funding from New York State Turfgrass Association (NYSTA), NYS IPM, and TriState Turfgrass Research Foundation was essential for providing a nonbiased evaluation of these products. Following is a brief review of some of these products together with the results of representative laboratory and field studies conducted by my research team over the past several years. It is important to remember that these studies were conducted under ideal conditions with regards to proper timing, equipment calibration, quality of control agent, and proper environmental conditions. ■

Bacillus thuringiensis

Bacillus thuringiensis (Bt) is a soil living bacterium common in nature that was first discovered in Japan in 1901. There are over 30 subspecies and varieties of Bt. This family of bacteria produces a toxic protein crystal that is active against a fairly narrow group of insect species. Certain Bt products have been used to control insects for many years. Various strains have been identified with activity against caterpillars, fly larvae, and beetle larvae. Current commercial Bt varieties include Bt *kurstaki* and Bt *aizawai* (caterpillars), Bt *israelensis* (mosquitoes), and Bt *tenbrionis* (potato beetles).

Because Bt bacteria can be produced in great quantities using artificial media, there is great interest in the commercialization of this bacteria for insect control. The bacteria normally do not reproduce in the insect host, persist

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Biorational Agents

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in the environment, or spread from the treatment site; for these reasons Bt typically has been used as a microbial insecticide for short term-control.

How Bt kills insects (mode of action) is fairly well understood. First, because it acts as a stomach poison, the toxic protein crystals must be eaten by an insect to be effective. In some cases, additional products produced by the living bacteria must be consumed for maximal activity. If the insect's gut content is of the proper acidity (pH) the crystals will dissolve in the insect's gut. Proteins released from the dissolved crystal binds to specific sites in the gut lining of susceptible insect species causing rapid paralysis of the gut. The major reasons for the lack of activity of a Bt strain against an insect species are first, improper gut pH that does not allow the toxic protein crystal to dissolve, and second, the inability of the toxic proteins to bind to the insect's gut. Almost immediately the insect stops feeding as the gut wall deteriorates. In most cases a susceptible insect will die 2-7 days after ingestion of the Bt toxin.

Although Bt products are registered for use against several turf-feeding caterpillars, including cutworms and sod webworms, these products have not been widely recommended or accepted in the turf industry, most probably due to their short residual, slow activity and inability to kill larger larvae.

Until recently there were no Bt varieties known to cause significant mortality in scarab grubs inhabiting turfgrass. In 1991, Bt variety *japanensis* strain *BuiBui* was discovered in Japan. Development and commercialization of Bt *BuiBui* has been undertaken by the biotechnology company Mycogen of San Diego CA, who expects registration of the product against certain scarab grubs in 1996, if formulation problems and consistent activity at competitive field rates can be addressed. Bt *BuiBui* has a spherical protein crystal that is toxic to certain kinds of scarab grubs. Unlike most commercial Bt products currently on the market, maximal activity of Bt *BuiBui* against scarab grubs occurs when formulations include both the toxic protein crystals and live spores produced by the bacteria. According to Mycogen, Bt *BuiBui* is nontoxic to all vertebrates, earthworms, honeybees, and plants.

Japanese beetle, Oriental beetle, northern and southern masked chafer, and green June beetle grubs have all been shown to be highly susceptible to Bt *BuiBui* in laboratory and field studies while June beetle, black turfgrass Ataenius beetle and Aphodius beetle grubs appear much less sensitive to this product. The results of four small plot field studies conducted in New York State that focused on the activity of this Bt against Japanese beetle grubs are outlined in Table 1. These results suggest a highly active

Table 1. Results of small plot field studies conducted in New York focusing on the activity of Bt against Japanese beetle grubs.

Rate (kg a. i./ha)	Mean Grubs/Ft²	Percent Control
1993 Marcellus, NY 2nd/3rd Instar Treated		
Control	12.4	—
36.4	1.33	89.3
72.9	0.58	95.3
145.8	1.67	86.6
1994 Horseheads, NY 1st/2nd Instar Treated		
Control	28.1	—
16.2	1.40	95.0
32.3	1.20	96.0
64.6	1.50	95.0
1995 Marcellus, NY 1st/2nd Instar Treated		
Control	12.5	—
2.9	4.5	64.0
5.9	0.6	95.0
11.8	0.3	98.0
23.5	0.0	100.0
1995 Schenectady, NY 3rd Instar Treated		
Control	33.8	—
2.9	17.3	49.0
5.9	26.0	23.0
11.8	17.8	47.0

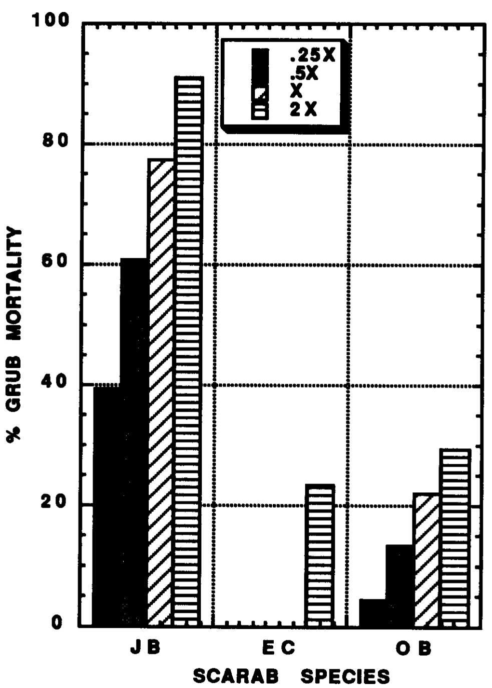
field product that appears to be more effective against earlier instars at lower field rates.

Insect Growth Regulators

Several chemical companies are developing novel classes of compounds with unique modes of action that interfere with the normal insect molting process by mimicking the action of the natural insect molting hormone ecdysone. High doses of these Insect Growth Regulator (IGR) products typically cause rapid insect mortality, while sublethal effects include rapid maturation to the adult stage, larvae showing deformities, and larvae undergoing additional larval molts instead of changing to pupa. Specific IGR products have shown activity against scarab grubs, cutworms and sod webworms.

Insect growth regulators typically require ingestion for optimum activity so it is important that the target insect is actively feeding when they are applied. The use of an IGR on scarab grub populations late in the fall as they prepare to move down into the soil for winter as well as the application to grubs in late spring as they prepare to pupate is ill advised for this reason. Laboratory and field studies indicate that early larval stages are susceptible to IGRs and also suggests that there is a fairly wide range of activity among closely related insects such as different species of scarab grubs (see Figure 1).

Figure 1. Species-Specific Activity of Insect Growth Regulator Against 3rd Instar Scarab Grubs.



Our studies have shown that one IGR shows truly impressive activity against Japanese beetle grubs but much less dramatic activity against either European chafer or Oriental beetle grubs. The specificity of these products demands that turfgrass managers determine which insect species is present, to avoid disappointing results.

The results of four small plot field studies conducted in New York State that focused on the activity of the experimental IGR, 0345, jointly developed by American Cyanamid and Rohm & Haas against Japanese beetle grubs are outlined in Table 2. These results suggest a highly active field product that appears to be more effective against earlier instars at lower field rates, as indicated in the two 1995 studies.

Our studies have shown that one IGR shows truly impressive activity against Japanese beetle grubs.

Table 2. Results of small plot field studies conducted in New York focusing on the activity of IGR 0345 against Japanese beetle grubs.

Formulation	Rate (kg a. i./ha)	Mean Grubs/Ft[2]	Percent Control
1994 Horseheads, NY 1st/2nd Instar Treated			
Control		28.1	—
2.5G (A)	1.5 lb AI/A	2.80	90.0
2.5G (A)	3.0 lb AI/A	0.50	98.2
2.5G (B)	1.5 lb AI/A	8.10	71.2
2.5G (B)	3.0 lb AI/A	4.40	84.3
2 (F)	1.5 lb AI/A	1.10	96.1
2 (F)	3.0 lb AI/A	0.00	100.0
1995 Marcellus, NY 1st/2nd Instar Treated			
Formulation	Rate (kg a. i./ha)	Mean Grubs/Ft[2]	Percent Control
Control		12.5	—
SC	1.5 lb AI/A	0.30	98.0
SC	2.0 lb AI/A	0.10	99.0
2.5 (G)	1.0 lb AI/A	0.90	93.0
2.5 (G)	1.5 lb AI/A	0.00	100.0
2.5 (G)	2.0 lb AI/A	0.50	96.0
5 (G)	1.0 lb AI/A	0.90	93.0
5 (G)	1.5 lb AI/A	2.00	84.0
5 (G)	2.0 lb AI/A	0.60	95.0
1995 Schenectady, NY 3rd Instar Treated			
Formulation	Rate (kg a. i./ha)	Mean Grubs/Ft[2]	Percent Control
Control		33.8	—
SC	1.5 lb AI/A	5.30	84.4
SC	2.0 lb AI/A	2.00	94.1
2.5 (G)	1.0 lb AI/A	9.30	72.6
2.5 (G)	1.5 lb AI/A	1.50	95.6
2.5 (G)	2.0 lb AI/A	1.30	96.3
5 (G)	1.0 lb AI/A	9.30	72.6
5 (G)	1.5 lb AI/A	3.30	90.4
5 (G)	2.0 lb AI/A	9.30	72.6

Entomogenous Nematodes

Entomogenous nematodes recently have received attention as alternatives to insecticides for turf insect control. There are many factors, in theory, that make nematodes the ideal microbial control agent: they have a broad host range, will not attack plants or vertebrates, are easy to mass produce, and can be applied with most standard

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Biorational Agents

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insecticide application equipment. Additionally, nematodes can search their hosts and kill them rapidly, multiply within the host and within several weeks release thousands of mobile progeny able to locate and infect new insect hosts. Because they are considered predators and not microbial insecticides, entomogenous nematodes are exempted from registration by the U.S. Environmental Protection Agency. This exemption from long term safety and water quality studies greatly reduced the costs and risks typically associated with bringing a new insecticide to the market .

Entomogenous nematodes enter through natural openings of the insect, most commonly the mouth or less commonly, through skin. They then move through the gut of the insect into the blood where they release a colony bacteria that lived within the nematode's body. Once inside the insect, the bacteria multiplies and produces toxins that rapidly kill the infected insect. Nematodes feed on bacteria and reproduce inside dead insects producing several thousand new nematodes. Under ideal conditions thousands of new infective nematodes will escape from the dead insect in as little as ten days and begin to search for new hosts to infect.

Table 3. Results of small plot field studies conducted in New York focusing on the activity of nematodes against white grubs.

Species	Rate	Mean Grubs/Ft^2
1993 Marcellus, NY 2nd/3rd Instar Treated		
Control		12.4
S. g.	0.5 B/A	4.70
S. c.	2.0 B/A	9.10
H. b.	1.0 B/A	6.30
1994 Horseheads, NY 1st/2nd Instar Treated		
Control		28.1
H. b. (O)	0.5 B/A	19.75
H. b. (O)	1.0 B/A	6.38
1995 Marcellus, NY 1st/2nd Instar Treated		
Control		12.5
H. b. (O)	1.0 B/A	1.60
Bt	2.9 kg a.i./ha	4.50
H. b. (O) + Bt	1.0 B/A + 2.9 kg a.i./ha	0.80

KEY: S. g.=*Steinemema glaseri;* S. c.=*Steinemema carpocapsae;* H. b.=*Heterorhabditis bacteriophora; O=Oswego strain*

Although there have been many successful field applications of entomogenous nematodes for turf insect control, problems with product quality, persistence and host specificity have led to unsatisfactory results is some instances. Although entomogenous nematodes generally are considered to have fairly broad host ranges in laboratory studies, different strains and species of nematodes vary in activity against different insect species in the field. Overall, the nematodes *Heterorhabditis bacteriophora (H. b.)* and *Steinernema glaseri (S. g.)* are more effective against white grubs than the more commonly marketed *Steinernema carpocapsae*, because the former two nematode species actively move down in the soil profile and search for insects (see Table 3). Conversely, *Steinernema carpocapsae*, has been effective for control of billbugs and caterpillars such as cutworms, webworms and armyworms.

Many unsuccessful field applications of entomogenous nematodes for scarab grub control can be traced to improper environmental conditions at the time of application and for several weeks post application. Nematodes are extremely sensitive to exposure to ultraviolet light and will last only a matter of minutes when exposed to full sunlight. They are also quite prone to desiccation requiring high relative humidity and a film of moisture on leaf and soil surfaces to survive and move. For these reasons nematodes should be applied very early in the morning or late in the day and be immediately irrigated with at least one half inch of water. Nematodes searching for grubs move over soil particles on thin water films and will not search efficiently in saturated soils. The inability to provide adequate soil moisture for several weeks post application limits the utility of nematodes in many situations.

One mistake often made by turfgrass managers is to save money by applying nematodes at rates lower than recommended. Extensive field studies on New York golf courses over the past five years confirm that there is a strong positive correlation between the number of nematodes applied and grub mortality (see Table 3 for 1994 data).

Although often treated as an insecticide, entomogenous nematodes are living organisms that must be treated with care to be effective. Only nematodes in the best condition will be able to successfully search for an insect host, overcome the defenses of the insect to infect and kill it, and multiply within the dead insect producing progeny to infect new hosts. As an example, excellent field results were obtained during 1995 using Hb nematodes (Oswego strain) that were reared in my laboratory (see Table 3). To maximize the probability of applying healthy nematodes it is critical that label directions related to nematode storage, application and rates are followed carefully.

Combinations of biological control agents may impart additive or synergistic mortality on grub populations. This may occur if the two agents attack different subpopulations of the pest infestation (i.e. grubs feeding at different depths in the soil or feeding vs. nonfeeding grubs). It may also occur if the one control agent stresses or predisposes an insect to a second agent. In 1994 we reported the synergistic interactions of fungal pathogens and a synthetic insecticide. During this past year we explored the possible interaction of Bt and the Hb nematode in the lab and in the field. Laboratory bioassays showed no activity of Bt against Japanese beetle grubs at the lowest field rate tested during 1995. Hb (Oswego) applied at an equivalent of 0.5 billion nematodes/A resulted in a 43% population reduction four weeks post treatment. The combination of Bt and Hb at the stated rates resulted in increased mortality of grubs for all dates evaluated (see Figure 2).

Fungal Pathogens

Virtually every group of turfgrass insect pests, including scarab grubs, chinch bugs, billbugs, annual bluegrass weevils, sod webworms, cutworms, and mole crickets, are susceptible to endemic populations of fungal pathogens such as *Beauveria bassiana* and *Metarhizium anisopliae.* Commercial interest in fungal pathogens as biological control agents have ebbed and flowed over the last decade as the promising results seen in laboratory and greenhouse tests have collided with the hard realities of producing an effective, consistent, price competitive and safe commercial field product. At present, there are no commercial fungal products available for management of turf pests.

Fungi differ from most other microorganisms because they do not have to be ingested to be effective. Infection is initiated by the adhesion of a fungal spore to the body of an insect. If conditions are correct, the spore will germinate and a tube will grow from the spore into the insect penetrating the circulatory system. After penetration, the fungus reproduces within the insect producing toxins that quickly kill the insect. After death of the host, hyphae emerge from the insect and develop into structures that produce more infective spores. These spores can then be spread through the environment infecting additional insects. Studies 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 isolates of *Metarhizium anisopliae* 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, have generated significant interest 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 were considerable differences in how well several fungal isolates performed under a variety of environmental conditions commonly found in northeastern golf courses (see Figure 3).

Fungal pathogens may also cause mortality of adult Japanese beetles. In a laboratory bioassay mating pairs of Japanese beetles were placed in a plexiglas chamber that contained grape leaves and soil for females to lay eggs. In one third of the chambers the *Metarhizium* isolate 2547 was incorporated into the soil, in the second third of the chambers soil without fungus was used, and in the last third of the chambers beetles had the choice of treated and untreated soil. As can be seen in Figure 4, high mortality was observed in both fungus containing treatments with male mortality lagging behind females in both treatments. This occurred because males do not contact the fungus directly in the soil, but through indirect contact as they mate with infected females.

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Combinations of biological control agents may impart additive or synergistic mortality on grub populations.

Figure 2. Interaction of Bt and Entomogenous Nematode Hb (Oswego).

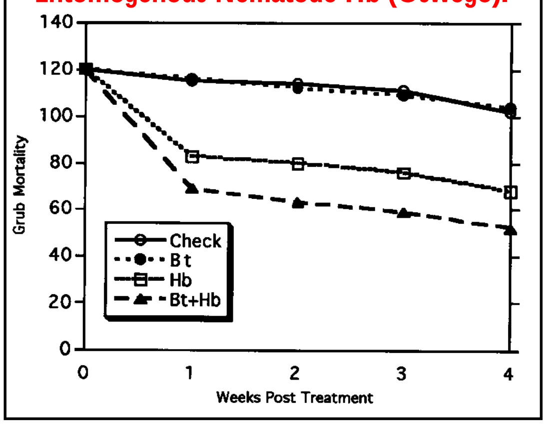

Figure 3. Comparative Activity of *Metarhizium* Isolates Against Japanese Beetle Grubs.

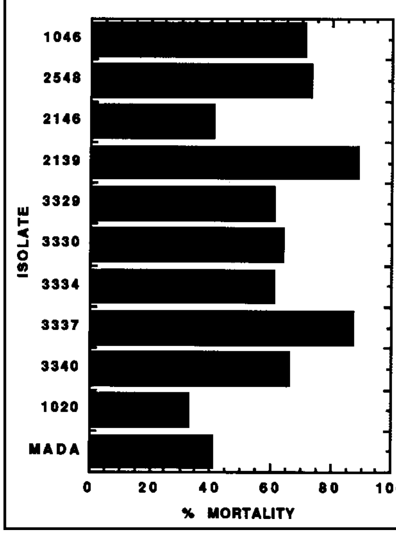

Figure 4. Japanese Beetle Oviposition Study, Adult Mortality.