TURFGRASS TRENDS

BIOLOGICAL PESTICIDES

Scientists Pursue Biological Control for Turfgrass Pests

By Sarah R. Thompson and Rick L. Brandenburg

No one will deny the pressures often placed on turfgrass managers to achieve perfection. Placing a dollar figure on the aesthetic quality of turfgrasses is difficult, and damage thresholds are usually low. As a result, many turfgrass managers rely on conventional pesticides. These pesticides, although effective, are often costly and require caution during application because of their chemical nature. By definition, pesticides are meant to kill the targeted pest. Unfortunately, some insecticides have the potential to cause deleterious effects to nontarget arthropods, wildlife or even humans.

Recently, the public has become more vocal over the potential risks involved with pesticide use and called for change. In Canada, for example, numerous cities will soon decide if pesticides should be banned when applied solely for cosmetic purposes. Isolated communities in the United States have followed suit.

Never before have turfgrass managers been asked to reduce or eliminate their pesticide choices more than now.

Those who support these bans feel chemicals should only be applied when human or animal health is at risk. Overall, societal concerns have increased because of greater awareness of pesticide use, combined with increased media coverage of the occasional problems.

Turfgrass managers are also plagued by the restricted use and loss of many of the older classes of chemicals. These products often provided broad-spectrum control quickly for little expense. The broad-spectrum nature of these chemicals posed problems for off-target organisms, and some of them contaminated groundwater sources. The newer products are typically more expensive (due to the long and rigorous research-and-development processes), may not have as long of a residual period and have a much narrower control spectrum.

Never before have turfgrass managers been asked to reduce or eliminate their pesticide choices more than now. As a result, all of the above factors support the need for research on biological control, which means using living organisms for pest management.

A number of biological control agents are available for insect management in turfgrass, including bacteria, fungi, nematodes and others. Historically, many of these agents showed promise under controlled laboratory conditions, but have failed to deliver in the field.

At North Carolina State University, we feel our resources are well spent exploring the factors that contribute to the lack of success of biological control agents in turfgrass. By determining why some biological control agents have not been successful in the field, we hope the limitations can be overcome. One area of recent research has focused on the evaluation of an entomopathogenic fungus, *Beauveria bassiana*, for mole cricket control.

Mole crickets are extremely damaging pests of turfgrasses in the southeastern United

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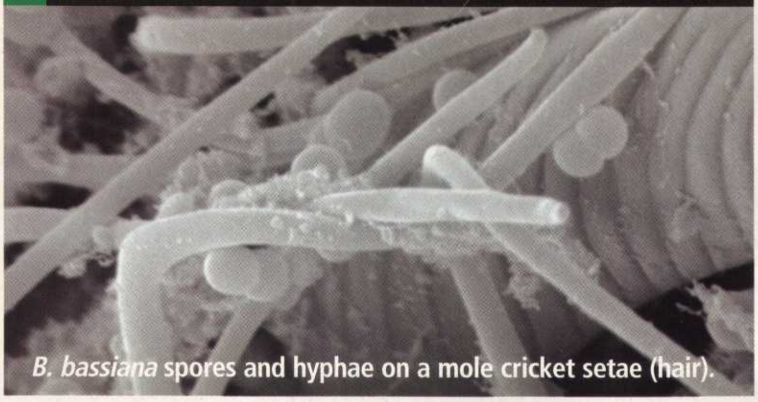
FIGURE 1



B. bassiana spores and hyphae on a mole cricket setae (hair).

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States. There are two major species of concern, introduced from South America around the turn of the 20th century (Walker and Nickle, 1981). Damage is caused when the crickets feed on the roots of turfgrass and produce surface tunnels. The root feeding can lead to total plant loss and eventual weed invasion. The production of surface tunnels and large megaphone-shaped calling chambers (in the spring) are unsightly, but also lead to desiccation and plant stress.

Most superintendents and sod farmers in the Southeast will agree that mole crickets are their No. 1 insect pest. We feel they make a great model insect for our studies because they are so large, mobile and damaging.

A number of studies conducted since 2001 explore the factors that may contribute to the disappointing results seen with entomopathogenic fungi in the field. *Beauveria bassiana* kills insects when the spores attach to the insect cuticle (Figure 1), penetrate into the body cavity and proliferate within the pest's body. Mortality results from toxemia from fungal metabolites or when the insect becomes depleted of nutrients (Jaronski and Goettel, 1997). Eventually, the fungus exits the cadaver and produces additional spores, capable of infecting other nearby crickets (Figure 2). Based on this mode of action, the spores need to come into contact with the pest for infection to occur.

There are a number of factors that may interfere with this necessary contact, including the efficacy of different strains towards the pest of interest, whether or not the spores can remain viable under harsh environmental conditions and any avoidance behaviors that may be exhibited by the pest. We examined these three factors in laboratory, field and greenhouse studies during 2001, 2002 and 2003.

Efficacy studies

Adult mole crickets were exposed to three different strains of *B. bassiana* in a topical bioassay. Each strain was originally isolated from a different host (darkling beetle, corn borer and grasshopper). This is important point because of the common belief that the most efficacious strain for a particular pest will be one isolated from that insect or another closely related species (Xu, 1988).

In our case, the grasshopper strain was isolated from its most closely related species, since mole crickets and grasshoppers are in the order *Orthoptera*.

Adult mole crickets were exposed to four different rates of each strain by coating them with a liquid/spore suspension. After treatment, the crickets were placed individually into separate containers and were examined daily for mortality. Cadavers were incubated at an optimal temperature for fungal growth and observed (Figure 2). Results from these studies support the theory concerning efficacy of original host strains since the grasshopper strain killed the most crickets in the shortest amount of time.

Current studies are examining the effects of pretreating mole crickets with sublethal doses of conventional and organic insecticides to test for synergies. Specifically, the crickets are being pretreated with imidacloprid and diatomaceous earth since literature suggests synergies between *B. bassiana* and these agents. Additionally, all strains currently being tested are isolated from *Orthoptera* and one specifically from a tawny mole cricket.

Spore viability studies

Another factor that determines the success of entomopathogenic fungi is the ability of the spores to remain viable in the field until contact occurs. Traditionally, viability studies have been conducted by plating spores on a growth agar, incubating the petri dishes for 24 hours or less and examining the spores under a microscope to determine the percentage germination (Schading et al, 1995).

In our studies, we used a different type of evaluation technique, since germination is not necessarily a measure of spore viability. During the summers of 2002 and 2003 at two different coastal North Carolina sites, we applied the spores in an emulsifiable oil formulation to the surface of bermudagrass plots. Post-application irrigation was applied to wash the spores into the thatch and soil.

At various dates after treatment (one, two,

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three, 14, 21 and 28 days), cup-cutter samples were taken, and we washed the spores from the sod samples. We exposed the spore suspension to a nucleic acid stain, which labels cells green or red (for viable and nonviable, respectively) when illuminated by fluorescent light (Schading et al 1995).

Data show there are significant differences between each strain in their ability to remain viable in the environment. Overall, spores from all three strains were able to persist and remain viable (although at a small percentage) for up to a month after treatment (Figures 3 and 4). There were no significant differences between the darkling beetle (DB-2) and corn borer (10-22) strains, but a greater percentage of spores from both remained at various dates after treatment compared to the grasshopper strain (BotaniGard). These studies give hope for the use of *B. bassiana* as a biological control agent in turfgrass since the spores are able to maintain viability over time. Most likely, practical usage of *B. bassiana* in the field would still require multiple treatments.

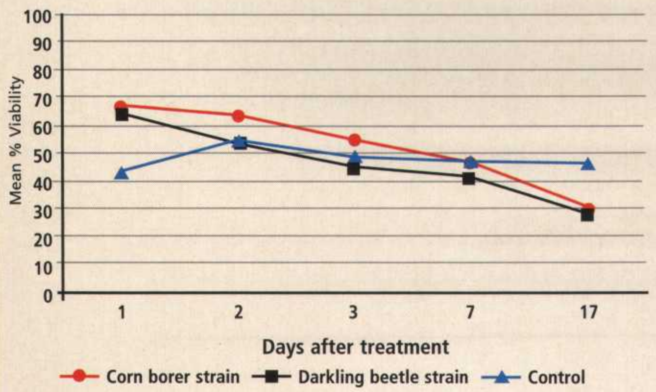
The objective of future research is to determine the most important environmental condition contributing to spore mortality. Studies will take place in the summers of 2004 and 2005, looking specifically at the effects of irrigation (available water) and ultraviolet (UV) degradation on spore viability. If this information is known, suggestions can be made on the best time for application, proper irrigation schedules and formulation choice for field treatments.

FIGURE 2

Mole cricket sporulating with *Beauveria bassiana.*

FIGURE 3

Mean percentage spore viability in 2002 field study (Sea Trails Plantation in Sunset Beach, N.C.) 1, 2, 3, 7 and 17 days after treatment.



Behavioral tests

For the critical contact to occur, the target pest needs to be incapable of detecting and avoiding the fungal spores.

We are all familiar with cases of repellency that exist between pests and chemical insecticides, but whether these same avoidance behaviors occur with biological control agents is something that we wished to explore. Previous work at Cornell University (Villani et al., 2002) has suggested that mole crickets are capable of detecting and avoiding contact with fungal spores of *B. bassiana* and *Metarhizium anisopliae* (another entomopathogenic fungus). Research was conducted in the fall and winter of 2001, 2002 and 2003 to quantify and classify these avoidance behaviors. Additionally, we wanted to determine if repellency existed for all strains of *B. bassiana* or if avoidance was a strain-specific occurrence.

Individual mole crickets were placed into large plastic containers filled with moist sand. On the sand surface, *B. bassiana* spores were sprayed and not watered in to create a spore layer. On top of this treated layer, an additional 2 inches of soil was added and smoothed flat. Bermudagrass sod pieces were added to the top of the new sand layer to serve as a food source (Figure 5). This setup encouraged the mole crickets to tunnel through the spore layer to reach the sod food source.

Various behaviors were measured after 24 hours to determine if the mole crickets were attempting to avoid contact with the fungus. By removing the sod, observations were made on the presence of 24-hour surface tunneling (confirming passage through the spore layer), the type of tunnels that were produced (horizontal vs. vertical

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FIGURE 4

Mean percentage spore viability in 2003 field study (Tri-State Sod Farm in Newton Grove, N.C.) 1, 3, 7, 14, 21 and 28 days after treatment.

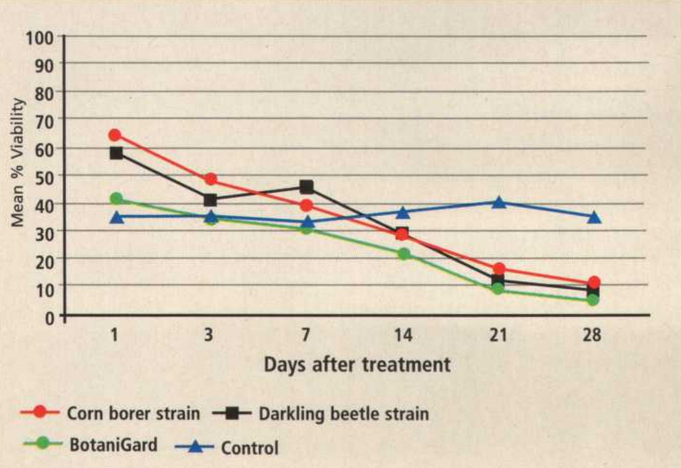
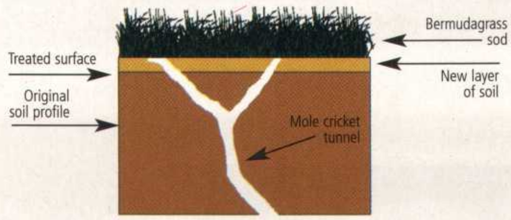


FIGURE 5



Design of mole cricket behavioral tests.

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tunnels), tunneling around the edges and the location of the cricket. Less vertical tunneling indicated that the crickets attempted to reduce exposure to the spores by minimizing the number of times that they passed through the spore layer. Little or no surface tunneling through the concentrated area of spores around the edges of the container also indicated an avoidance response. Additionally, finding the cricket in the top (read: new) layer of soil showed an attempt to reduce passages through the spore layer. The amount of new surface tunneling was also quantified.

Results from these studies suggest that mole crickets can detect the presence of *B. bassiana* and alter their behavior to minimize contact and infection. The changes in behavior were only significant with the darkling beetle strain and a conventional insecticide, bifenthrin. The corn borer strain invoked less-significant avoidance behaviors, while the grasshopper strain appeared to increase mole cricket activity.

Future research at North Carolina State includes an investigation of the influence of rates, formulation (baits), and time on mole cricket avoidance of biological control agents. Because the avoidance behaviors, like the spore viability and efficacy studies, appear to be strain-specific, we are optimistic that a strain, which is virulent, persists in the environment and remains undetected by the crickets will be isolated.

Conclusions

Studies like ours involving biological control agents for turfgrass insects are important to help tear down the barriers that exist with using these products in the field.

As societal concerns and environmental protection laws continue to increase, it's critical that we are prepared to offer alternatives for pest control in turfgrass that are reliable, practical, safe and cost-effective. Although many biological agents are not yet ready to put into use today, we are hopeful, based on our studies, that they may be a viable option in the near future.

Thompson is a graduate research assistant, and Brandenburg is a turfgrass entomologist at North Carolina State University in Raleigh.

REFERENCES

Jaronski, S.T. and M.S. Goettel. 1997. "Development of *Beauveria bassiana* for control of grasshoppers and locusts." *Mem. Entomol. Soc. Can.* 171: 225-237.

Schading, R.L., R.I. Carruthers, and B.A. Mullin-Schading. 1995. "Rapid determination of conidial viability for entomopathogenic hyphomycetes using fluorescence microscopy techniques." *Biocon. Sci. and Tech.* 5: 201-208.

Villani, M.G., L.L. Allee, L. Preston-Wilsey, N. Consolie, Y. Xia, and R.L. Brandenburg. 2002. "Use of radiography and tunnel castings for observing mole cricket (Orthoptera: Gryllotalpidae) behavior in soil." *Am. Entomol.* 48(1): 42-50.

Walker, T.J. and D.A. Nickle. 1981. "Introduction and spread of pest mole crickets: *Scapteriscus vicinus* and *S. acletus* reexamined." *Ann. Entomol. Soc. Am.* 74: 158-163.

Xu, Q.F. 1988. "Some problems about study and application of *Beauveria bassiana* against agricultural and forest pests in China," pp. 1-9. *In* Y.W. Li, Z.Z. Li, Z.Q. Liang, J.W. Wu, Z.K. Wu, and Q.F. Xu (eds.), *Study and Application of Entomogenous Fungi in China*, Vol. 1. Academic Periodical Press, Beijing, China.