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Scientists at the University of Kentucky continue to explore biological control options for insect pests of golf courses. Their studies involving a baculovirus indicates that this method of biological control of black cutworms is better suited for targeted knock-down of small cutworms than for season-long residual control that would require several applications of the baculovirus throughout the growing season.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 400 projects at a cost of $31 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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USGA Turfgrass and Environmental Research Committee

Biological Control of Black Cutworms Using a Virus

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SUMMARY

Scientists at the University of Kentucky continue to explore biological control options for insect pests of golf courses. They evaluated a naturally-occurring baculovirus, *Agrotis ipsilon* multiple nucleopolyhedrovirus (AgipMNPV), as a potential biological insecticide for black cutworms (*Agrotis ipsilon*). Because the virus isn't commercially available yet, thousands of already-infected cutworms containing millions of virus particles were ground up, strained, and diluted to make a liquid suspension that was sprayed on the turf. Their findings include:

- One week after a September application, 50-60% of mid-sized introduced cutworms became lethally infected.
- In another trial done in summer, 3-day-old virus residues gave 50–60% control on a sand-based putting green and its surrounds, but no residual control after 2 or 4 weeks.
- Using an optical brightener or lignin, a natural plant pollymer, to the spray mix did not synergize or prolong infectivity of suspension sprays.
- In trials conducted on whole tees and surrounds at two central Kentucky golf courses, 10-day-old virus residues gave 76% and 82% control of newly-hatched larvae on the two golf courses, but only 41% and 33% cutworm suppression after one month. This suggests that as a biological insecticide, AgipMNPV is better suited for targeted knockdown of small cutworms than for season-long residual control.
- UK scientists also discovered four species of parasitic wasps and a flies attacking black cutworms that could be useful for biological control.

Like humans and other animals, insects are susceptible to lethal diseases caused by bacteria, fungi, viruses, or other microbes. Fortunately, the pathogens of plant-feeding insects are almost always specific to their particular six-legged victims or hosts. One group, the baculoviruses, attacks mainly caterpillars, each strain infecting one or a few closely related host species (2).

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These viruses are good candidates for use as biological insecticides because they have no adverse effects on plants, mammals, birds, fish, and bees or other non-target insects. This is desirable when trying to conserve beneficial predatory insects to aid in overall pest management or when treating ecologically sensitive areas. The US Forest Service, for example, currently uses a baculovirus, registered as Gypchek®, to aerially spray thousands of acres of forest for gypsy moth control (6). But, the specificity of baculoviruses can be a drawback for agriculture, where growers want one product to control a variety of pests. Currently, researchers are attempting to use genetic engineering to expand virus host ranges to include desired pest species.

In the golf industry's drive toward sustainability, using pest-specific pathogens could be a way to reduce reliance on chemical insecticides.

The black cutworm is a worldwide pest of golf course putting greens and tees, as well as sport fields.

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Black cutworm larvae are active at night, chewing down the grass surrounding their burrows in thatch and soil and causing brown pock marks that reduce smoothness and uniformity of playing surfaces.

The black cutworm, a worldwide pest of putting greens and other closely-mowed playing surfaces, is a logical target for a virus-based biological insecticide. Even if relatively expensive, such a product could be cost-effective for putting greens if one application could provide extended control. Using a biological insecticide in rotation with conventional insecticides could also discourage pests from becoming resistant to conventional insecticides, as has occurred with annual bluegrass weevils on golf courses in the Northeast (5). But virus-based insecticides have not yet been developed for the turfgrass market.

Cutworms' Worst Nightmare

In 2003 a former graduate student, Callie Prater-Freeman, discovered a naturally-occurring baculovirus, *Agrotis ipsilon* multiple nucleopolyhedrovirus (AgipMNPV), killing black cutworms on Kentucky golf courses. The caterpillars ruptured at death, releasing fluid filled with millions of virus particles into the turf that infected other cutworms. A project was launched, supported by the United States Golf Association's Turfgrass and Environmental Research Program, to evaluate the virus as a potential biological insecticide (4). Initial experiments showed that AgipMNPV rapidly kills young larvae, but larger ones require higher dosages and feed for several days before death occurs (3).

Spraying a suspension of the virus controlled mid-sized cutworms in small-plot trials, including one on a putting green collar where 90-94% infection was achieved. Virus spray residues in fairway-height bentgrass remained infective for at least several weeks (1). Those findings suggested that applying the virus to putting green surrounds or tees might suppress successive generations of cutworms or even provide season-long control. This current project sought to test that approach in golf course settings including whole tees under play.

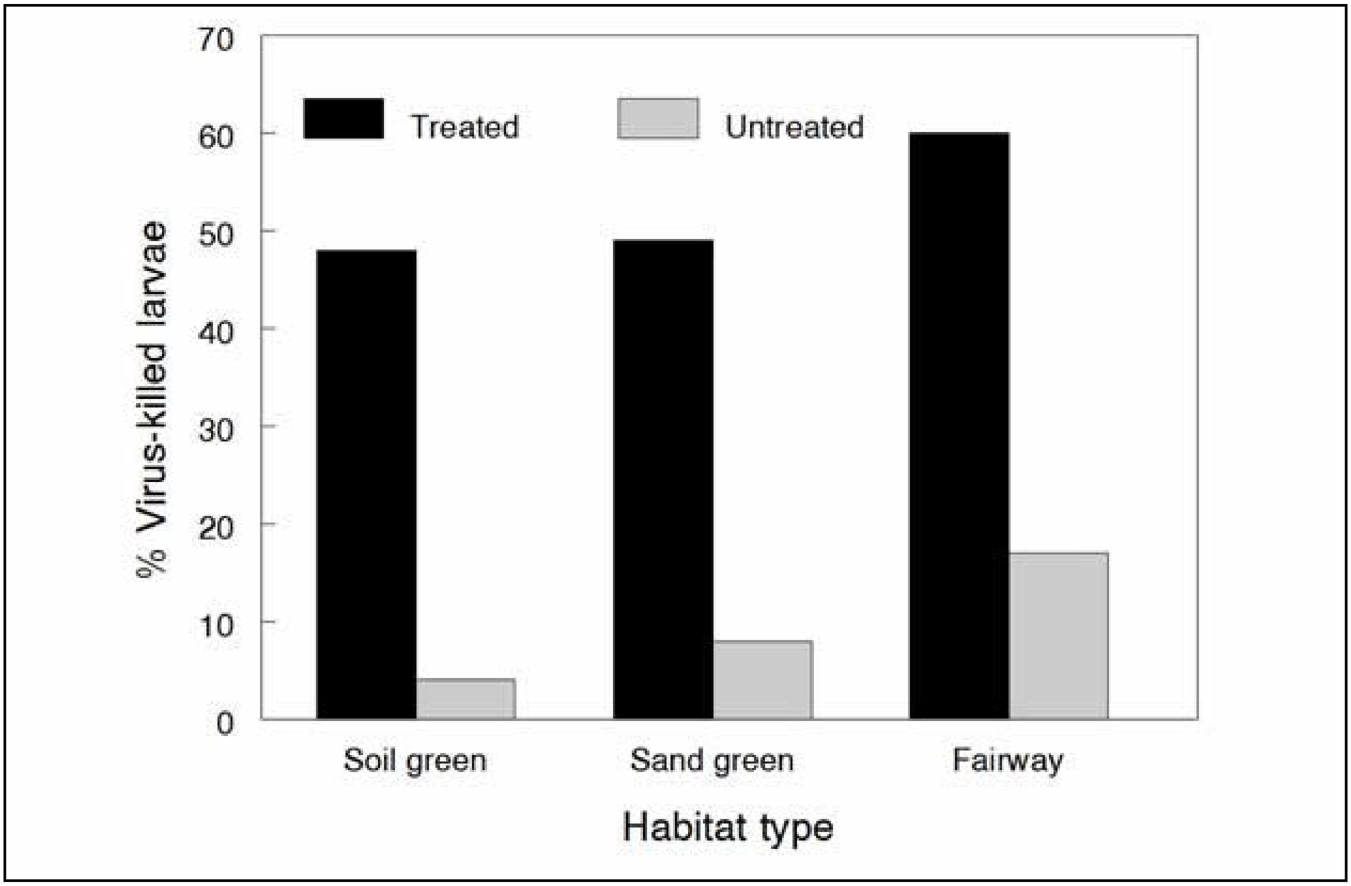

Figure 1. Percentage of larvae killed by virus infection was higher in treated than in untreated plots in all three golf course-type settings following a 4-day exposure period to 1-week-old residues of AgipNMPV.

Mass Producing the Virus

Because AgipMNPV is not commercially available we needed to "brew" enough for field trials, a feat requiring about 15,000 virus-killed caterpillars that occupied a student worker for a whole winter. Virus preparation entailed feeding cutworms on virus-contaminated grass clippings so they became diseased, grinding up the corpses and straining out the skins and guts, mixing the virus-infected blood in water, and finally diluting the virus suspension for spraying in the field. Fortunately, scientists at other institutions are studying ways to mass-produce baculoviruses on artificial media which would greatly reduce their cost.

Field Trials in Golf Course Settings

In the first trial, AgipMNPV was applied to plots on soil- and sand-based greens and in fairway-height creeping bentgrass in September to test short-term control and also see if the virus would establish and suppress cutworms the following spring. Baculoviruses can be degraded by sunlight, so we thought the residues might persist longer in the higher-mowed grass where they would be better sheltered from exposure.

When mid-sized cutworms were introduced into the turf one week after application and left to feed four days, 50-60% became lethally infected on all sites (Figure 1). But there was no infection, even in the higher turf, when the sites were challenged with cutworms six weeks after application or the following spring. Because of cool night temperatures, cutworms fed only sparingly in the autumn challenges which probably limited the amount of virus ingested and contributed to the modest short-term control. Still, the data suggest that virus residues that are infective in autumn do not overwinter at high enough levels to control cutworms the following spring.

Six tees, as well as a six-foot buffer of fairway-height grass surrounding them, were sprayed with suspensions of virus in water on each golf course.

In another trial done in summer, 3-day-old virus residues gave 50–60% control on a sand-based putting green and its surrounds, but no residual control after 2 or 4 weeks.

Studies using baculoviruses to control caterpillars on crop plants have shown that residual activity can sometimes be extended by adding small amounts of a commercial optical brightener or lignin, a natural plant polymer, to the spray mix (7). The adjuvants protect the virus particles from ultra-violet (UV) degradation and can also aid their penetration through the insect's gut wall (8).

We tested that concept by applying AgipMNPV alone or with one or the other adjuvant to fairway-height creeping bentgrass and challenging the turf with cutworms introduced one, three, or five weeks after treatment. In that trial, the virus alone gave 86, 60, and 20% lethal infection at 4 days, 3 weeks, and 5 weeks after application, but the adjuvants did not synergize or prolong infectivity.

Trials on Tees Under Play

The most extensive trials involved treating whole bentgrass tees and surrounds at two central Kentucky golf courses to see how long the virus would suppress cutworms in turf under normal maintenance and play. There were six pairs of treated and non-treated tees per course, each comprised of the front and back men's tees on a particular hole. One randomly assigned tee on each hole, and a six-foot buffer of surrounding grass, was sprayed with AgipMNPV in mid-May. Naturally-occurring cutworms were sampled and eggs and larvae were introduced every few weeks to ensure adequate cutworm infestations for evaluation. Ten-day old virus residues gave 76% and 82% control of newly-hatched larvae on the two golf courses, but only 41% and 33% suppression after one month (Table 1). When the tees were challenged by introducing mid-sized cutworms, fresh (1-week-old) virus residues gave at most 43% control, but there was no residual activity beyond one month (Figure 2).

The Bottom Line

Our experiments suggest that as a biological insecticide, AgipMNPV is better suited for targeted knock-down of small cutworms than for season-long residual control. Golf courses are a severe environment for a baculovirus. The frequent mowing and clipping removal, irrigation, and other management practices continually remove virus particles from the grass foliage, so several applications per growing season would likely be required to manage cutworms on putting greens. Better control may be gained by selecting for AgipMNPV strains having higher potency or by formulating the virus with synergists. For example, certain fungicides used on golf courses

AgipMNPV effectiveness was determined by sampling natural densities of black cutworm populations using a soap drench one week after virus application.

Golf course	Weeks after virus treatment	Average number of cutworms per sample		% reduction
		Virus-treated	Non-treated	
University Club	1	1.7	7.3	76%
	4	6.0	10.3	41%
Cherry Blossom	1	0.8	4.5	82%
	4	5.8	8.7	33%

Table 1. Average numbers of larvae recovered 10 days after implanting golf course tees under play with black cutworm eggs. There were six treated and six non-treated tees on each golf course. Separate groups of eggs were introduced 1 and 4 weeks after treated tees had been sprayed with AgipMNPV.

have been shown to disrupt the lining of the insects' gut (the cutworm's first line of defense from pathogens), so applying the virus in combination with such a fungicide might boost its activity. We are currently testing that possibility.

Despite these hurdles, efforts to further develop and commercialize AgipMNPV or other biological insecticides for sustainable golf course management are warranted. More than 800 golf courses world-wide now hold a certification with the Audubon Cooperative Sanctuary Program. Golf courses are increasingly recognized as important to landscape conservation in urban areas, and interest in organic golf has increased. The golf industry's commitment to environmental stewardship will likely provide opportunities for biological insecticides to play a greater role in insect pest management.

Enemies in the Grass

One value-added aspect of our project was discovery of several previously unknown beneficial insects that attack cutworms on golf courses. Collectively these natural enemies killed 22–31% of the cutworms introduced onto tees of our cooperating golf courses on particular dates. We found three species of parasitic wasps that lay eggs inside the caterpillar, a wasp that deposits its eggs into cutworm eggs, and a fly that is attracted by cutworm feces and deposits its live maggots

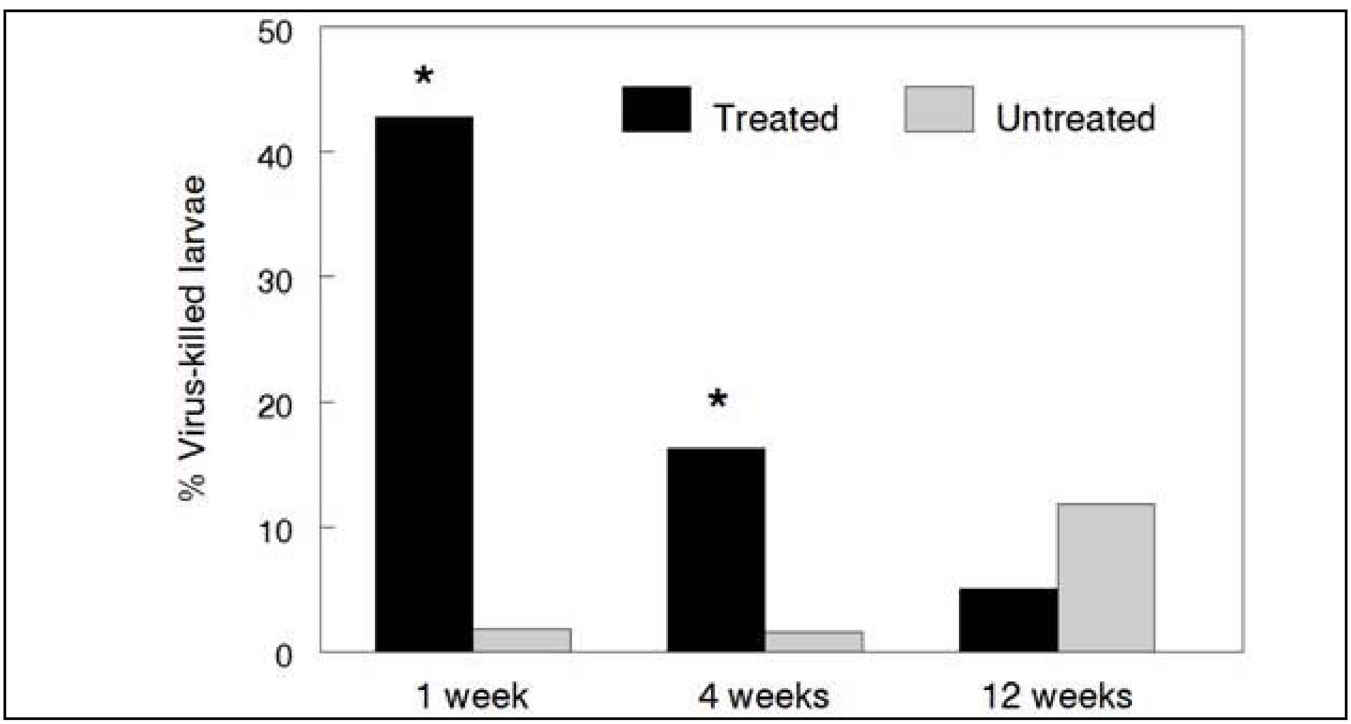

Figure 2. Percentage of larvae killed by the virus in groups of mid-sized cutworms introduced onto treated and untreated golf course tees at 1, 4, or 12 week after the virus was applied. Asterisks denote challenges in which there were significantly more virus-killed larvae on the treated tees than on untreated tees.

around the cutworm's burrow. When the cutworm comes out to feed, maggots ambush it, burrow through its skin, and consume its internal organs. Clearly those parasitic insects, and likely others, contribute to biological control, which is another reason why developing selective biological insecticides that do not harm beneficial species would be useful for sustainable pest management on golf courses.

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