

Season-Long Biological Control of Black Cutworms

A recently discovered baculovirus offers promise for season-long biological control of this common turfgrass pest.

BY DANIEL A. POTTER AND ANDREA BIXBY



(Left) The black cutworm (BCW), *Agrotis ipsilon*, is a major pest of golf courses and sports fields in the U.S. and throughout the world. (Right) In 2003, numerous black cutworms collected from golf courses in Kentucky exhibited disease symptoms including necrotic spots, milky appearance, and liquefaction of larval tissues. A virus isolated from the cadavers offers promise as a season-long biological control for this turfgrass pest.

Objectives:

1. Evaluate AgipMNPV, a naturally occurring baculovirus, as a bio-insecticide for season-long and multi-year preventive control of black cutworms (BCW) on golf courses.
2. Compare infection rates and persistence of AgipMNPV to BCW in sand-based and soil-based putting greens and fairway-height creeping bentgrass.
3. Investigate compatibility and possible synergism of AgipMNPV with soil insecticides used for grub control on golf courses.
4. Investigate compatibility of endophytic and other insect-resistant turf-

grasses with biological control of black cutworms by AgipMNPV.

Start Date: 2007

Project Duration: Three Years

Total Funding: \$60,000

In 2003, a former University of Kentucky graduate student, Callie Prater, discovered that numerous black cutworm larvae collected from Kentucky golf courses exhibited disease symptoms, including necrotic spots, milky appearance, and liquefaction of larval tissues. A virus isolated from the cadavers was identified as *Agrotis ipsilon* multiple nucleopolyhedrovirus (AgipMNPV).

The USGA-funded research at the University of Kentucky was the first to evaluate use of a baculovirus to suppress an insect pest in turfgrass. It showed that AgipMNPV quickly controls young larvae, but larger ones require higher dosages and continue to feed for several days before being killed. Virus-infected black cutworms rupture in death and spread millions of virus particles onto foliage and thatch that persist and infect subsequent larvae. Spraying a suspension of the virus in water gave good control of third-instar BCW in field trials in creeping bentgrass, including one on a putting green collar where 90-94% infection was achieved. Virus spray residues continued

Once infected with the baculovirus, the insect liquefies internally and dies. The outer covering of the insect body soon ruptures, releasing the liquefied contents and spreading virus particles onto foliage and thatch that infect other larvae.



Once infected by the virus, black cutworm larvae can be used to prepare virus suspensions and applied to the field to further infect resident larvae.



to infect third instars for at least four weeks in the field. That study suggested that establishing a reservoir of the virus in putting green surrounds or other areas could suppress successive generations of black cutworms on golf courses. This new project will evaluate that approach in realistic turfgrass settings.

AgipMNPV was applied to replicated plots on a soil-based green, a sand-based green, and fairway-height creeping bentgrass in the fall of 2007 to evaluate potential for its residues to provide residual control on golf course sites. Third-instar larvae were introduced one week after application, and

when those larvae were collected four days later, 50–60% had become infected with the virus on all sites. Additional challenges with black cutworms will be done six weeks after application and in the spring of 2008 to evaluate if the virus remains infective after the winter. Smaller larvae will be used and left in the turf for seven days, which is expected to provide higher infection rates.

A larger study will be conducted on tees and surrounds at two central Kentucky golf courses to evaluate the virus for season-long suppression of black cutworms under field conditions. Six tees, as well as a six-foot buffer

of fairway-height grass surrounding them, will be treated with the virus on each golf course. Black cutworms crawl onto tees from adjacent turf, so treating a buffer zone may significantly reduce populations. Six untreated tees on each course will be used for comparison. Virus efficacy will be determined by sampling natural densities of black cutworm populations and also implanting sentinel larvae into the turf. The virus suspension for this whole-tee trial requires about 7,000 virus-killed black cutworms, which are being cultured in the lab, but we are hopeful that methods will be developed to mass-produce the virus on artificial media. We also plan to investigate the compatibility of endophytic and other insect-resistant turfgrasses with biological control of black cutworms by AgipMNPV.

SUMMARY POINTS

- AgipMNPV has the potential to provide season-long or multi-year black cutworm control from a single application. Studies to determine virus persistence on sand-based and soil-based putting greens, fairway-height creeping bentgrass, and whole tees are underway.
- AgipMNPV may be compatible or have a synergistic interaction with insecticides used for grub control, as well as endophytic and other insect-resistant turfgrasses. These interactions will be determined in greenhouse and field experiments planned for 2008.

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CONNECTING THE DOTS

An interview with DR. DAN POTTER regarding the use of the AgipMNPV baculovirus to control white grubs.

Q: How was the AgipMNPV baculovirus identified and does it infect insects that are pests of other crops?

A: The virus was accidentally discovered about five years ago when we saw inordinately high mortality of black cutworms collected from Kentucky golf courses. The larvae soon became blackened, flaccid, and liquefied, symptoms indicative of infection by a group of insect pathogens called baculoviruses. Blood samples were sequenced by PCR, a technique that enables organisms to be identified by comparing their DNA sequences with those of known ones archived in a "gene bank." The results matched a virus called AgipMNPV that had recently been described from black cutworms collected from field corn in Illinois. Our discovery was the first documentation of a virus infecting a turfgrass pest. AgipMNPV and other baculoviruses are specific for certain insects. AgipMNPV is highly virulent to black cutworm, slightly infective to a few other closely related caterpillars (e.g., fall armyworm), but does not infect beneficial insects or vertebrates.

Q: Since the AgipMNPV baculovirus more readily infects young larvae of black cutworms and requires higher dosages to kill more mature black cutworms, how important do you think application timing will prove to be? Or is it more a matter of inoculating black cutworm-infested turf areas anytime during the growing season and let natural infection take its course?

A: We envision the virus being used to inoculate greens, tees, and surrounds for extended, season-long, or even multi-year control. Baculoviruses can persist for many years once the spore-like occlusion bodies are present in the turf. Larvae that die from the virus spread it to others. So, once established, the virus would suppress infestations by killing many larvae soon after they hatch. Timing would be important if AgipMNPV were used as a curative insecticide against smaller larvae. The virus alone is too slow-acting to be a good knockdown remedy for large cutworms, but we are studying using it with synergists that cause lesions in the insect gut lining and may increase virus efficacy and speed of kill.

Q: Do you have data or observations of how much different infectivity and death of black cutworms are depending on age of the larvae? Have you performed experiments that compare different developmental stages of black cutworms?

A: Yes, we have done those tests; they were published in an article in the *Journal of Economic Entomology* 99:1129-1137 (August 2006). Doses that killed 100% of newly hatched larvae (first instars) in a few days caused only 42% and 30% mortality of third and fifth instars, respectively. Higher doses were needed to kill the late instars. But again, we think that combining the virus with synergists can increase speed of kill of even large larvae.

Q: Your approach for black cutworm control on greens and tees involves treating buffer areas surrounding those areas so that the black cutworms will be exposed to the baculovirus as they move onto greens and tees from the surrounding area. If this approach proves successful, viral suspensions could be used very efficiently. What do you feel is the feasibility of treating whole fairways with this biological control agent?

A: Currently, production of insect-pathogenic viruses is expensive because most of them are produced by mass-rearing caterpillars, inoculating them, and grinding up the cadavers to make a biological insecticide. There is progress, however, toward being able to mass-produce viruses in artificial media, much like the biological

insecticide *Bacillus thuringiensis* (Bt). That technology would reduce cost and make it feasible to inoculate whole fairways. Right now, however, it would probably be too expensive to produce sufficient virus to treat such large areas.

Q: You suggest that the long-range success for golf courses to use this baculovirus for black cutworm control depends, in large part, on methods being developed for mass production of the baculovirus. Do you know of other cropping systems where mass production systems for biological agents have been produced? How hopeful are you that such mass-production methods can be developed for the AgipMNPV baculovirus?

A: Yes, baculoviruses are already used to control caterpillar pests on 2 to 3 million hectares per year worldwide. For example, they currently are manufactured on a commercial scale for field application against corn earworm/tobacco budworm in the USA and caterpillar pests of soybean and sugar cane in Brazil. A baculovirus is now a cornerstone of codling moth control in both organic and integrated apple production in Europe. There is much ongoing research on producing baculoviruses in insect cell culture, a process already being used not just for mass production of insect-pathogenic viruses, but also human vaccines. A cell line that grows the black cutworm virus is already available. We plan to test if the virus grown by that method is as infective as wild-type virus.

Q: Of all the biological control measures that have been investigated for controlling turfgrass insect pests (i.e., parasitic wasps, milky spore disease, entomopathogenic nematodes, etc.), how does the AgipMNPV baculovirus rank as far as the level of control and feasibility as a realistic alternative to conventional insecticides?

A: I think a commercially available virus would be at the top of the list because of the potential for extended control and reduction of pesticide inputs to high-profile areas such as putting greens and surrounds.

Q: Do you know if this baculovirus can infect other insect larvae such as armyworms? Are you planning future research to test this virus on larvae of other turfgrass insects?

A: Studies addressing that question have been done. The virus is slightly infective to a few other pest caterpillars in the same family as black cutworms, but like other baculoviruses, it has a narrow spectrum and has no adverse effects on plants, mammals, birds, fish, or beneficial insects.

Q: This seems to be a very promising area of research. What would you tell golf course superintendents regarding where this research may lead?

A: Restrictions on synthetic pesticides are increasing worldwide despite the fact that modern insecticides are much more selective and safer than ones used in the past. Interest in "organic" golf courses is on the rise. This research provides groundwork for developing the first virus-based biological insecticide for turf. Such a product potentially could allow superintendents to permanently suppress cutworms below action thresholds from one application. Many superintendents now treat cutworms multiple times per growing season. Cutting back on pyrethroids and other insecticides around greens and tees may also delay resistance in pests, e.g., annual bluegrass weevil, that inhabit the same golf course settings as cutworms.

JEFF NUS, PH.D., manager, Green Section Research.