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University of Kentucky scientists evaluated *Agrotis ipsilon* nucleopolyhedrovirus (AgipMNPV) for suppressing black cutworms (*Agrotis ipsilon* Hufnagel) in turf representative of golf course habitats and on whole tees under actual play. The results of these studies suggest AgipMNPV is better suited for targeted control of early instars than for season-long control of black cutworms.

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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 450 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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Evaluating a Naturally-Occurring Baculovirus for Extended Biological Control of Black Cutworms in Golf Course Habitats

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SUMMARY

Golf courses are a potential market for microbial insecticides, but how intensive management of such sites interacts with efficacy of entomopathogens is poorly known. University of Kentucky scientists evaluated *Agrotis ipsilon* nucleopolyhedrovirus (AgipMNPV) for suppressing black cutworms (*Agrotis ipsilon* Hufnagel) in turf representative of golf course habitats and on whole tees under actual play. Results include:

- In independent trials on sand- or soil-based putting greens and surrounds or fairway-height creeping bentgrass, less than 1 week-old AgipMNPV residues [10 X 10^8 occlusion bodies (OBs) per m^2] typically resulted in 50–60% lethal infection of introduced third-instars. In most cases, however, there was no residual control beyond 2–4 weeks.
- Spraying fairway-height bentgrass with AgipMNPV alone (10 X 10^9 OBs per m^2) gave 90, 85, and 7% infection of second-instars introduced 4 days, 3 weeks, or 5 weeks later, respectively, but adjuvants (optical brightener, lignin, or both) intended to synergize and protect the virus from UV degradation did not extend infectivity.
- Fresh (less than 1 week) AgipMNPV residues killed 76–86% of neonates hatching from eggs on tees under play, but levels of control plummeted within a few weeks. Three species of braconids, an encyrtid *Copidosoma bakeri* (Howard), and a tachinid, *Bonnetia comta* (Fallen) collectively killed 24–31% of larvae recovered from those tees.
- AgipMNPV seems better suited for targeted control of early instars than for season-long control of black cutworms. Golf turf is a severe environment for baculoviruses, so several applications per growing season would likely be needed to effectively control caterpillar pests.

Microbial products currently constitute less than 0.1% of insecticides used on golf courses, lawns, and sport fields (16). Although modern chemical insecticides labeled for turfgrass are less broadly toxic than many used in the past, some still have the potential to intoxicate pollinators and other beneficial invertebrates, leach or run off into surface or ground water, and impact aquatic organisms (34). Over-reliance on insecticides also has led to resistance in certain turfgrass insect pests (36). Because of those issues, and especially in response to societal concerns and increased restrictions on pesticides, it is imperative for the turfgrass industry to move toward sustainable, reduced-risk tactics for pest management (32, 33).

Turfgrass insect pests are naturally infected by various pathogens (10, 20), but of those, only *Paenibacillus popilliae* (Dutky), the causal agent of milky disease in Japanese beetle (*Popillia japonica* Newman) grubs, and entomopathogenic nematodes presently are marketed in the United States (33). *Bacillus thuringiensis* Berliner (Bt) products are labeled against grass-feeding caterpillars but rarely used by the turf industry because of their short residual and poor activity against larger larvae (20). A product with the fungus *Beauveria bassiana* (Balsamo) was briefly mar-

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keted in the 1990s but withdrawn due to inconsistent performance. Hurdles to commercialization of microbial turfgrass insecticides include their narrow spectrum of activity, relatively high cost, short shelf-life, problems with *in vitro* production, and competition from highly effective synthetic insecticides (16).

Baculoviruses (family Baculoviridae), because of their virulence, speed-of-kill, and persistence on protected plant parts and in soil (9), seem suited for controlling lepidopteran (catepillar-type) turfgrass pests. Baculoviruses have been used to control insect pests in agricultural and forest settings (5, 11, 25), but none have been developed or marketed for use on turf. Prater et al. (35) documented a natural epizootic of *Agrotis ipsilon* nucleopolyhedrovirus (AgipMNPV) decimating populations of the black cutworm, *Agrotis ipsilon* (Hufnagel), on central Kentucky golf courses. In field trials in fairway-height creeping bentgrass, freshly-sprayed residues of AgipMNPV gave about 80% lethal infection of third- or fourth-instars after 1–4 days, and about 50% lethal infection after 4 weeks. That groundwork suggests that AgipMNPV may provide short-term and residual control of black cutworms in turfgrass settings (35).

Agrotis ipsilon (black cutworm) is a logical target for a baculovirus insecticide because it is a nearly worldwide pest of golf course putting greens and tees, as well as sport fields (34, 51, 52). The 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 (34). Birds foraging on the caterpillars pull up tufts of grass, compounding the injury. Golf course superintendents often apply insecticides several times each growing season for cutworm control.

Viral occlusion bodies (OBs) can persist on leaf undersides, in protected plant parts, and in the underlying soil (9, 31). Turfgrass is a perennial system with dense foliage and thatch, so once a reservoir of virus is established, it seemingly would be protected from UV degradation and

In earlier studes conducted by University of Kentucky scientists on field trials in fairway-height creeping bentgrass, freshly-sprayed residues of AgipMNPV gave about 80% lethal infection of third- or fourth-instars after 1–4 days, and about 50% lethal infection after 4 weeks.

weather. It is not known, however, if the frequent irrigation, mowing, and chemical inputs typical on golf courses affect virus efficacy on tees or putting greens under play.

Optical brighteners used as fluorescent stains for microbes (12) have been used to protect entomopathogenic viruses, fungi, and nematodes from inactivation by solar radiation (19, 27, 40). Lignin, a natural plant polymer found in vascular plants and trees, is also an effective UV protectant (3, 31, 45). Fluorescent brighteners can also synergize viruses, facilitating their arrival at the host midgut epithelial cells (their initial target site) by disrupting the peritrophic membrane (26, 29, 47). However, most studies showing the benefits of adjuvants were done in laboratory or greenhouse settings (24, 26, 40), and none have involved turfgrass.

This study evaluated AgipMNPV as a bioinsecticide for short-term and extended control of black cutworms in turfgrass settings representative of golf course habitats and on whole golf tees under actual play. Our working hypothesis was that preventively applying the virus would deposit enough infective occlusion bodies in the thatch and soil to suppress successive generations of *A. ipsilon* during the growing season. Adjuvants intended to enhance viral performance and protect the virus from UV degradation were also tested in combination with field applications of AgipMNPV. New information on the parasitoid complex impacting *A. ipsilon* populations on Kentucky golf courses was obtained from recovery of sentinel larvae in the various experiments.

Materials and Methods

Insects and Virus

Agrotis ipsilon eggs and larvae from a colony maintained on soybean-based diet were shipped from a commercial insectary (Benzon, Carlisle, PA) via overnight mail and transferred to our assays within a few hours of arrival. The AgipMNPV isolate used was originally obtained from naturally-infected, late-instar *A. ipsilon* from central Kentucky golf courses (35). Frozen infected caterpillars were macerated in 0.1% sodium dodecyl sulfate (SDS) for 10 minutes, filtered through five layers of cheese cloth, and centrifuged at 900 X g for 10 minutes. The pellet contents were resuspended in 0.5% SDS and centrifuged again. Re-suspension and centrifugation were repeated with 0.5M NaCl with the final suspension in distilled water. Sodium azide was added at 0.02% concentration to prevent bacterial growth. This purified occlusion bodies (OB) suspension was stored at 4º C.

Small-Plot Field Evaluations in Golf Course Settings

Field trials evaluating AgipMNPV against *A. ipsilon* were conducted on three types of sites representative of golf course settings at the University of Kentucky's Turfgrass Research Center (UKTRC), Spindletop Farm, near Lexington. Independent replicated trials were run concurrently on a soil-based (push up) putting green, a sand-based putting green, and in an adjacent stand of fairway-height grass. The turfgrass at all three sites was 'Penncross' creeping bentgrass, *Agrostis stolonifera* L., on a Maury silt loam (fine, mixed, mesic typic Paleudalf) with a pH of 6.3. The push-up green and fairway stand were on homogeneous, original soil where no sand topdressing had been applied. The sand-based green was constructed with a 95% sand content rootzone mix according to United States Golf Association Method guidelines (4).

Both greens were mowed at 4.0 mm (0.157 inches) five times per week and irrigated from a permanent sprinkler system to prevent visible stress. Nitrogen fertilizer (urea; 46-0-0) was applied to all sites in September, October, and November at 0.48 kg actual N per 100 m^2 (1.0 lb N per 1,000 ft^2) per application. Fungicides were applied curatively for control of fungal diseases, and dithiopyr (0.07 kg AI/ha) was applied for crabgrass control. The fairway-type turf was mowed at 1.6 cm (0.63 inches) three times per week and irrigated as necessary to prevent drought stress.

Purified virus suspension was applied at 10 X 10^8 occlusion bodies per treated plot in 162 ml of distilled water using a hand sprayer.

Six pairs of treated and untreated 1-m^2 plots were marked on each site. Purified virus suspension was applied at 10 X 10^8 OBs per treated plot in 162 ml of distilled water using a hand sprayer (Solo, Newport News, VA) on September 11, 2007. Three passes were made in alternating directions to ensure even coverage. Light post-treatment irrigation (162 ml per plot) was applied to move virus residues to lower grass blades, stems, thatch, and upper soil.

Twenty, third-instar *A. ipsilon* were introduced onto each plot 1 week and 6 weeks after application (September 18 and October 26, 2007) and again on May 13, 2008 to determine if the virus had remained infective in the thatch layer. Larvae were confined in circular metal enclosures (39.0 cm diam × 10.2 cm height) driven 2 cm into the ground. We used different quadrants of each plot for the successive infestations. Enclosures were covered with 0.64-cm mesh wire hardware cloth to prevent bird predation. Grass was not mowed while cutworms and enclosures were in the plots.

Surviving larvae were recovered after 4 days by using a soap flush consisting of 1.3 ml lemon-scented Joy dishwashing detergent (Proctor & Gamble, Cincinnati, OH) per liter of water (34). Larvae were rinsed with distilled water as soon as they surfaced, placed in individual capped 30-ml cups with soybean-based noctuid diet (6) and monitored until death or pupation. Death due to viral infection was verified by examining blood for viral occlusion bodies using a phase-contrast microscope.

Residual Activity on a Closely-Mowed Putting Green Versus Surrounds

On golf courses, *A. ipsilon* developing from eggs laid in peripheral areas (surrounds) often crawl onto putting greens as late instars (51). Because higher-mowed turf seemingly would be a less harsh environment for virus longevity, applying AgipMNPV to surrounds might provide longer-lasting control than treating only greens (35). That hypothesis was tested on a sand-based creeping bentgrass green at the UKTRC, maintained as described above, and in the irrigated surrounds consisting of a mixture of creeping bentgrass, clover, and tall fescue mowed at 16 mm (0.63 inches) three times per week.

Six pairs of treated and untreated 1-m^2 plots were marked on both the green and about 2 meters into the adjacent surrounds. Purified virus suspension was applied at 10 X 10^9 OBs per treated plot in 162 ml of distilled water using a hand sprayer. Twenty, early third-instars were introduced onto each plot 4 days after application, followed by groups of 20 second-instars at 14 and 36 days after application. Larvae were confined in metal enclosures, as described earlier, with different plot quadrates used for each challenge. Surviving larvae were recovered 4 days post-treatment using a soap disclosing solution, rinsed, and placed individually in cups with artificial diet, and death from viral infection was assessed as described above.

Residual Activity With or Without Adjuvants

An experiment initiated in August 2008 tested whether increased protection and residual activity is provided to AgipMNPV by an optical

brightener (Blankophor P167- Bayer Corp., Pittsburgh, PA) a lignin (Polyfon, MeadWestvaco, Charleston, SC), or a combination of the two. The study site consisted of fairway-height creeping bentgrass maintained as described above. Virus suspensions were prepared as described earlier, with adjuvants added at 1% of the final volume of solution. Combination treatments contained 1% of each adjuvant. Treatments included brightener and virus; lignin and virus; brightener, lignin, and virus: virus alone; and untreated controls.

Virus solutions (40 ml per plot) were applied with a hand sprayer to small (0.25 m^2) plots at 10×10^9 OB per m^2. Four replicates of each treatment were applied 5 weeks, 3 weeks, and 3 days before larvae were introduced, except the brightener, lignin and virus combination, which was only applied at 5 and 3 weeks before challenge because available virus was limited at the time. Metal enclosures, as described earlier, were driven into the turf, and all plots were simultaneously challenged with 20 second-instars on September 18, 2008. The larvae were left to feed for 5 days before being recovered using a soap flush, rinsed, placed individually in 30-ml cups with soybean diet, and monitored until death or pupation. Death from virus infection was assessed as before.

Residual Efficacy of AgipNMPV on Golf Course Tees under Play

Field tests were conducted on whole tees and surrounding areas at two central Kentucky golf courses (University Club Golf Course, Lexington; Cherry Blossom Golf Course, Georgetown, KY) in 2008. Six holes (i.e., the composite unit made up of tees, fairways, and greens) were chosen on each course and two tees per hole were selected for use. Both courses have 4–5 oval or rectangular tees per hole; only back and front tees were used because they receive less play. All tees were composed of creeping bentgrass, which was mowed at 1.3 cm (0.5 inches) three times per week, and irrigated to prevent visible drought stress.

One tee of each hole, as well as a 2-meter buffer of surrounding higher-mowed turf, was sprayed with virus (10×10^8 OBs per m^2) on each course. The AgipMNPV suspension needed to treat the 12 total tees and surrounds required about 16,000 virus-killed late-instar *A. ipsilon* (8,000 per golf course) which were cultured in the lab in winter 2007–08 and frozen until enough virus was produced. Virus suspensions were delivered using a backpack sprayer (Solo, Newport News, VA) at a spray volume of 162 ml/m^2 between 1800–2200 hours. *A. ipsilon* crawl onto tees from surrounds (52), so treating a buffer zone may reduce populations. Treating in the evening may reduce UV light degradation. Six similarly-sized tees on the same holes served as controls on each course. Tees were treated in early May and maintained by the golf course staff under their normal management regimes, except that no surface insecticides were applied.

Virus residual efficacy was determined by sampling naturally-occurring *A. ipsilon* populations and by inoculating the tees with eggs or larvae. The former were sampled by soap flush once per month on three 1-m^2 areas of each tee. In addition, each tee was inoculated with 20 second-instars and 150 eggs three times (mid-May, mid-June, early August 2008). Neonates (120 per tee) also were implanted once, 2 months after applica-

Larvae were confined in circular metal enclosures driven 2 cm into the ground.

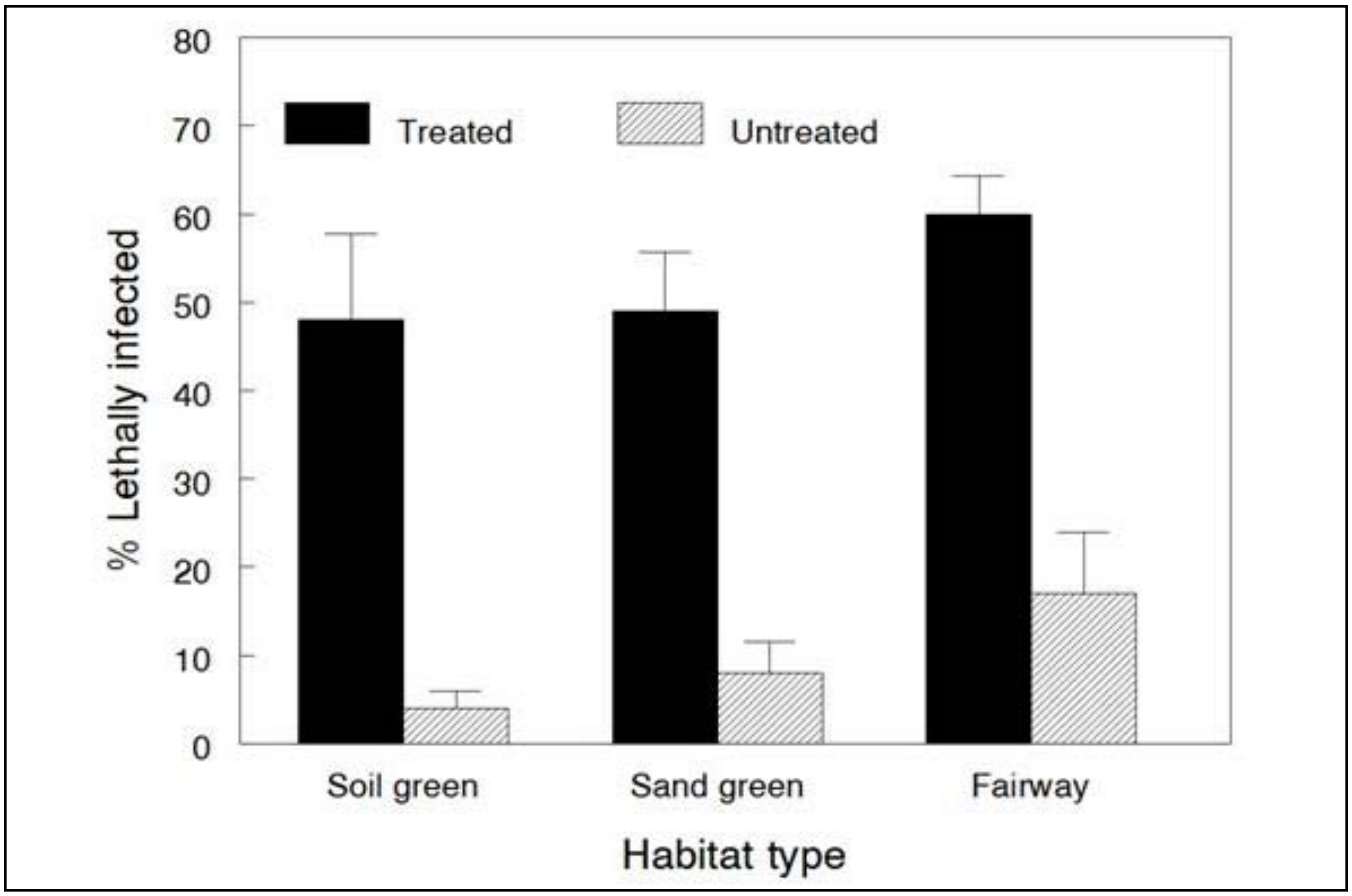

Figure 1. Mean (± SE) percentage lethal infection of 3rd-instar *A. ipsilon* following 4-day exposure to 1-week-old residues of AgipNMPV applied at 10 X10^8 occlusion bodies per m^2 in three different golf course-type habitats. Lethal infection was significantly greater in treated than in untreated plots in all settings.

tion on July 27 and August 2 at Cherry Blossom and University Club, respectively.

Second-instars were confined in 39-cm diameter metal enclosures covered with wire mesh, as described earlier. Eggs and neonates were confined in white PVC cylinders (10.0-cm diam × 10.2-cm height) driven 2 cm into the ground to prevent escape and predation by non-flying insects. Different areas of each tee were used on each sample or inoculation date, and those dates were on alternate weeks for the two golf courses because of the large amount of time required to treat, inoculate, and sample the 12 tees on each course.

Surviving larvae from the egg and larval challenges were recovered by soap flush 10 days post-introduction. All naturally occurring or inoculated larvae recovered in the samples were held on diet until death or pupation, which was followed by evaluation of incidence of virus infection, evaluated by microscopic examination of hemolymph as described earlier. Cutworms recovered from all experiments were held until death or pupation. Any larva showing signs of parasitism was watched closely until adult parasitoids had emerged. Wasps and flies were identified by C. A. Boring (University of Kentucky) and E. R. Hoebeke (Cornell University), respectively. Voucher specimens are deposited in the University of Kentucky Insect Collection.

Because all assays were done in the field, recovery of larvae following egg or larval challenges was variable. In most cases, 5–20 larvae were recovered per plot. Analyses for numbers of healthy or virus-infected larvae recovered were based on counts, so all replicates were included. Lethal virus infection in the small-plot trials simulating different golf course settings, the comparison of putting green turf versus surrounds, and the trials on whole tees under play was analyzed

using one-tailed paired t-tests. Percentage data were normalized by arcsine square-root transformation before analysis, and those few plots from which fewer than 5 larvae were recovered were treated as missing values to avoid calculating percentages on inadequate sample sizes.

For evaluation of efficacy with or without spray adjuvants, percentages of larvae dying from virus were examined by weighted factorial analysis of variance (ANOVA) for main effects and interaction of treatments and virus residue age. Percentages of larvae dying from virus within 3 days after recovery from the field also were analyzed to test the hypothesis that adjuvants would accelerate lethal infection.

Within each residue age, a weighted two-way ANOVA and one-sided Dunnett's tests were used to compare treatments in the absence of the control, and to compare treatments versus control, respectively. The brightener/lignin combination was excluded from ANOVA factorial analyses because it had not been applied on all dates, creating an unbalanced experimental design. It was, however, included for analyses of infection in larval cohorts exposed to 3- or 5-week-old residues.

Results

Small-Plot Field Evaluations in Golf Course Settings

One-week-old AgipNMPV residues resulted in 50–60% lethal infection when challenged with third-instars (Figure 1). Infection rates from the 1-week challenge were higher in treated than in untreated plots regardless of type of site. However, there was almost no infection on any of the sites when the treated turf was challenged with third-instars 6 weeks after application, or again the following May. Hemolymph smears revealed only 11 virus-infected larvae (4.9 %) among those recovered from treated plots after 6 weeks, and only one virus-killed larva the following May.

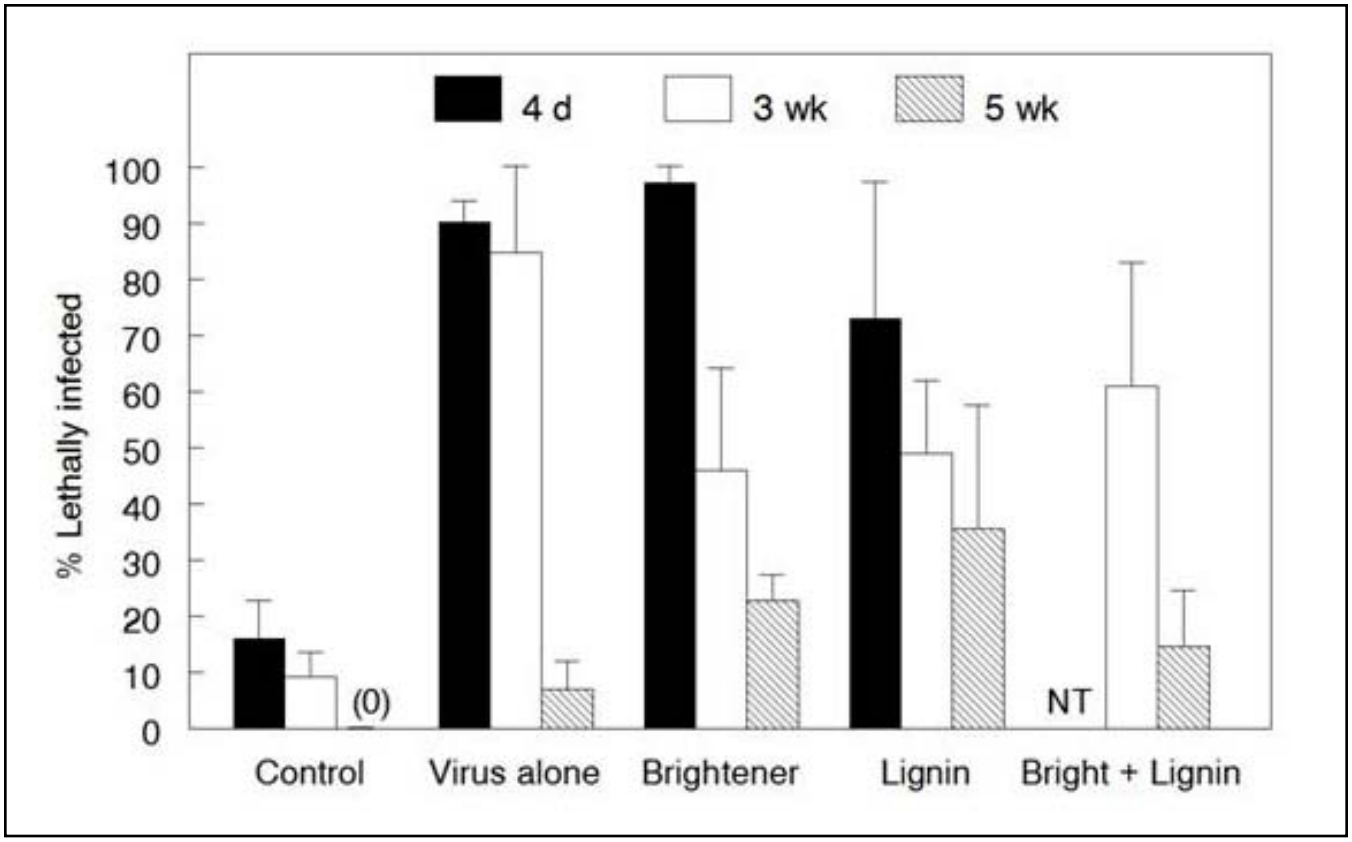

Figure 2. Residual infectivity of AgipMNPV applied at 10 X 10^9 occlusion bodies per m^2 either alone or in combination with 1% optical brightener, lignin, or both. Data are means (± SE). The legend denotes the age of residues on September 18 when each plot was challenged with 20 second-instars for 5 days. The treatment with both adjuvants was not tested (NT) with 4-day-old residues because stocks of virus were limited at that time. Adjuvants did not synergize or prolong infectivity.

Residual Activity on Closely-Mowed Putting Green and Surrounds

Percentages of larvae killed by 3-day-old virus residues did not significantly differ between paired plots on the sand-based creeping bentgrass putting green surface or higher-mowed mixed-grass surrounds (59% versus 50%, respectively). Infection in treated plots was lower in the 2-week challenge (15% versus 95% lethal infection, respectively), again with no difference between putting green plots and surrounds. Mortality from exposure to 3-day-old residues was higher in treated plots than in controls in both settings (putting green: 59% versus 7 % infection, respectively; surrounds: 50% versus 0% infection, respectively). Infection rates were similarly low in treated and untreated plots on the green and in the surrounds at 2 weeks after application (putting green: 15% versus 2.5%, respectively; surrounds: 8.6% versus 0.8% infection, respectively). No larval death due to virus infection occurred at any site when larvae were exposed to 4-week-old AgipMNPV residues.

Residual Activity With or Without Adjuvants

Overall infection was 86, 60, and 20% at 4 days, 3 weeks, and 5 weeks after application, respectively, with significant decline over time (Figure 2). The adjuvants, however, did not synergize or prolong infectivity. Four-day-old residues of all treatments gave significantly higher rates of lethal infection than occurred in controls. Virus alone was the only treatment that provided significantly higher lethal infection 3 weeks after application when compared to the control, however, its efficacy was similar to all other treatments.

Five weeks after application, infection levels were low, highly variable, and did not significantly differ in any of the treatments. Percentage of larvae dying from virus within 3 days after recovery from the field decreased as virus residues aged, but did not differ between treatments, nor was there date by treatment interaction (Figure 2).

Residual Efficacy of AgipMNPV on Golf Course Tees under Play

Fresh (10-day-old) virus residues reduced numbers of healthy (i.e., not lethally infected by virus) larvae recovered from tees that had been inoculated with eggs in mid-May by 76% and 82% at University Club and Cherry Blossom golf courses, respectively (Figure 3). Similarly, 41% fewer healthy larvae were recovered from treated than from non-treated tees at University Club in June, 10 days after the second inoculation with eggs at 4 weeks after treatment. However there was no significant reduction at Cherry Blossom (Figure 3).

Numbers of non-infected larvae recovered following the third inoculation with eggs, 12 weeks after treatment, did not differ between treated and non-treated tees at University Club (11.5 versus 3 , respectively), and in fact, were somewhat higher on the treated than on the non-treated tees. Very few larvae were recovered following the same challenge at Cherry Blossom. No significant treatment effect was evident when the tees were challenged with cohorts of neonates two months after treatment at University Club (9.5 versus 17.8 healthy cutworms recovered from treated versus non-treated tees, respectively, or at Cherry Blossom, 3.2 versus 6.5, respectively).

Lethal infection of introduced second-instars was significantly higher in treated plots at 1 week and 4 weeks after virus application at University Club (Figure 4). At Cherry Blossom, however, percent lethal infection of second-instars did not differ between treated and non-treated tees at 1 week (15 verus 7, respectively) or 4 weeks (9.7 versus 10, respectively) after application. Natural infestation of the tees by *A. ipsilon* was quite low, with only a few larvae recovered by soap-drench sampling, so those data were not analyzed.

Parasitism

Parasitoids emerged from 40 of the 164 neonate larvae (24%) that had been introduced onto golf tees at University Club in June and

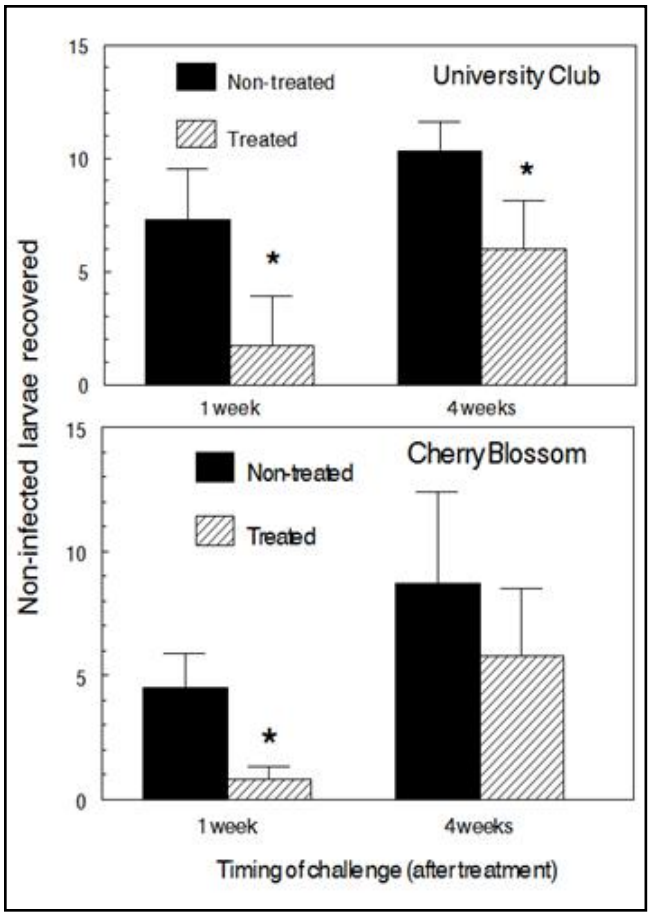

Figure 3. Mean (± SE) numbers of viable second- and third-instars (excluding ones lethally infected by virus) recovered 10 days after inoculating golf course tees under play with *A. ipsilon* eggs. There were six treated and six non-treated tees on each golf course. Separate cohorts of eggs were introduced 1 and 4 weeks after treated tees had been sprayed with AgipMNPV (10 X10^9 occlusion bodies per m^2). Asterisks denote challenges in which there were fewer viable larvae on the treated tees.

recovered by soap flush after 10 days. Of those, 30 were killed by the tachinid fly, *Bonnetia comta* (Fallen), seven by braconid wasps, and the other three were parasitized by *Copidosoma bakeri* (Howard), a polyembryonic encyrtid wasp.

At Cherry Blossom, 20 of the 65 larvae (35%) recovered following the neonate introductions were parasitized, 10 by braconid wasps (two *Meteorus spp.* and one *Microplitis sp.*), six by *C. bakeri*, and four by *B. comta*. *C. bakeri* parasitizes eggs of *A. ipsilon* (39), so its recovery in samples of larvae presumed to have originated from larval inoculations indicates that some larvae recovered in those samples were from the natural population.

Seven of the 87 larvae recovered from the tees at Cherry Blossom following the July13 egg inoculation were parasitized, six by braconid wasps and one by *C. baker*i. At University Club, only one of the 87 cutworms recovered from the July 10 egg inoculation was parasitized by a braconid, but 22% (19 of 87) of those recovered following the August 28 egg inoculation of the same tees were parasitized, 15 by braconids, three by *C. bakeri*, and one by *B. comta*. No parasitoids emerged from other inoculations at either golf course.

Discussion

Baculoviruses could realistically compete with chemical insecticides for managing *A. ipsilon* or other grass-feeding caterpillars on golf courses or sport fields if one application provided extended suppression once the virus became established in the turfgrass (34, 35). Potential markets for AgipMNPV or other biological insecticides include sustainably or organically-managed golf courses, courses participating in environmental stewardship programs (e.g., as wildlife sanctuaries; 2, 14, 23), sites where surface runoff of chemicals into ponds or streams is a concern (48), and sport fields or other sites where the use of chemical insecticides is prohibited or poses unacceptable risk.

Turfgrasses typically have a thatch layer composed of living and dead stems, stolons, and roots and partially decomposed organic matter that accumulates between the living plants and soil (18). Thatch binds and retains pesticide residues (13, 28, 43), so we speculated it would retain a virus reservoir that would induce lethal infections for many weeks or months. Virus occlusion bodies can also persist on the underside of leaves, in protected plant parts, and in the topsoil (9).

The hypothesis that higher-mowed grass would protect the virus longer from UV degradation was not supported by infection rates in larval

challenges. Fresh virus residues killed a high percentage of *A. ipsilon* in all heights of turf, but efficacy lasted only a few weeks regardless of whether the residues were on or around greens, on tees, or in fairway-height grass. The frequent mowing, clipping removal, and irrigation on golf courses doubtless contribute to movement of virus out of the turfgrass and, together with UV degradation, account for loss of residual activity within a few weeks.

Commercially produced baculoviruses have been used to manage insect pests in agricultural and forest settings (5, 11, 25, 44) and, in some cases, one application has provided season-long or multi-year control (15, 53). Examples of this include inoculative releases of a non-occluded virus of the rhinoceros beetle, *Oryctes rhinoceros* L., in coconut palms (53), and releases of the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, baculovirus in soybean (15). Our trials on whole creeping bentgrass tees are the first to evaluate a baculovirus for season-long control of a pest on golf courses under actual maintenance and play. Although 1-week-old virus residues reduced larval populations resulting from implanted eggs by 76–82%, elevated infection of implanted larvae lasted no more than a few weeks.

Combining insect-pathogenic viruses with optical brighteners (19, 40) or lignin (3, 30, 45) may screen them from UV degradation and enhance their longevity. In this study, however, such adjuvants failed to accelerate or extend efficacy of AgipMNPV against *A. ipsilon* in fairway-height creeping bentgrass. Frequent mowing and clipping removal of golf course fairways, tees, and greens would soon remove viral occlusion bodies deposited on grass blades, so residual virus would be mostly on lower portions of the plants or in the thatch or upper soil. Any screening by adjuvants might be less evident in a relatively dense turfgrass canopy than in more exposed settings.

Stilbene optical brighteners also bind to the chitin in the caterpillar midgut, disrupting peritrophic membrane formation and increasing larval susceptibility to baculovirus infection (47). Thus their use as adjuvants can provide comparable infection of target pests at lower virus application rates (7, 40, 41). The relatively high application rate used in our field trial, where the virus alone gave 80-90% infection for as long as 3 weeks, may have masked any short-term synergism that might have been apparent at a lower rate.

Most of the previous studies documenting synergism of a baculovirus by optical brighteners were in systems where the plant canopy offered significant protection from sunlight (49, 54). Benefits of adding a brightener might be less evident in more exposed turfgrass systems, since some brighteners themselves can be degraded within a few days of application (46). Boughton et al (7), for example, found that the optical brightener M2R, which reduced the LD_{50} of AgipMNPV to *A. ipsilon* in the laboratory, failed to enhance its efficacy against the same pest in greenhouse- or field-grown corn.

In caterpillars, a defense mechanism known as midgut cell sloughing, triggered by virus-challenged midgut epithelial cells, becomes increasingly developed in later instars (17). Neonate *A. ipsilon* are especially vulnerable to AgipMNPV whereas later instars must ingest higher dosages to become lethally infected (35). Black cutworm moths deposit eggs on grass blades in turfgrass settings (51), so neonates would be exposed to virus residues and ideally are killed before reaching destructive size.

First-instars are highly susceptible to insect predation (22) and difficult to sample by soap drench and recover from turf field plots, so second- or third-instars were used for most trials herein. Mortality from virus would likely have been higher had neonates been used, a supposition supported by the 76-82% reduction in larval population when the virus-sprayed tees were inoculated with eggs. Lethal infection of the second- and third-instars probably also would have been higher had the larvae been left to feed longer in the treated turf, rather than being sampled, brought back, and transferred to virus-free diet for assessment after a few days .

Golf putting greens receive more pesticides per unit area than any other turfgrass sites (42), so they are a focal point for concerns about

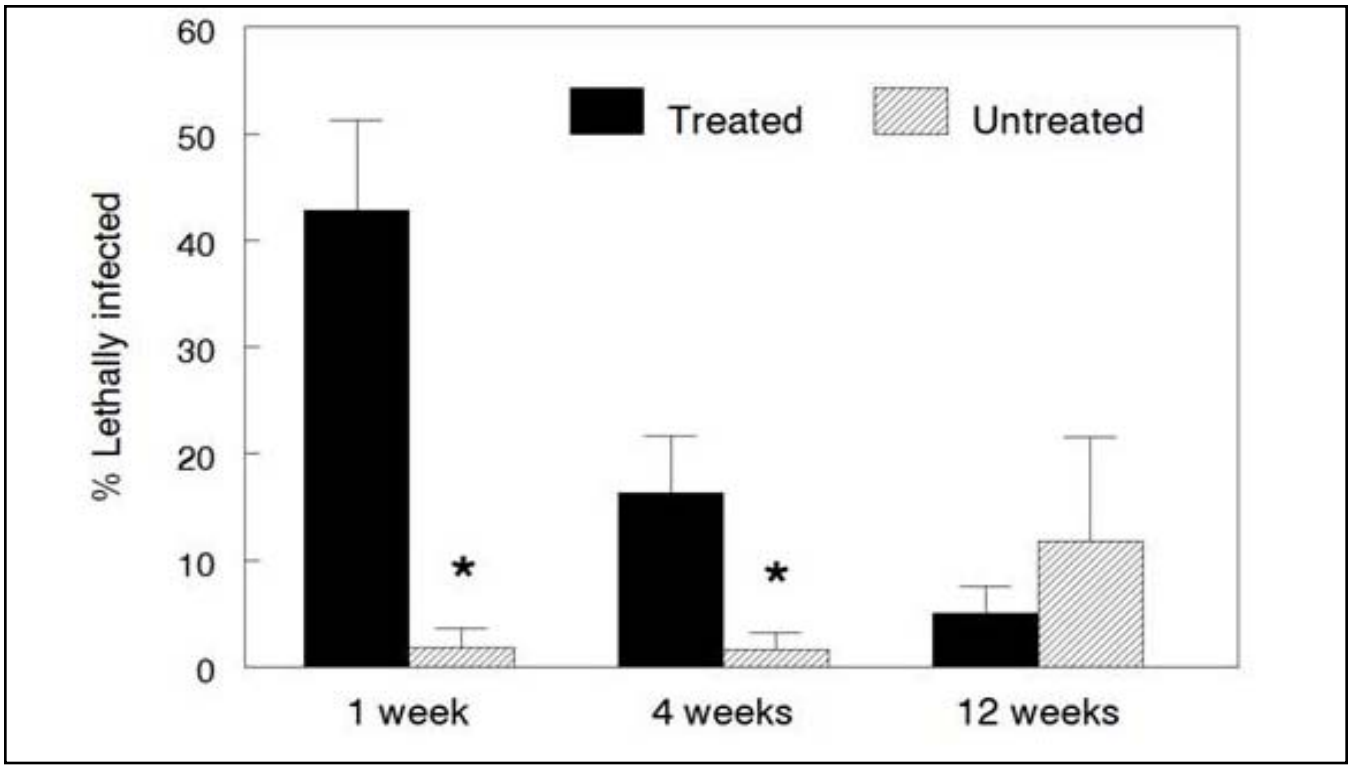

Figure 4. Mean (± SE) percentage lethal infection by AgipMNPV in cohorts of second-instar *A. ipsilon* introduced onto treated and untreated golf course tees at 1, 4, or 12 weeks after the virus was applied. These data are from University Club only. Asterisks denote challenges in which virus residues provided significantly higher infection than occurred on the non-treated tees.

insecticide leaching, surface runoff, and potential exposure to golfers. Golf, however, requires smooth uniform playing surfaces, especially on greens, so many golf superintendents treat for cutworms multiple times per growing season. In the northeastern United States, another key pest, the annual bluegrass weevil (*Listronotus maculicolis* Lietz; Coleoptera: Curculionidae), is increasingly resistant to pyrethroids (36), which likely has been exacerbated by reliance on the same chemical class for cutworm control. By diversifying options for cutworm control, an AgipMNPV-based bio-insecticide could be useful in resistance management of *L. maculicolis* and other turf pests (e.g., billbugs, chinch bugs) having similarly limited dispersal and out-crossing capability.

Little is known about the identity of black cutworm parasitoids in turfgrass habitats or how much they contribute to biological control. Although parasitism was not the focus of this study, parasitoids emerged from 24% and 31% of the cutworms recovered following neonate larval introductions on tees of the two golf courses in June, and from 22% of larvae recovered from egg inoculation sites at University Club in August. Of those, *Meteorus spp.* and *Microplitis sp.* oviposit into larvae (Bixby and Potter, unpublished data), whereas *C. bakeri* is a polyembryonic encyrtid that oviposits in the host egg (39). Females of the tachinid *B. comta* are attracted to host frass and larviposit near the cutworm burrow; the planidial maggot then burrows into the host larva (8, 37, Bixby, unpublished data). Clearly those parasitoids, and likely others, contribute to biological control of *A. ipsilon* in turfgrass, so conservation of their benefits is another reason why a selective, baculovirus-based insecticide would be useful for managing cutworms on golf courses.

Conclusions

In summary, while it was hoped that AgipMNPV could provide season-long control of *A. ipsilon* in golf course settings, our results suggest it may be better suited for targeted knock-

down of early instars than for season-long residual control. Golf courses are severe environments for entomopathogens. Daily or frequent mowing and clipping removal, irrigation, and other intensive management practices are not conducive to maintaining lethal levels of baculoviruses on grass foliage. Multiple applications per growing season may be required to manage cutworms or other grass-feeding caterpillars on such high-profile sites as putting greens. Nonetheless, we were limited to spraying a self-made crude AgipMNPV suspension. Better control possibly could be gained by selecting for AgipMNPV strains having higher virulence, or by formulating the virus with synergists or performance-enhancing adjuvants other than those tested.

As with most entomopathogens, commercial success of AgipMNPV would be facilitated by advances in *in vitro*-production methodology allowing the virus to be produced more economically and in greater amounts (5, 21, 50). Despite those hurdles, turf provides a strong potential market for biological insecticides, and efforts to develop AgipMNPV or other baculoviruses for sustainable golf course management are warranted.

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Literature Cited

1. Analytical Software. 2008. Statistix version 8, User's manual. Analytical Software, Tallahassee, FL.

2. Anonymous. 2006. Golf and the environment. Audubon International. Selkirk, NY. (TGIF Record 146786)

3. Arthurs, S. P., L. A. Lacey, and R. W. Behle. 2006. Evaluation of spray-dried lignin-based formulations and adjuvants as solar protectants for the granulovirus of the codling moth, *Cydia pomonella* (L.). *J. Invertebr. Pathol.* 93:88–95.

4. Beard, J. B. 2002. Turf management for golf courses. Ann Arbor Press. Chelsea, Ml. (TGIF Record 73347)

5. Black, B. C., L. A. Brennan, P. M. Dierks, and I. E. Gard. 1997. Commercialization of baculoviral insecticides. Pages 341-387. *In* L. K. Miller (ed.) The Baculoviruses. Plenum, New York, NY.

6. Blanco, C. A., M. Portilla, C. A. Abel, H. Winters, R. Ford, and D. Streett. 2009. Soybean flour and wheat germ proportions in artificial diet and their effect on the growth rates of the tobacco budworm, *Heliothis virescens*. *J. Insect Sci.* 9:59.

7. Boughton, A. J., Lewis, L. C. and Bonning, B.C. 2001. Potential of *Agrotis ipsilon* nucleopolyhedrovirus for suppression of the black cutworm (Lepidoptera: Noctuidae) and effect of an optical brightener on virus efficacy. *J. Econ Entomol.* 94:1045–1052. (TGIF Record 76776)

8. Clement, S. L., W. L., Rubink, and D. A., McCartney. 1982. Larviposition response of *Bonnetia comta* to a kairomone of *Agrotis ipsilon*. *Entomophaga* 31:277-284.

9. Cory, J. S., R. S. Hails, and S. M. Sait. 1997. Baculovirus ecology. Pages 301–309. *In* L. K. Miller (ed.) The Baculoviruses. Plenum, New York, NY.

10. Cranshaw, W. S., and M. G. Klein. 1994. Microbial control of insect pests of landscape plants. Pages 503–520. *In* A. R. Leslie (ed.) Handbook of Integrated Pest Management for Turf and Ornamentals. Lewis, Boca Raton, FL. (TGIF Record 30866)

11. Cunningham, J. C. 1995. Baculoviruses as microbial insecticides. Pages 261–292. *In* R. Reuveni (ed.) Novel Approaches to Integrated Pest Management. Lewis, Boca Raton, FL.

12. Darken, M. A. 1961. Applications of fluorescent brighteners in biological techniques. *Science* 133:1704-1705.

13. Dell, C. J., C. S. Throssell, M. Bischoff, and R. F. Turco. 1994. Estimation of sorption coefficients for fungicides in soil and turfgrass thatch. *J. Environ. Qual.* 23:92-96. (TGIF Record 30956)

14. Dodson, R. G. 2000. Managing wildlife habitat on golf courses. Wiley, New York, NY. (TGIF Record 62907)

15. Fuxa, J. R. 1991. Insect control with baculoviruses. *Biotech. Adv*. 9:425–442.

16. Grewal, P. S. 1999. Factors in the success and failure of microbial control in turfgrass. *Integr. Pest Manage. Rev.* 4:287-294. (TGIF Record 69659)

17. Hoover, K., J. O. Washburn, and L. E. Volkman. 1999. Midgut-based resistance of *Heliothis virescens* to baculovirus infection mediated by phytochemicals in cotton. *J. Insect Pathol.* 46:999-1007.

18. Hurto, K. A., A. J. Turgeon, and L. A. Spomer. 1980. Physical characteristics of thatch as a turfgrass growing medium. *Agron. J.* 72:165-167. (TGIF Record 527)

19. Ignoffo, C. M., and O. F. Batzer. 1971. Microencapsulation of ultraviolet protectants to increase sunlight stability of an insect virus. *J. Econ. Entomol.* 64:850-853.

20. Klein, M. G. 1995. Microbial control of turfgrass insects. Pages 95-100. *In* R. L. Brandenburg and M. G. Villani (eds.) Handbook of Turfgrass Insect Pests. Entomological Society of America, Lanham, MD. (TGIF Record 36024)

21. Kompier, R., J. Tramper, and J. M. Vlak. 1988. A continuous process for the production of baculovirus using insect-cell cultures. *Biotechnol. Lett.* 10:849-854.

22. López, R .L., and D. A. Potter. 2000. Ant predation on eggs and larvae of black cutworm (Lepidoptera: Noctuidae) and Japanese beetle (Coleoptera: Scarabaeidae) in turfgrass. *Environ. Entomol.* 29:116–125. (TGIF Record 63975)

23. Lyman, G. T., C. S. Throssell, M. E. Johnson, G. A. Stacey, and C. D. Brown. 2007. Golf course profile describes turfgrass, landscape and environmental stewardship features. *Appl. Turfgrass Sci.* doi:10.1094/ATS-2007-1107-01-RS. (TGIF Record 130525)

24. Martin, P. A. W. 2004. A stilbene optical brightener can enhance bacterial pathogenicity to gypsy moth (Lepidoptera: Lymantriidae) and Colorado potato beetle (Coleoptera: Chrysomelidae). *Biocontrol Sci. Tech.* 14: 375–385.

25. Moscardi, F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. *Annu. Rev. Entomol*. 44:257–289.

26. Mukawa S., M. Nakai, S. Okuno, J. Takatsuka, and Y. Kunimi. 2003. Nucleopolyhedrovirus enhancement by a fluorescent brightener in *Mythimna separata* (Lepidoptera: Noctuidae). *Appl. Entomol. Zool.* 38:87-96.

27. Nickle, W. R., and M. Shapiro. 1994. Effects of eight brighteners as solar radiation protectants for *Steinernema carpocapsae*, A-ll strain. *J. Nematol.* 26:782-784. (suppl).

28. Niemczyk, H. D., and R. A. Chapman. 1987. Evidence of enhanced degradation of isofenphos in turfgrass thatch and soil. *J. Econ. Entomol.* 80: 880-882. (TGIF Record 15065)

29. Okuno, S., J. Takatsuka, M. Nakai, S. Ototake, A. Masui, and Y. Kunimi. 2003. Viral-enhancing activity of various stilbene-derived brighteners for a *Spodoptera litura* nucleopolyhedrovirus. *Biol. Control* 26:146-156.

30. Peng, F., J. R. Fuxa, A. R. Richter, and S. J. Johnson. 1999. Effects of heat-sensitive agents, soil type, moisture, and leaf surface on persistence of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus. *Environ. Entomol.* 28:330–338.

31. Peng, P. P., and D. S. Argyropoulos. 2007. On the interaction of UV screens with the lignocellulosic matrix. *Photochem. Photobiol*. 71:149–156.

32. Potter, D. A. 2008. Managing insect pests of sport fields: What does the future hold? *Acta Hort.* 783:481–498. (TGIF Record 136450)

33. Potter, D. A. 2005. Prospects for managing destructive turfgrass insects without protective chemicals. *Intl. Turfgrass Soc. Res. J.* 10:42-54. (TGIF Record 105292)

34. Potter, D. A. 1998. Destructive turfgrass insects: biology, diagnosis, and control. Wiley, New York, NY. (TGIF Record 43046)

35. Prater, C. A., C. T. Redmond, W. Barney, B. Bonning, and D. A. Potter. 2006. Microbial control of the black cutworm (Lepidoptera: Noctuidae) in turfgrass using *Agrotis ipsilon* multiple nucleopolyhedrovirus. *J. Econ. Entomol*. 99: 1129-1137. (TGIF Record 114656)

36. Ramoutar, D., S. R. Alm, and R. S. Cowles. 2009. Pyrethroid resistance in populations of *Listronotus maculicollis* (Coleoptera: Curculionidae) from southern New England golf courses. *J. Econ. Entomol.* 102:388-392. (TGIF Record 147053)

37. Rubink, W. L., and S. L. Clement. 1982. Reproductive biology of *Bonnetia comta*, a parasitoid of the black cutworm. *Environ. Entomol.* 11:981-985.

38. SAS Institute. 2003. SAS, version 9.1. SAS Institute, Cary, NC.

39. Saeki, Y., P. H. Crowley, C. W. Fox, and D. A. Potter. 2009. A sex-specific size-number trade-off in clonal broods. *Oikos* 188:1552–1560.

40. Shapiro, M. 1992. Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. *J. Econ. Entomol.* 85:1682-1686.

41. Shapiro, M., and J. L. Robertson. 1992. Enhancement of gypsy moth (Lepidoptera: Lymantriidae) baculovirus activity by optical brighteners. *J. Econ. Entomol.* 85:1120–1124.

42. Smith, A. E., and W. R. Tillotson. 1993. Potential leaching of herbicides applied to golf course greens. Pages 168-181. *In* K. D. Racke and A. R. Leslie (eds.) Pesticides in Urban Environments. ACS Symp. Ser. 522. American Chemical Society, Washington, DC. (TGIF Record 37692)

43. Spieszalski, W. W., H. D. Niemczyk, and D. J. Shetlar. 1994. Sorption of chlorpyrifos and fonofos on four soils and turfgrass thatch using membrane filters. *J. Environ. Sci. Health. B*. 29: 1117-1136. (TGIF Record 37414)

44. Szewczyk, B., L. Hoyos-Carvajal, M. Paluszek, I. Skrzecz, and M. Lobo de Souza. 2006. Baculoviruses - re-emerging biopesticides. *Biotechnol. Adv.* 24:143-160.

45. Tamez-Guerra, P., M. R. McGuire, R. W. Behle, J. J. Hamm, H. R. Sumner, and B. S. Shasha. 2000. Sunlight persistence and rainfastness of spray-dried formulations of baculoviruses

isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 93:210–218.

46. Vail, P. V., D. F. Hoffmann, and J. S. Tebbets. 1999. Influence of fluorescent brighteners on the field activity of the celery looper nucleopolyhedrovirus. *Southwest. Entomol.* 24:87-97.

47. Wang, P., and R. R. Granados. 2000. Calcofluor disrupts the midgut defense system in insects. *Insect Biochem. Mol. Biol.* 30:135-143.

48. Watschke, T. L., R. O. Mumma, D. T. Linde, J. A. Borger, and S. A. Harrison. 2000. Surface runoff of selected pesticides applied to turfgrasses. Pages 94–105. *In* J. M. Clark and M. P. Kenna (eds.) Fate and Management of Turfgrass Chemicals. ACS Symp. Ser. 743. American Chemical Society, Washington, DC. (TGIF Record 64578)

49. Webb, R. E., N. H. Dill, J. D. Podgwaite, M. Shapiro, R. L. Ridgway, J. L. Vaughn, L.Venables, and R. J. Argauer. 1994. Control of third- and fourth-instar gypsy moth (Lepidoptera: Lymantriidae) with Gypchek combined with a stilbene disulfonic acid additive on individual shade trees. *J. Entomol. Sci.* 29:82-91.

50. Weiss, S. A., D. W. Thomas, B. F. Dunlop, R. Georgis, and P. V. Vail. 1994. *In vitro* production of viral pesticides: key elements. Pages 57-63. *In* C. J. Monsour, S. Reid, and R. E. Teakle (eds.) Proc. Symp. Biopesticides: Opportunities for Australian Industry, University of Queensland, Brisbane, Australia.

51. Williamson, R. C., and D. A. Potter 1997. Oviposition of black cutworm (Lepidoptera: Noctuidae) on creeping bentgrass putting greens and removal of eggs by mowing. *J. Econ. Entomol.* 90:590–594. (TGIF Record 40204)

52. Williamson, R. C., and D. A. Potter. 1997. Nocturnal activity and movement of black cutworms (Lepidoptera: Noctuidae) and response to cultural manipulations on golf course putting greens. *J. Econ. Entomol.* 90:1283-1289. (TGIF Record 44069)

53. Zelazny, B., A. Lolong, and B. Pattang. 1992. *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) populations suppressed by a baculovirus. *J. Invertebr. Pathol.* 59:61–68.

54. Zou, Y., and S. Y. Young. 1996. Use of fluorescent brightener to improve *Pseudoplusia includens* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus activity in the laboratory and field. *J. Econ. Entomol.* 89:92-96.