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Biological and Microbial Control

Microbial Control of Black Cutworm (Lepidoptera: Noctuidae) in Turfgrass Using Agrotis ipsilon Multiple Nucleopolyhedrovirus

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ABSTRACT Agrotis ipsilon multiple nucleopolyhedrovirus (family Baculoviridae, genus Nucleopolyhedrovirus, AgipMNPV), a naturally occurring baculovirus, was found infecting black cutworm, Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae), on central Kentucky golf courses. Laboratory, greenhouse, and field studies investigated the potential of AgipMNPV for managing black cutworms in turfgrass. The virus was highly active against first instars (LC50 = 73 occlusion bodies [OBs] per μl with 2-μl dose; 95% confidence intervals, 55–98). First instars that ingested a high lethal dose stopped feeding and died in 3–6 d as early second instars, whereas lethally infected fourth instars continued to feed and grow for 4–9 d until death. Sublethal doses consumed by third or fifth instars had little or no effect on subsequent developmental rate or pupal weight. Horizontal transmission of AgipMNPV in turfgrass plots was shown. Sprayed suspensions of AgipMNPV (5 × 108–6 × 109 OBs/m²) resulted in 75 to 93% lethal infection of third or fourth instars in field plots of fairway-height creeping bentgrass, Agrostis stolonifera (Huds.), and on a golf course putting green collar. Virus spray residues (7 × 109 OBs/m²) allowed to weather on mowed and irrigated creeping bentgrass plots significantly increased lethal infection of implanted larvae for at least 4 wk. This study, the first to evaluate a virus against a pest in turfgrass, suggests that AgipMNPV has potential as a preventive bioinsecticide targeting early instar black cutworms. Establishing a virus reservoir in the thatch and soil could suppress successive generations of that key pest on golf courses and sport fields.

KEY WORDS Agrotis ipsilon, black cutworm, baculovirus, microbial control, turfgrass

Biological insecticides constituted <0.1% of the estimated $500 million U.S. turfgrass insecticide market in 1998 (Grewal 1999). Receptivity to such products nevertheless is increasing in response to societal concerns about pesticide use on lawns, golf courses, and sport fields (Potter 2004, 2005). Baculoviruses, especially nucleopolyhedroviruses (NPVs), have promise as bioinsecticides for caterpillars because of their specificity of action, high virulence, and ability to replicate in the host (Moscardi 1999). Infected larvae rupture in death, contaminating foliage with millions of occlusion bodies (OBs), the infectious form of the virus, that then are ingested by other larvae. OBs, consisting of virions embedded in a protein matrix, are relatively stable in the environment when protected from UV light (Cory and Myers 2003). Formulating OBs with optical or fluorescent brighteners often enhances their activity (Shapiro 1995, Dougherty et al. 1996). Baculoviruses therefore have potential to provide long-term suppression once introduced among a pest population (Moscardi 1999, Cory and Myers 2003).

The black cutworm, Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae), a worldwide pest of turfgrass, is especially damaging to golf course putting greens and other closely mowed sites (Potter 1998). Late instars burrow in the soil or thatch, surfacing at night to chew grass blades and stems and cause depressed spots or pockmarks that reduce the smoothness and uniformity of playing surfaces (Williamson and Potter 1997). Birds compound the damage by pulling up tufts of grass as they forage on cutworms. Golf superintendents typically treat their greens with insecticides multiple times each growing season to control A. ipsilon.

Many black cutworms collected from central Kentucky golf courses in 2002–2004 showed symptoms of viral infection, including necrotic spots and swollen milky appearance followed by rupture and release of liquefied body contents (Prater and Potter 2004). Presence of OBs in the hemolymph confirmed viral infection. This, to our knowledge, is the first report of a viral epizootic impacting a pest population in turfgrass. Polymerase chain reaction (PCR)-produced polyhedron gene sequences from assayed field-collected larvae (Prater 2005) matched profiles of A. ipsilon multiple NPV (family Baculoviridae, genus...
Nucleopolyhedrovirus, AgipMNPV), a baculovirus previously isolated and described from infected black cutworms in Illinois (Boughton et al. 1999). Several baculoviruses have been registered as bioinsecticides against insect pests in agricultural and forest settings (Cunningham 1995, Moscardi 1999). Black cutworms are susceptible to at least five different baculoviruses (Johnson and Lewis 1982, El-Salamony et al. 2003), but no one has previously evaluated a virus for managing them, or any other insect pest, in turfgrass. We report here on laboratory, greenhouse, and field trials to evaluate potential of the Kentucky isolate of AgipMNPV as a bioinsecticide for controlling A. ipsilon on golf courses, sports fields, and similar sites.

Materials and Methods

Insects. A colony of A. ipsilon was maintained on pinto bean diet (Hendrix et al. 1991) at 25 ± 0.5°C and a photoperiod of 14:10 (L:D) h by methods similar to Reese et al. (1972). Laboratory experiments were conducted under those conditions. Additional larvae were obtained from corporate or commercial insectaries (Dow AgroSciences, Indianapolis, IN; Benzon, Carlisle, PA) when field trials required hundreds of developmentally synchronized caterpillars.

Virus. The original Kentucky isolate was obtained from naturally infected late instars of A. ipsilon from central Kentucky golf courses (Prater 2005) and identified as AgipMNPV (GenBank accession no. AY136484, GenBank, Los Alamos, NM) by PCR amplification of the polyhedron gene (Lange et al. 2004). It was amplified and purified by methods similar to those described in Entwistle (1998). Briefly, frozen caterpillars were macerated in 0.1% sodium dodecyl sulfate (SDS) for 10 min and then filtered through five layers of cheesecloth. OBs were then pelleted by centrifugation at 900 × g for 10 min. The pellet was resuspended in 0.5% SDS and centrifuged again. Resuspension and centrifugation were then repeated with 0.5 M NaCl. OBs underwent final resuspension in distilled water, and sodium azide was added at 0.02% concentration to prevent bacterial growth. Virus stocks were stored at 4°C. OB concentrations were quantified with a phase-contrast microscope and a Neubauer bright-line hemocytometer (Fisher, Pittsburgh, PA). The Kentucky AgipMNPV isolate was used for all trials.

Lethal Concentration (LC)50 and Time to Death for Infected First Instars. Neonate (≤1-d old) A. ipsilon were infected by droplet-feeding (Hughes et al. 1986) to estimate the oral LC50 for Kentucky-isolate AgipMNPV and time to death from viral infection. Larvae were allowed to drink from suspensions containing one of six concentrations of virus (0, 5, 25, 125, 625, or 3,125 OBs per µl) in distilled water. Larvae that ingested this exposure dose (≈2 µl) were transferred to a pinto bean diet-containing well (17 mm in diameter) of a Falcon 24-well tissue culture plate (Fisher, Pittsburgh, PA). Well plates were sealed with Parafilm M film (Pechiney, Menasha, WI). There were five replicates per concentration and 24 larvae per replicate. Larvae were monitored for mortality for 10 d, by which time survivors were late fourth instars that, when transferred to individual cups with fresh diet, developed to pupation. Data were analyzed by probit analysis (Finney 1971) as adapted for PC use (SAS Institute 1990). Chi-square statistics were calculated to determine the goodness-of-fit of the log concentration–probit mortality regression lines, and 95% confidence intervals (CI) also were calculated.

Another experiment was run to clarify how quickly first instars consuming food contaminated with a lethal dose of virus cease development and die. Pinto bean diet, as described above, was added to 24-well tissue culture plates. Once it had set, a 5-µl droplet containing AgipMNPV at 8,748 OBs per µl (estimated LC100 from the preceding assay) or distilled water was evenly applied to the diet surface (227 mm2) within each well. A 2-d-old first instar was then placed in each well. Four replicates (plates) were used for each treatment with 24 larvae per replicate. Percentage mortality and instar attained were monitored daily until all treated larvae had died. Mortality was compared within dates by two-sample t-tests on arcsine square root-transformed percentages. Analysis for instar was by one-sample t-test because of absence of variance in the virus-treated group.

Lethal and Sublethal Effects on Third and Fifth Instars. Lethal and sublethal effects of AgipMNPV on third and fifth instars were evaluated across a range of acute dosages. Virus suspensions (0, 5, 25, 125, 625, or 3,125 OBs per µl) in distilled water were prepared as described previously. Larvae were starved for 4 h, and then droplet-fed 5 µl of the appropriate suspension. Larvae that consumed the full droplet within 15 min were moved to individual 30-ml diet cups containing 20 ml of pinto bean diet and monitored daily for mortality for 10 d. There were four replicates of 30 larvae per dose (0, 25, 125, 625, 3,125, or 15,625 OBs per larva) for both instars. Pupal weight (1 d after pupation), and days to pupation and moth eclosion also were recorded for survivors from each cohort. Death due to viral infection was confirmed by examining hemolymph for OBs by using phase-contrast microscopy. Percentage mortality was arcsine square root-transformed and analyzed using one-way analysis of variance (ANOVA), within cohorts, for treatment (dose) effects, followed by polynomial contrasts to test for linear or quadratic trends (Analytical Software 2000).

First instars were included in the aforementioned trial for general comparison and to verify potency of the virus suspensions. They were dosed by swabbing 5 µl of the appropriate AgipMNPV suspension (the same amount ingested by the third and fifth instars as a single acute dose) onto the surface of diet in 24-well culture plates, as described above. One neonate was transferred to each well and observed daily for mortality. Dose–mortality response was analyzed by probit analysis as described above.
Cessation of Feeding and Growth of Older Larvae. Another trial evaluated how quickly late instars stop feeding and growing after ingesting a lethal dose of virus. Fourth instars (1 d postmolting) were starved overnight and then provided a 3-μl droplet containing either 3000 OBs per μl or distilled water. Larvae that consumed the full 9000-OB dose were transferred to individual 30-ml cups with perforated snap lids and provided a 6-g disk (wet weight) of pinto bean diet every other day. There were four replicates of 20 larvae per treatment. Larvae and remaining diet were weighed at 1, 3, 5, and 7 d after infection. Means for controls and still-living larvae that subsequently died from AgipMNPV were compared within intervals by two-sample t-tests.

Horizontal Transmission in Turfgrass. Greenhouse studies were performed to determine capacity for horizontal transmission of AgipMNPV in turf. On 3 October 2004, 20 polyvinyl chloride (PVC) cylinders (20.3 cm in diameter by 10.2 cm in height) were driven 8 cm deep into a 1,675-m² stand of ‘Penncross’ creeping bentgrass, *Agrostis stolonifera* (Huds.), on a Maury silt loam soil (fine, silty, mixed, mesic Typic Paleudalf, soil pH 6.2) at the University of Kentucky Spindletop Research Farm, near Lexington. The turf was managed similarly to golf course fairways, i.e., fertilized with 146 kg N/ha/yr from urea, irrigated from a permanent sprinkler system to prevent drought stress (2.54 cm/wk), and mowed at 16 mm three times per week. The cylinders with enclosed turf and soil were pried up and brought to a greenhouse. Treatments were zero, one, three, or six AgipMNPV-killed fifth instars of black cutworm added to each enclosure, with five replicates blocked by location on the greenhouse bench. Those larvae had been infected by droplet-feeding (1,500 OBs), held on diet until death, and frozen at −80°C until use. The cadavers were placed on the turf surface, where naturally infected larvae typically die, and allowed to disintegrate for 4 d. Twenty healthy, laboratory-reared fourth instars were then added to each cylinder and allowed to burrow into the turf. Surviving larvae were recovered after 6 d by using a soap drench consisting of 10 ml Joy Ultra dishwashing detergent (Proctor and Gamble, Cincinnati, OH) per 7.75 liters of water (Potter 1998). Larvae were rinsed with distilled water to remove the soap, placed in individual capped 30-ml cups with diet, and observed for mortality as before. Viral infection of dead larvae was verified as described above. Percentage mortality was analyzed by two-way ANOVA on arcsine square root-transformed values, followed by polynomial contrasts to test for linear response to dose (i.e., number of cadavers).

Field Efficacy, 2003. An initial field trial was conducted to investigate potential for a sprayed AgipMNPV suspension to control *A. ipsilon* in turf. The study site was one end of the previously described stand of creeping bentgrass at Spindletop farm. PVC rings (10.2 cm in height, 39 cm in diameter, 0.12-m² enclosed area) were cut from sewer pipe and sharpened on one edge. Twenty-four such enclosures were partially driven (7 cm) into the turf in a randomized complete block with six replicates and 0.3 m between enclosures. Each enclosure was inoculated with 20 laboratory-reared fourth instars on 24 September 2003. Larvae were allowed to burrow in and acclimate for 24 h. Enclosures were individually covered with 0.64-cm mesh wire hardware cloth to prevent bird predation and petroleum jelly was applied to their inner lip to discourage larval escapes. The next evening, the turf within each enclosure was sprayed with one of four suspensions: high rate (5 × 10⁸ OBs per m²), high rate with immediate watering-in (1 cm, from a watering can), low rate (5 × 10⁶ OBs per m²), or control (distilled water). The siphon tube of a hand-held pump-type sprayer (Sprayco, Detroit, MI) was inserted into a 50-ml centrifuge tube containing 20 ml of virus suspension; 15 ml of the suspension was then sprayed onto each plot. Different sprayers and tubes were used for each treatment. A 61-cm-tall bottomless cardboard box was placed around each plot while treating to prevent spray drift and cross-contamination.

Larvae were recovered 4 d after treatment by the aforementioned soap flush method, rinsed with distilled water, and placed into individual cups with diet in the field. Cups from each enclosure were placed in separate plastic trays, taken to the laboratory, and checked daily until death or pupation. Death from viral infection was verified by examining hemolymph for OBs. Percentage mortality with AgipMNPV was arcsine square root-transformed and then analyzed by two-way ANOVA followed by orthogonal contrasts to compare treatments versus the control, and the high rate of virus with or without watering in.
ments. Larvae were recovered by soap drench after 6 d. Procedures for spraying, handling larvae, rearing, and mortality assessment and for data analysis were as for the Field Efficacy, 2003 trial.

**Field Residual Efficacy, 2005.** This trial evaluated potential for AgipMNPV residues to provide extended control of black cutworms in fairway-height turf. The study site was the opposite end of previously described creeping bentgrass stand used for the 2003 field trial. Thirty-six plots (45.7 by 45.7 cm) were marked with spots of turf paint. Individual plots were separated by 3 m and arranged in a randomized complete block with six replicates. Treatments were AgipMNPV (7 × 10⁹ OBs per m²) applied 28, 21, 14, 7, or 1 d before cutworms were introduced, plus an untreated control. Treatment dates were 19 and 26 May and 2, 9, and 16 June, respectively. Spray methodology was as described above except that 25-ml spray volume was applied to each plot. PVC enclosures (39 cm in diameter by 10.2 cm in height) were driven into each plot, and 1,080 total larvae (30 per enclosure) were introduced on 17 June. Enclosures were covered with hardware cloth and greased as before. Surviving larvae were recovered after 4 d and individually reared and evaluated as in earlier field trials. Percentage mortality from viral infection was verified and analyzed as described above.

**Results**

**LC₅₀ and Time to Death for Infected First Instars.** The probit–dose relationship for *A. ipsilon* infected as first instars by droplet-feeding did not depart from linearity ($\chi^2 = 140.0, P < 0.0001$; slope = $1.75 \pm 0.15$; intercept = $-3.25$). The calculated LC₅₀ value was 73 OBs per μl (95% CI, 54.5–97.7; 2-μl dose). Most larvae that died did so 6–9 d after ingesting the relatively higher dosages of virus (Fig. 1).

First instars placed on diet swabbed with a lethal rate of virus began dying within 3 d, and 100% had died within 6 d (Fig. 2). All virus-treated larvae died as second instars, whereas controls had high survival with normal growth. Reduction in survival ($t = 4.6, df = 6, P < 0.005$) and stadium ($t = 2.25, df = 7, P < 0.05$) were evident within the treated cohorts by 3 and 4 d, respectively.

**Lethal and Sublethal Effects on Third and Fifth Instars.** Mortality of droplet-fed third and fifth instars increased linearly with increasing viral concentration ($F = 49.5, 12.5$, respectively; $df = 5, 20, P < 0.05$; polynomial contrasts, $P < 0.05$) but averaged only 42 ± 2 and 30 ± 2%, respectively, at the highest dose (Fig. 3). That amount of virus applied to the diet surface resulted in 91 ± 3% mortality of first instars (Fig. 3). The probit–concentration relationship for first instars was linear ($\chi^2 = 111.0, P < 0.0001$; slope = $1.22 \pm 0.12$; intercept = $-2.24$). The calculated LC₅₀ value was 67 OBs per μl (95% CI, 45.2–97.8), similar to the estimate from the previously described droplet-feeding assay.

Survivors from third-instar cohorts that had ingested relatively high amounts of virus produced slightly smaller pupae ($F = 8.2, df = 5, 554; P < 0.001$). Mean ± SE pupal weights were 463 ± 5, 447 ± 4, 444 ±
5, 434 ± 5, 431 ± 5, and 422 ± 6 mg for dosages of 0, 25, 125, 625, 3,125, and 15,625 OBs, respectively, with a significant linear trend (polynomial contrasts, \( P < 0.001 \)). There also were small nonlinear sublethal effects on developmental rate. Days to pupation averaged 11.7 ± 0.2, 11.8 ± 0.1, 12.1 ± 0.2, 12.2 ± 0.1, 12.1 ± 0.2, and 11.7 ± 0.2 (\( F = 2.81, \text{df} = 5, 554, P < 0.02 \)), and days to eclosion averaged 26.6 ± 0.2, 26.7 ± 0.2, 27.3 ± 0.2, 27.4 ± 0.2, 26.9 ± 0.2, and 26.4 ± 0.3 (\( F = 3.11; \text{df} = 5, 543; P < 0.01 \)) for the aforementioned six dosages, with quadratic response trends (\( P < 0.005 \)) for both parameters.

Pupal weights of survivors that ingested the aforementioned dosages as fifth instars were 438 ± 5, 437 ± 5, 436 ± 5, 445 ± 5, and 431 ± 6 mg, respectively (\( F = 2.36; \text{df} = 5, 634; P < 0.05 \)) with nonsignificant linear or quadratic response to dose. Days to pupation for those larvae averaged 9.4 ± 0.1, 9.3 ± 0.1, 9.4 ± 0.1, 9.5 ± 0.1, 9.2 ± 0.1, and 8.7 ± 0.1 (\( F = 4.56; \text{df} = 5, 634; P < 0.01 \)), suggesting slightly accelerated development at the highest dose. Days to eclosion, however, was not affected (\( F = 1.43; \text{df} = 5, 614; P > 0.05 \)). Means were 23.4 ± 0.2 and 23.0 ± 0.2 d, respectively, for controls and the highest viral dose.

Cessation of Feeding and Growth of Older Larvae. Lethally infected and control groups started with 50 and 71 larvae, respectively; numbers of infected larvae still living after 1, 3, 5, 7, or 9 d were 50, 48, 47, 24, and 0, respectively. There was no mortality of controls. Lethally infected fourth instars weighed significantly less than healthy controls at 1.5, and 7 d after ingesting the virus (\( t = -4.6, -5.9, -4.9; \text{df} = 119, 116, 93; P < 0.001 \); Fig. 4). Their feeding, however, was only slightly reduced until close to death (\( t = -18.1, \text{df} = 116, P < 0.01; t = -13.9, \text{df} = 93, P < 0.01 \) for the 3–4 and 5–6-d intervals, respectively; Fig. 4).

Horizontal Transmission of AgipMNPV. Lethal infection of implanted third instars increased linearly in response to numbers of virus-killed cadavers disintegrating on the plot surface (\( F = 5.98; \text{df} = 3, 12; P = 0.01 \); polynomial contrasts, \( P < 0.05 \)). Mean ± SE percentage mortality with AgipMNPV was 29 ± 9, 39 ± 10, 51 ± 6, and 73 ± 13 for plots upon which zero, one, three, or six cadavers, respectively, had been placed. Virus infection in the controls was similar to that observed from naturally occurring AgipMNPV in the turfgrass stand from which the cores were obtained (see below).

Field Efficacy, 2003. Mean ± SE number of larvae recovered per plot (of the original 20) ranged from 10.1 ± 2.3 to 13.7 ± 1.1, with no difference between treatments (\( F = 0.64; \text{df} = 3, 15; P = 0.60 \)). There was ≈34% background mortality from naturally occurring AgipMNPV in control plots (Fig. 5). Both spray rates of virus significantly increased the incidence of lethal infection (\( F = 5.95; \text{df} = 2, 9; P = 0.02 \); orthogonal contrasts, \( P < 0.05 \)). Watering-in the virus spray before it dried on the foliage reduced its efficacy (orthogonal contrast, high rate with or without water, \( P = 0.06 \)).

Field Efficacy with or without Adjuvants on a Golf Course Putting Green. All treatments resulted in higher incidence of lethal infection than occurred in

![Fig. 4. Larval weight gain (top) and food consumption during each of three successive 2-d intervals (bottom) after fourth instars of A. ipsilon were droplet-fed a lethal dose (9,000 OBs) of AgipMNPV, or distilled water. Only still-living larvae that subsequently died from AgipMNPV are shown for the infected group. Asterisks denote significant within-interval differences (\( t \)-tests, \( P < 0.05 \)).](image1)

![Fig. 5. Mean ± SE percentage mortality of fourth instars of A. ipsilon in AgipMNPV-sprayed fairway-height creeping bentgrass field plots. Low and high rates of virus were 5 × 10^6 and 5 × 10^6 OBs per m², with posttreatment irrigation (1 cm) applied to one set of high-treated plots before residues had dried. Asterisks denote means differing significantly from the control (two-way ANOVA, Dunnét’s test, \( P < 0.05 \)).](image2)
control plots ($F = 22.9; df = 4, 16; P < 0.001; Dunnett’s test, $P < 0.01$). Mean ± SE percentage mortality with AgipMNPV averaged 90 ± 2, 90 ± 5, 94 ± 3, and 91 ± 2, respectively, for AgipMNPV alone or with the optical brightener M2R, a spreader sticker, or both adjuvants in the spray mix. Mortality from naturally occurring AgipMNPV in the control plots averaged 27 ± 5%. Neither adjuvant increased the incidence of lethal infection from AgipMNPV over spraying the virus alone (orthogonal contrasts, $P < 0.001$).

### Field Residual Efficacy, 2005

Mean ± SE numbers of larvae recovered per plot of the (original) 30 ranged from 240.0 ± 1.4–25.8 ± 1.2, with no difference between treatments ($F = 0.47$; df = 5, 24; $P = 0.79$). Percentage of recovered larvae the eventually died from virus ranged from 74.8% for plots having 1-d-old AgipMNPV residues to 8.9% for larvae from control plots ($F = 14.7$; df = 5, 24; $P < 0.001$; Fig. 6). Incidence of lethal infection ranged from 44 to 51% in plots with 7-, 14-, 21-, or 28-d-old virus residues, higher in each of those treatments than in control plots (Dunnett’s test, $P < 0.05$) but lower than for plots with 1-d-old residues (orthogonal contrasts, $P < 0.05$). Age of the virus residues on the field plots did not affect how quickly infected larvae died from virus. Days to death, calculated from when larvae were introduced into the field plots, ranged from 9.1 ± 0.5 to 9.5 ± 0.4 d ($F = 1.01$; df = 4, 20; $P = 0.42$).

### Discussion

This is the first study to investigate use of a virus to suppress an insect pest in turfgrass. AgipMNPV was isolated and amplified from naturally infected larvae from golf courses where viral epizootics were observed. Laboratory assays showed AgipMNPV to be highly active against early instars with a positive dose–response. Later instars required higher lethal dosages and continued feeding for several days to a week after consuming a lethal dose. Horizontal transmission was demonstrated. Sprayed virus suspensions provided 75 to >90% mortality of implanted third instars in turfgrass field plots, providing residual suppression in creeping bentgrass for at least 28 d. AgipMNPV therefore seems promising as a biological control agent for *A. ipsilon* on golf courses and sport fields.

Turf settings, particularly golf course greens and surrounds, seemingly are a good match for viral insecticides. Turfgrass is a perennial system with dense foliage, so once a virus is established in the thatch and soil it should be less vulnerable to UV degradation than in agricultural crops. Frequent watering may promote viral transmission by moving inoculum from the soil to plants, which in nonirrigated crops depends largely on rainfall (Fuxa and Richter 2001). Black cutworms tend to be the primary insect pest on golf greens (Potter 1998). The females deposit their eggs on grass blades (Williamson and Potter 1997), so viral infection should debilitate early instars before they reach destructive size. Finally, golf greens and surrounds receive more pesticides per unit area than any other turfgrass sites (Smith and Tillotson 1993). For that reason, and their frequent proximity to ponds and streams, greens are a focal point for concerns about human and environmental exposure to insecticides. The golf industry is increasingly receptive to biological insecticides (Kenna and Snow 2000). Golf courses, and also sport fields, therefore are a potential market for a selective baculovirus having potential to provide extended suppression of a key insect pest.

It is unclear why epizootics of AgipMNPV occurred in *A. ipsilon* populations on central Kentucky golf courses in 2002–2004. Average cumulative rainfall for April to September 2002–2004 was 86.4 cm, well above the average of 61 cm for that period in the previous decade (http://wwwagwx.ca.uky.edu/cgi-public/wxlist days www.ehtm). Abundant rain might have brought virus in the soil and thatch into contact with foliage (Fuxa and Richter 2001) and helped disseminate OBs throughout the turf.

Baculovirus epizootics occur irregularly in field populations of agricultural pests, including various noctuid species (Cory and Myers 2003). Horizontal transmission depends on susceptible larvae encountering and ingesting a discrete patch of viral OBs that resulted from the death of an infected individual (Cory and Myers 2003). It depends in part on the relative density of infected to susceptible hosts. Larval densities in our horizontal transmission plots were high but not unrealistic given that individual *A. ipsilon* females deposit as many as 1,600 eggs patchily in turf and that early instars feed in aggregations on grass foliage before dispersing and feeding both from burrows and by grazing on the turf surface as late instars (Williamson and Potter 1997). Fluctuations in temperature and relative humidity, plant secondary chemicals, cultural practices (e.g., cultivation), chemical stress, parasitism, secondary pathogens, and other factors also may play a role in triggering viral epizootics. For example, baculovirus epizootics in populations of *Wiseana* spp. (Lepidoptera: Hepialidae), a soil-dwelling pasture pest in New Zealand, may be affected by pasture age, grazing history, and cultivation as well as larval density, rainfall, and dissemination of OBs in bird droppings (Crawford and Kalmokoff 1977, Flem-
ing et al. 1986). Understanding why AgipMNPV has greater or lesser impact on A. ipsilon in some years, or at some sites, will require better understanding of both host and virus population dynamics in turfgrass.

None of the main textbooks on turf entomology (Brandenburg and Villani 1995, Potter 1998, Vittum et al. 1999) mention baculoviruses associated with A. ipsilon or any other specific pest. There have been no published life tables or natural enemy surveys for A. ipsilon in turf so it is understandable that a virus that is most infective to early instars has been overlooked. Background AgipMNPV accounted for 34 and 27% mortality of fourth and third instars of cutworms introduced into control plots at our 2003 and 2004 field sites, despite those larvae being in the turf for just a few days. Mortality in controls probably did not result from contamination from treated plots, because the enclosures were sprayed from close range, a barrier was used to prevent drift, and the enclosures themselves would have minimized any runoff or passive movement of the pathogen. We expect that Agip-MNPV is a widespread mortality agent for A. ipsilon, especially early instars, on golf courses, sports fields, and similar sites.

Susceptibility to baculovirus infection typically decreases with increasing larval age (Cory and Myers 2003), a phenomenon related in part to the greater capacity of later instars to slough off virally infected midgut tissues (Keddie et al. 1989, Hoover et al. 2000). A one-time AgipMNPV dose that was lethal to first instars resulted in only 46 and 30% mortality of third and fifth instars in trials. Sprayed virus suspensions nevertheless resulted in eventual 75-94% mortality of third or fourth instars in our field trials, probably because the larvae consumed greater total amounts of virus while feeding in the turfgrass for several days. Defensive response to baculoviruses also may involve activation of the phenyloxidase cascade (Trudeau et al. 2001). Surviving viral challenge can be costly and may cause arrested development and reduced vigor or reproductive fitness (Rothman and Myers 1996, Cory and Myers 2003). We found, however, at most only small differences in developmental time and pupal weight of sublethally dosed third and fifth instars.

Boughton et al. (2001) estimated median survival times of 4–5 d for third instars of A. ipsilon droplet-fed lethal doses of the Illinois AgipMNPV isolate. Spray or bait formulations of AgipMNPV reduced damage by introduced third instars to corn seedlings in their small-plot greenhouse and field trials. Those reductions were relatively small (<30%), however, and seedlings in treated plots still sustained considerable damage. In our study, fourth instars infected with Kentucky-isolate AgipMNPV fed and grew for several days to a week after consuming a lethal dose. Slow speed-of-kill of third and fourth instars also occurred in our field trials where infected larvae that later died from virus caused noticeable damage during the 4–6 d they were in the turf, as well as normal surfacing response to the soap flush. Lethally infected first instars, in contrast, showed arrested development and little feeding. AgipMNPV therefore seems better suited for preventive application targeting early instars than as a curative or rescue treatment for damage-causing late instars.

Unlike Bacillus thuringiensis, which is pathogenic to early instars of many caterpillar species, baculoviruses usually are relatively host specific (Cory and Myers 2003). Boughton et al. (1999) showed the Illinois Agip-MNPV isolate to be moderately infective to Heliothis virescens (F.) and Helicoverpa zea (Boddie) but essentially noninfective to six other noctuids, including fall armyworm, Spodoptera frugiperda (J.E. Smith), and the one crambid species that was tested. Home lawns therefore are probably not a potential market for an AgipMNPV-based bioinsecticide because their main caterpillar pests tend to be sod webworms (Pyralidae) and fall armyworm rather than A. ipsilon (Potter 1998).

Guidelines for chemical control of black cutworms usually suggest withholding irrigation and mowing for 12–24 h after spray applications to keep residues on the blades and stems where larvae will consume them during the night (Potter 1998). Watering-in the high-rate virus spray before it dried reduced efficacy in our 2003 field trial, so postponing irrigation also might be advisable if AgipMNPV were applied as a bioinsecticide. Sprayed AgipMNPV suspensions nevertheless provided infection of third instars for at least 4 wk in our residual field trial despite the turf being mowed and irrigated several times per week. They also provided high rates of infection in the 2004 trial on a putting green collar where the site was irrigated daily. Formulating AgipMNPV with a spreader-sticker or the M2R optical brightener provided no added value in our 2004 field trial where high efficacy of the virus suspension alone would have obscured any potential benefit of the adjuvants. The greatest value of formulating baculoviruses with optical brighteners is to obtain similar levels of pest suppression at lower virus application rates (Shapiro 1995, Boughton et al. 2001). Such synergism might have been apparent had we used a lower application rate of virus.

In summary, the virulence, particularly to early instars, and persistence of AgipMNPV in turfgrass make it a good candidate as a bioinsecticide for A. ipsilon on golf course putting greens and surrounds, and sports fields. We envision that the virus would be applied preventively, early in the growing season, causing high mortality in the first generation and creating a reservoir of OBs in the thatch that suppresses subsequent generations throughout the growing season. Once established at a site, the virus could potentially give multiple years or even permanent suppression of the target pest. Commercial production of baculoviruses currently is accomplished primarily in vivo (Moscardi 1999), although there is progress toward large-scale in vitro production (Slavicek et al. 1996, Black et al. 1997). This study provides groundwork for understanding how naturally occurring AgipMNPV suppresses black cutworm populations in turfgrass, and hopefully it will encourage interest in developing viral insecticides for use on turf.
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