Research Article

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Can a chitin-synthesis-inhibiting turfgrass fungicide enhance black cutworm susceptibility to a baculovirus?

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Abstract

BACKGROUND: Developmental resistance, i.e. reduced virulence and speed of kill of late instars, is a limiting factor in the use of baculoviruses for caterpillar control. *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (*Agip*MNPV) is highly infective to young black cutworms, *Agrotis ipsilon*, but too slow-acting against late instars for effective curative control on golf courses or sports fields. Chitin-synthesis-inhibiting fungicides containing the active ingredient polyoxin-d are used to control fungal diseases in turfgrass, and similar compounds have been shown in the laboratory to synergize baculoviruses by disrupting peritrophic membrane function. This study tested whether applying the virus together with such a fungicide can synergize *Agip*MNPV activity against *A. ipsilon* in turfgrass.

RESULTS: The addition of a chitin synthesis inhibitor failed to increase *Agip*MNPV infectivity to *A. ipsilon* in the field. Rather, delayed and slightly reduced mortality from viral infection was seen when larvae fed on fungicide/virus-treated grasses as opposed to virus-only treatments. Choice tests revealed the fungicide residues to be a mild feeding deterrent.

CONCLUSION: Because polyoxin-d does not deactivate *Agip*MNPV, the two substances are compatible. However, combination applications of polyoxin-d and *Agip*MNPV on turfgrass might interfere with larval ingestion of a lethal virus dose, resulting in prolonged larval feeding in the field.

Keywords: baculovirus; *Agip*MNPV; fungicide; polyoxin-d; chitin synthesis inhibitor; *Agrotis ipsilon*; turfgrass

1 INTRODUCTION

Baculoviruses (family Baculoviridae, genus Nucleopolyhedroviruses), present a seemingly good alternative to broad-spectrum insecticides because of their efficacy, specificity and safety to humans and other non-target organisms. They have been used worldwide to manage pests in various cropping systems and forests.[1–5] It is striking, however, given that >400 insect species, mostly members of the orders Lepidoptera and Hymenoptera, have been reported as hosts for baculoviruses, how infrequently they are successfully used in integrated pest management programs.[3,6,7]

One of the limitations of baculovirus-based insecticides is their relatively slow speed of kill, especially of late instars.[3–5,8] As larvae mature, they typically become less susceptible to virus infection and may continue to feed for several days after ingesting a lethal dose, so targeting early instars is necessary to avoid economic damage to plants.[9–11] Most research to enhance the usefulness of baculoviruses has focused on using optical brighteners to protect them from degradation by ultraviolet light.[12–14] Another approach is to increase the virulence of the virus itself. For an insect to become infected, it must first ingest virus occlusion bodies (OBs) while feeding. After ingestion, the OBs release virions in the host midgut, which then must pass through the peritrophic membrane to initiate virus infection in the midgut.[10] This chitinous membrane is the insect's first line of defense against a virus, so a compound that disrupts its function may help facilitate infection and increase speed of kill. Additives such as optical brighteners may work this way,[12] but chitin synthesis inhibitors, too, have been shown to synergize baculoviruses and dramatically increase their activity by disrupting peritrophic membrane function.[15,16]

Polyoxins are *Streptomyces*-derived antibiotics that inhibit fungal and insect chitin syntheses.[17–19] Polyoxin-d strongly affected peritrophic membranes *in vitro* in adult blowflies, *Calliphora erythrocephala*, by inhibiting chitin synthesis and by changing the fine structure of the membrane.[20] Nucleopolydrovirus (NPV) susceptibility was increased in larvae of the silkworm, *Bombyx mori*, when commercially available polyoxin fungicidal agents were incorporated into the insect's artificial diet.[15,16] Enhanced biological activity of *Spodoptera litura* NPV by a chitin-synthesis-inhibiting compound was attributed to obvious ruptures on the outer surfaces of the peritrophic membrane, which potentially facilitated the passage of virions through the peritrophic membrane.[21] These compounds have been validated as synergists in laboratory experiments but have not been tested in the field.

The black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae), is nearly a worldwide pest of golf-course putting greens and tees, as

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well as sports fields and various garden crops.[22,23] In turf, the night-active larvae chew down the grass surrounding their burrows, causing brown pock marks that reduce smoothness and uniformity of playing surfaces.[23] Prater *et al*.[11] documented a natural epizootic of *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (*Agip*MNPV) decimating black cutworm populations on Kentucky golf courses, established dose–mortality relationships and demonstrated that a sprayed viral suspension can provide short-term control in the field.[11] When sprayed suspensions of *Agip*MNPV were evaluated for season-long control of black cutworm on creeping bentgrass (*Agrostis stolonifera* L.) golf-course tees under actual maintenance and play, one-week-old virus residues reduced larval populations resulting from introduced eggs by 76–82%. Residual control, however, lasted no more than a few weeks.[24] *Agip*MNPV quickly controls young larvae, but larger late instars require higher dosages and continue to feed for several days before being killed.[11,24] Combinations of *Agip*MNPV with adjuvants, such as optical brightener and lignin, failed to accelerate or extend efficacy of the virus against *A. ipsilon* in the field.[24] Even if they had worked, such adjuvants likely would be too expensive to use in synergizing virus applications targeting grass-feeding caterpillars on golf courses or sports fields.

If baculovirus efficacy could be enhanced by something already being used in the turf or crop system, land managers would incur no additional cost. For example, fungicides containing the active ingredient polyoxin-d are already being used, sometimes several times per season, to control turfgrass diseases such as brown patch, *Rhizoctonia* spp. An overlapping application of polyoxin-d fungicide and baculovirus would be a practical combination in golf-course settings, because fungal diseases and cutworm infestations often occur on the same tees, greens and other highly maintained sites. The purpose of this study was to determine whether the combined use of a chitin-synthesis-inhibiting substance, polyoxin-d, could enhance or synergize *Agip*MNPV activity against *A. ipsilon* in turfgrass.

2 MATERIALS AND METHODS

2.1 Insects, virus and fungicide

Agrotis ipsilon eggs and larvae were obtained from a commercial insectary (Benzon, Carlisle, PA) where they had been maintained on soybean-based diet. They were shipped in cups of diet by overnight mail and transferred to the present assays within a few hours of arrival. The *Agip*MNPV isolate used in all experiments was originally obtained from naturally infected late-instar *A. ipsilon* from central Kentucky golf courses.[11] Frozen infected caterpillars were macerated in 0.1% sodium dodecyl sulfate (SDS) for 10 min and filtered through five layers of cheesecloth. Virus OBs were then centrifuged at 900 × *g* for 10 min. The pellet was resuspended in 0.5% SDS and centrifuged again. Resuspension and centrifugation were repeated with 0.5 M NaCl with the final suspension in distilled water. Sodium azide was added at 0.02% concentration to prevent bacterial growth. This purified OB suspension was stored at 4 °C. OB concentrations were determined using a phase contrast microscope and a Neubauer bright-line hemocytometer (Fisher, Pittsburgh, PA).

The polyoxin formulation evaluated as a synergist for *Agip*MNPV was Endorse® wettable powder fungicide (Arysta LifeScience, Cary, NC), containing 2.5% active ingredient polyoxin-d zinc salt (equivalent to 2.2% polyoxorim and 0.3% metallic zinc), zinc 5-{[2-amino-5-*O*-(aminocarbonyl)-2-deoxy-L-xylonoyl]amino}-1-[5-carboxy-3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl]-1,5-dideoxy-β-D-allofuranuronate. Endorse® is a group-19 fungicide and is labeled for controlling fungal diseases on golf courses, residential lawns, parks and commercial and institutional grounds. The wettable powder was dissolved in distilled water for all applications.

2.2 Evaluating virus/fungicide combinations in small field plots

An experiment initiated in July 2010 tested whether increased activity is provided to *Agip*MNPV residues by the fungicide. The trial was conducted in a stand of 'Penncross' creeping bentgrass on a Maury silt loam (fine, mixed, mesic typic Paleudalf; pH = 6) at the University of Kentucky's Turfgrass Research Center (UKTRC), Spindletop Farm, near Lexington, Kentucky. The turfgrass, representative of a golf-course fairway, was mowed at 1.6 cm 3 times per week, irrigated as necessary to prevent drought stress and fertilized in September, October and November at 0.48 kg actual N per 100 m^2 per application from urea (46-0-0). Fungicides (non-polyoxin) had been applied curatively, as needed, for control of fungal diseases, but were not used for at least 4 weeks before the present trials.

Individual plots were 0.5 m^2, with 1 m^2 buffers, and arranged in a randomized complete block with six replicates of each treatment. Virus suspensions were prepared as described above. Treatments included high, medium and low rates of virus (5×10^8, 5×10^7 and 5×10^6 OB m^{-2}) with and without fungicide, fungicide alone and an untreated control. Fungicide treatments were at a high label rate for golf-course fairways [1.2 g (product) m^{-2}]; virus rates were based on previous field experiments.[11,24] Larvae were confined in circular metal enclosures (39.0 cm diameter, 10.2 cm height) which were twisted and pressed to seat their lower edge about 1 cm into the ground. Each solution was dissolved in 50 mL of water and applied using a hand-pump sprayer inserted into a 50 mL plastic vial. The area inside each enclosure (0.12 m^2) was treated, and larvae were introduced as soon as the residues had dried.

Twenty third-instar *A. ipsilon* were introduced into each of the metal enclosures, which were then 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 of lemon-scented dishwashing detergent (Joy®; Proctor & Gamble, Cincinnati, OH) per liter of water.[23] Larvae were rinsed with distilled water as soon as they surfaced, placed in individual capped 30 mL cups with soybean-based noctuid diet,[25] held at 25 °C and monitored until death or pupation. Death due to viral infection was verified by examining blood for viral OBs by using a phase contrast microscope at 400× magnification.

2.3 Testing for direct insecticidal effects of fungicide

The soap drench brought up relatively few cutworms from fungicide-treated plots in the above experiment, suggesting that there had been a disproportionately high number of escapes from those enclosures, or mortality from the fungicide itself. Therefore, a follow-up trial was conducted at the same field site to determine whether the fungicide alone reduced cutworm survival. Treatments included high and low rates of fungicide (1.2 and 0.6 g m^{-2}) and an untreated control. Plots were again 0.5 m^2 with a 1 m^2 buffer, and set up in a randomized complete block design with six replicates of each treatment. The experiment was carried out as described above; however, the metal enclosures were driven more deeply (3 cm) into the turf to ensure that larvae could not escape by burrowing beneath their edges.

2.4 Testing fungicide/virus synergism; greenhouse trials

In August 2010, creeping bentgrass cores (15.2 cm diameter, 6.5 cm deep) were harvested with an oversized golf-course cup cutter from the aforementioned creeping bentgrass stand. Grass cores were placed in pots with a small amount of potting mix below and around them to help maintain moisture. The potting mix consisted of 3 : 1 Pro-Mix BX (Premier Horticulture, Quakertown, PA) and autoclaved topsoil. Plants were watered as needed. The turfgrass was maintained in a glasshouse under a 14 h photoperiod with supplemental lighting from 1000 W sodium vapor bulbs unless ambient light was $\geq$450 L mol m^{-2} s^{-1}, and watered as needed to maintain vigor. Day and night temperatures were set at 22 and 18 °C respectively.

The treatments (virus/fungicide combinations) included high, medium and low rates of virus (5×10^9, 5×10^6 and 5×10^3 OB m^{-2}) with or without fungicide at high, medium and low rates (2.1, 0.21 and 0.012 g m^{-2}), plus an untreated control. Each solution was dissolved in 50 mL of water and applied using a separate hand-pump sprayer inserted into a disposable tube containing the treatment combination. Six replicates of each treatment were arranged on greenhouse benches in a randomized complete block design. Treatments dried for 20 min before third-instar *A. ipsilon* (12 per pot) were introduced into pots. Larvae were allowed to feed on treated grasses for 24 h. Cutworms were recovered by removing the grass plugs from their containers and examining the soil, roots, thatch and grass. The grass plug was then placed back into the pot, and those few remaining larvae were extracted using a soap disclosing solution and immediately rinsed with fresh water to remove soap as soon as they surfaced. All larvae were placed individually in 30 mL rearing cups with artificial diet and monitored until death or pupation, as above. Days until death were recorded. Death due to viral infection was verified by examining blood for OBs using a phase contrast microscope.

The above experiment was repeated to determine how varying the duration of exposure by feeding cutworms might affect virus synergism by the fungicide. Two virus rates (1×10^8 and 5×10^8 OB m^{-2}) and one fungicide rate (2.1 g m^{-2}) were applied alone and in combination, plus an untreated control. Cohorts of five replicates per treatment were set up in a randomized complete block design to be sampled at three different times (after 1, 2 and 4 days of feeding and exposure).

2.5 Fungicide effects on consumption of treated grass

Feeding preference of neonates and third instars was compared between fungicide-treated and untreated grass to try to reconcile results from the field and greenhouse experiments. More specifically, the hypothesis was tested that reduced consumption of fungicide-treated grass might interfere with cutworm ingestion of a lethal virus dose, thus resulting in lower infection rates. Creeping bentgrass cores were collected from the UKTRC on 13 September and maintained in a glasshouse as described above. Grass clippings were cut into 2.5 cm sections. The clippings were treated with the label rate of fungicide (2.1 g m^{-2}) by dipping them into the mixed fungicide solution for 5 s and then allowing the residues to air dry. Three treated and three untreated clippings were placed in an alternating, spoke-like arrangement on a moistened filter paper in the bottom of a polystyrene petri dish (90 mm $\times$ 15 mm). Ten neonates were placed in the center of each dish before replacing the lid. For the no-choice tests, one treated or untreated grass blade and one neonate were placed in each arena. There were 20 replicates for each test. Larvae were left to feed in the dark for 17 h at room temperature (about 22 °C). The total area of leaf tissue consumed in each treatment was visually estimated to the nearest 10% by two independent observers whose ratings were averaged, and the number of larvae actively feeding was also scored for each dish and treatment.

The trials were repeated with third instars, using larger arenas (styrofoam bowls, 115 mm $\times$ 50 mm). Grass blades were held in place on moistened filter paper using insect pins to prevent them from being scattered by the larvae. A single larva was added to each bowl; bowls were then capped with plastic wrap, covered with another styrofoam bowl and placed in a dark growth chamber (27 °C). The percentage of each grass blade that had been consumed was visually estimated, as above, at 1, 4 and 18 h.

2.6 Statistical analysis

Larval recovery and weights, percentage mortality from virus and other variables were analyzed by a 2 $\times$ 4 (small-plot field experiment) or a 4 $\times$ 4 (greenhouse experiment) factorial analysis of variance (ANOVA) for main effects and interaction of fungicide and virus rate (weighted ANOVA was used for field experiment percentages). The effect of virus rate was analyzed by polynomial contrasts for significance of linear or quadratic trends. A three-way repeated-measures ANOVA also was conducted on cumulative percentage mortality for greenhouse experiments. Fixed factors were fungicide rate and virus rate (between-subject factors) and time after exposure to treatments (within-subject factor), with repeated measure (mortalities) on the time factor, as mortalities were recorded on groups of larvae within the same replicates over time. Dunnett's tests were performed to compare virus mortalities in control groups (virus alone) to fungicide/virus combinations. The percentage of fungicide-treated or untreated leaf tissue consumed in the choice and no-choice tests was compared by Wilcoxon signed-rank tests or two sample *t*-tests for no-choice tests respectively. Replicates were omitted from analysis if there was no feeding on either treatment. Chi-square tests also were used to compare total proportions of treated or untreated blades with some feeding damage. Statistix 8[26] was used for all statistical analyses except for weighted ANOVAs, for which SAS[27] was used. Percentage data were normalized by arcsine square root transformation for analysis. All data are reported as original (non-transformed) means $\pm$ SE.

3 RESULTS

3.1 Evaluating virus/fungicide combinations in small field plots

Few larvae were recovered from fungicide-treated plots, regardless of whether or not virus was included in the treatment (Table 1). The percentage of recovered larvae that ultimately died from viral infection increased at higher virus rates with a significant linear trend for rate. Lower rates of virus infection occurred in combination treatments than with virus alone, resulting in a significant fungicide by virus interaction; however, there was no main effect of fungicide on percentage mortality (Table 1).

3.2 Testing for direct insecticidal effects of fungicide

Unlike the first experiment, in which the relatively small number of larvae recovered from fungicide-treated plots had suggested mortality from the fungicide itself, or proportionately more escapes from those enclosures, similar numbers of larvae were recovered from fungicide-treated and untreated plots (control = 15.3 $\pm$ 0.6;

Table 1. Numbers of *A. ipsilon* recovered from small-plot field experiments, and percentage that died from virus infection

Rate (OB m^{-2})	Fungicide	Larvae recovered	% mortality[a]
0	−	11.3 ± 2.3	1.5 ± 1.5
	+	3.3 ± 1.7	4.8 ± 4.8
5×10^6	−	4.1 ± 2.0	8.5 ± 4.7
	+	2.5 ± 1.3	0 ± 0
5×10^7	−	8 ± 3.2	24.3 ± 8.9
	+	3.3 ± 1.9	7.8 ± 5.1
5×10^8	−	6.5 ± 1.9	61.7 ± 14.3
	+	1.0 ± 1.0	47.7 ± 21.4
ANOVA (*F*-values)[b]			
Fungicide		11.7**	0.2
Virus rate		1.6	18.4**
F × V		0.8	6.1*

[a] Weighted ANOVA.
[b] df = 1, 3, 3 and 35 for fungicide, virus rate, interaction and error respectively.
* and ** denote significance at $P \leq 0.05$ and 0.01 respectively.

high fungicide rate = 18.0 ± 0.9; low fungicide rate = 15.7 ± 1.5; $F_{2,10} = 2.9$; $P = 0.1$). This indicated that the fungicide itself did not have direct adverse effects on larval survival.

3.3 Testing fungicide/virus synergism; greenhouse trials

When larvae were exposed to treated turfgrass in the greenhouse for 24 h, the number recovered from the pots was similar for all treatments. Percentage mortality from virus infection increased as virus rate increased, but was similar for the two lowest virus rates. There was no significant main effect of fungicide (Table 2). A virus × fungicide interaction was seen; however, when fungicide/virus combinations were compared with comparable rates of virus alone, the percentage mortality from virus was similar regardless of whether or not fungicide was included.

When larvae were exposed to treated grasses for 1, 2 and 4 days, the number recovered from the pots again was similar for all treatments. Longer duration of feeding on treated grasses and exposure to the higher virus rates corresponded to greater mortality from virus infection ($F_{2,67} = 4$; $P = 0.02$ and $F_{2,67} = 115$; $P \leq 0.01$ respectively) for all exposure durations (Table 3). Within rates, larvae exposed to the low rate of virus alone experienced significantly higher mortality (41.8 ± 4.7 versus 22.1 ± 5.5; $F_{1,24} = 10.1$; $P \leq 0.01$) and died more quickly compared with larvae feeding on grasses treated with the low-virus/fungicide combination for all exposure times (Fig. 1 and Table 3). Larvae also died more quickly at the high virus rate compared with high-virus/fungicide combinations when exposed for 2 and 4 days; however, the rate of death was similar when exposed for only 1 day (Fig. 1 and Table 3).

Table 2. Numbers of larvae recovered from virus/fungicide-treated pots after 24 h of feeding, and percentage infected by virus in greenhouse trials

Rate (OB m^{-2})	Fungicide	Larvae recovered	% mortality[a]
0	High	10.3 ± 0.8	1.5 ± 1.5
	Medium	9.8 ± 0.9	4.8 ± 4.8
	Low	10.0 ± 0.4	4.8 ± 3.4
	0	11.2 ± 0.3	0 ± 0
5×10^3	High	10.7 ± 1.0	17.1 ± 4.7
	Medium	8.8 ± 0.5	1.3 ± 1.3
	Low	7.1 ± 0.8	17.5 ± 9.2
	0	10.1 ± 0.5	4.8 ± 3.1
5×10^6	High	7.8 ± 1.3	6.5 ± 3.1
	Medium	9.7 ± 0.7	4.5 ± 2.9
	Low	9.3 ± 1.0	5.3 ± 2.5
	0	9.1 ± 1.0	16.3 ± 6.2
5×10^9	High	12.0 ± 0.8	70.3 ± 4.9
	Medium	7.1 ± 1.5	78.5 ± 9.5
	Low	9.7 ± 1.5	87.5 ± 5.6
	0	9.7 ± 1.4	77.1 ± 4.3
ANOVA (*F*-values)[b]			
Fungicide		1.8	1.2
Virus rate		1.4	126.5**
F × V		1.9	2.1*

[a] ANOVA.
[b] df = 3, 3, 9 and 75 for fungicide, virus rate, interaction and error respectively.
* and ** denote significance at $P \leq 0.05$ and 0.01 respectively.

Table 3. Analysis of variance for main effects and interaction of virus rate, fungicide and days of exposure on percentage of black cutworms that died from viral infection

		ANOVA (*F*-values) for cohorts exposed for		
		1 day[a]	2 days[b]	4 days[b]
Main effects	Virus	34.1**	3.9	37.3**
	Fungicide	0.1	16.3*	0.4
Interactions	Virus × fungicide	3.5*	2.7	1.6
	Virus × time	37.9**	0.9	19.5**
	Fungicide × time	0.9	2.6**	1.17
Contrasts[c]	VL versus VLF	17.11**	3.14**	19.17**
	VH versus VHF	0.47	9.24**	65.7**

[a] df = 2, 1, 2, 10, 5 and 120 for virus rate, fungicide, interactions and error respectively.
[b] df = 2, 1, 2, 8, 4 and 96 for virus rate, fungicide, interactions and error respectively.
[c] Preplanned single-degree-of-freedom contrasts.
* and ** denote significance at $P \leq 0.05$ and 0.01 respectively.

3.4 Fungicide effects on consumption of treated grass

In choice tests with neonates, the total number of grass blades with some damage caused by cutworm feeding was similar for the treated and the non-treated grasses (52 versus 58; $\chi^2 = 0.33$; $P = 0.56$). Larvae consumed proportionately less of the treated than of the non-treated grass tissue (17.1±1.8% versus 24.7±1.7% respectively; Wilcoxon signed-rank test, $P \leq 0.01$). The numbers of larvae feeding on treated versus untreated grass blades were similar at the time of assessment, however. In no-choice tests with neonates, the percentage feeding damage on treated grass blades (8.5 ± 2.6%) was significantly lower than for non-treated blades (22 ± 3.9%; $t_9 = -2.76$; $P = 0.01$), but the number of blades with some cutworm damage was similar regardless of treatment. Third

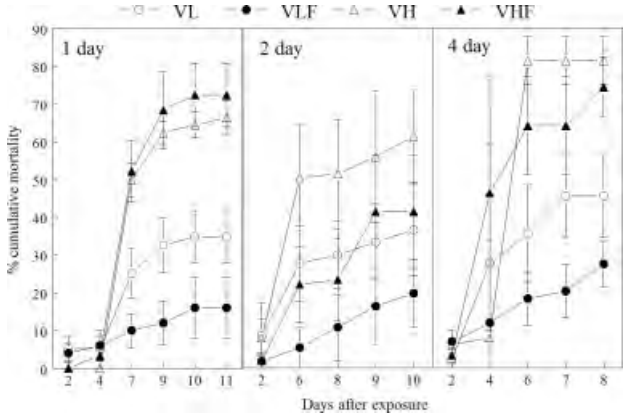

Figure 1. Cumulative lethal virus infection for *A. ipsilon* fed on bentgrass cores treated with two rates of *Agip*MNPV (VL = low virus, 1×10^8; VH = high virus, 5×10^8 OB m^{-2}) and one fungicide rate (F = 2.1 g m^{-2} of formulated product), applied alone and in combination. Larvae were exposed to treated grasses for 1, 2 and 4 days. Data are means (± SE). Delayed and slightly reduced mortality from *Agip*MNPV occurred when larvae fed on fungicide/virus-treated grasses as opposed to virus-only treatments for all cases, except those in which larvae were exposed to the high virus rate for 1 day.

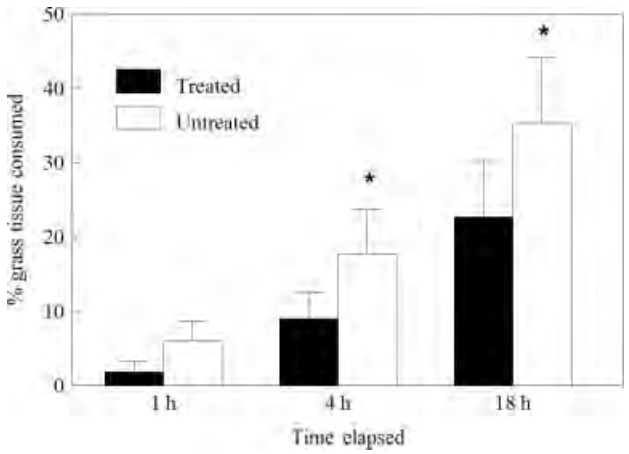

Figure 2. Mean (± SE) percentage of fungicide-treated versus non-treated grass leaf tissue consumed by third-instar *A. ipsilon* in choice tests. Asterisks denote significant feeding preference for untreated grass (Wilcoxon signed-rank tests, $P < 0.05$). The trend after 1 h was significant at $P = 0.08$.

instars showed significant preference for non-treated grass blades in choice tests (Fig. 2).

4 DISCUSSION

Combined or overlapping applications of a labeled polyoxin-d fungicide and *Agip*MNPV would be practical in turfgrass settings, so it was hoped that the combination would enhance infectivity of the virus against the black cutworm, an important golf-course pest, compared with levels of control provided by virus alone. That hypothesis is reasonable given previous laboratory studies with other insect species in which chitin-synthesis-inhibiting agents facilitated passage of virions through the chitinous peritrophic membrane, enhancing viral infection.[15,16,20,21] However, no synergism by the chitin-synthesis-inhibiting fungicide was seen; instead, there was delayed and slightly reduced mortality from *Agip*MNPV when larvae fed on fungicide/virus-treated grasses compared with virus-only treatments.

Poor recovery of larvae from fungicide-treated plots in the first field experiment initially suggested that polyoxin-d might have an insecticidal effect on cutworms. However, in a second experiment, when metal enclosures were driven deeper into the turf, similar numbers of larvae were recovered from fungicide- and non-fungicide-treated plots, revealing that the fungicide does not kill the cutworms. In choice tests, cutworms avoided feeding on polyoxin-d-treated grass. This suggests that larvae disproportionately escaped from the fungicide-treated turf by crawling beneath the shallow-driven enclosures used in the first field experiment. Because polyoxin-d does not deactivate *Agip*MNPV, and high virus rates can knock down and overwhelm cutworm populations in the short term,[24] the two substances are compatible and can be used together in the field. However, polyoxin-d residues on treated grass might interfere with larval ingestion of a lethal virus dose by inhibiting feeding or repelling larvae from putting greens, tees or other treated sites.

Previous studies examining the insecticidal effects of chitin synthesis inhibitors have all been done in laboratory settings and involved direct injection of the compound into the insect or incorporating it into artificial diet.[28–32] To the present authors' knowledge, this is the first study to examine the use of a chitin synthesis inhibitor as a synergist to an entomopathogen on living plants in greenhouse or field settings. Adjuvants such as stilbene optical brighteners, which have been shown to protect baculoviruses from UV degradation, enhance their longevity or act as synergists to virus infection in laboratory studies, may or may not provide the same benefits in the field.[33,34] The optical brightener M2R, for example, reduced the LD_{50} value of *Agip*MNPV to *A. ipsilon* in the laboratory but failed to enhance its efficacy against the same pest in greenhouse- or field-grown corn (*Zea mays* L.),[35] and also failed to accelerate or extend the efficacy of *Agip*MNPV against *A. ipsilon* in turfgrass field plots.[24] Optical brighteners can also deter feeding, and therefore results from a laboratory experiment may not translate to field settings where insects can disperse away from treated plant material.[36] Possibly, polyoxin chitin synthesis inhibitors consumed on plant tissue are less disruptive to caterpillar peritrophic membranes than when ingested in artificial diet. Plant secondary chemicals can alter the susceptibility of insects to naturally encountered pathogens as well as to microbial insecticides applied for biological control.[37] Caterpillar mortality, for example, can differ by as much as 50-fold, depending on the species of host plant upon which baculoviruses are consumed.[38–40]

The authors are still optimistic that *Agip*MNPV has potential as a microbial insecticide for managing black cutworms on golf courses, sports fields and in garden crops. Selecting for more virulent strains, or formulating the virus with adjuvants that enhance its persistence in field settings, could be productive. Testing *Agip*MNPV in combination with other chitin-synthesis-inhibiting fungicides suited for golf-course use is warranted, because some may be more disruptive to peritrophic membranes without discouraging feeding on treated grass as occurred with polyoxin-d. Another approach might be to combine a high dose of virus with a short-lived natural feeding stimulant[41,42] so that targeted larvae more rapidly ingest a lethal dose. The commercial success of *Agip*MNPV, like most entomopathogens, largely depends on future development of *in vitro* production methodology allowing the virus to be produced more economically and in greater amounts.[2,43]

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