Title: Biological control of black cutworm in turf with baculovirus

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Objectives:

1) Determine effective application rates and formulations of the virus required for efficacious control of larvae,
2) Compare baculovirus treatments with alternative control treatments when applied under field conditions,
3) Evaluate compatibility of virus applications to fit with integrated management strategies for pest control within the golf-turf environment.

Start Date: Spring 2015

Project Duration: 3

Total Funding: $60,000

Bullet Points

- Experimental treatments of baculovirus to creeping bentgrass successfully killed medium-sized black cutworm larvae when applied at a rate of 2.3×10^{10} virus particles per 1000ft^2, but not when applied at a lower rate of 4.6×10^9 virus particles per 1000ft^2.
- Successful treatments of baculovirus formulations provided levels of control similar those provided by the chemical standard, Talstar S (bifenthrin).
- Cutworm feeding damage in baculovirus treated plots demonstrated that the slow speed of kill by the experimental virus treatments may require applications that target younger larvae
- Initial evaluations suggest reasonable storage stability of experimental formulations of *Agip*MNPV

Summary text:

A newly discovered baculovirus, *Agip*MNPV, may be developed as a biological insecticide for control of black cutworm (BCW) larvae in turf. Only BCW and a few closely related insects are susceptible to infection by the virus, and this specificity makes it a desirable biological control agent. The first year of this research focused on two objectives; to determine efficacious rates of virus and compare virus formulations applied under field conditions.

Virus production and formulation

*Agip*MNPV was produced in the laboratory by infecting BCW larvae, harvesting disease-killed larvae, and grinding the cadavers to from a slurry containing high concentrations of virus particles known as occlusion bodies (OBs). Initial laboratory experiments demonstrated that

virus OBs may be processed to create a variety of liquid and dry formulations with little effect on insecticidal activity. These formulations were prepared as indicated in Table 1.

Laboratory evaluations for storage stability

Experimental formulations are being evaluated for stability when refrigerated or stored at room temperature. Bioassays evaluate a single dosage of virus that is expected to cause 80% mortality of newly-hatched BCW larvae. With the exception of the clay formulation, initial activity for experimental formulations was near expected levels (Figure 1). After four months storage, refrigerated samples showed no loss of activity and only the glycerin formulation displayed reduced activity when stored at room temperature. These evaluations will continue for up to one year of storage.

Field efficacy experiments – Purdue University

Treatments were applied to field plots of creeping bentgrass measuring 0.3 × 2.1 m. Plots were maintained at 3/16 inch height and were arranged in a randomized complete block design with four replications. All treatments were applied as aqueous sprays using a hand-held CO_2 boom sprayer calibrated to deliver 2 gallons/1000 ft^2. After applications dried, a PVC cage (8 inch diameter) was installed in each plot and artificially infested with five 2^{nd}- 3^{rd} instar BCW larvae. Infestations were created in a different section of each plot at 0, 3 and 14 DAT (Figure 2). Larvae were allowed to feed within the cages for seven days before being flushed from the turf using a standard soapy water solution. Larval survival in treated plots was compared with that in untreated control plots and plots treated with a chemical standard, Talstar S (bifenthrin) (Tables 2 and 3).

To determine the effective field application rate, two formulations (glycerin and lignin) were applied to field grown bentgrass plots at three rates. The low rate of virus (2×10^{11} PBs/A) did not control artificial infestations of medium-sized larvae. Medium (1×10^{12} OBs/A) and high rates (5×10^{12} OBs/A) of virus provided level of control equal to the Talstar S treatment. Control diminished over time for all treatments with only the chemical and lignin formulated virus providing significant control relative to the untreated plots at14 DAT. The expected slow speed of kill by virus treatments (3-5 days after ingestion) allowed larvae to cause significant damage to the grass and demonstrates the need to target younger larvae with virus applications.

Table 1. Ingredients used to prepare experimental formulations of the black cutworm baculovirus intended for aqueous spray application to turfgrass

Formulation	Form	Reasoning
Unformulated	Liquid	Technical product used to prepare other formulations included for comparison
Glycerine	Liquid	Added to reduce growth of contaminating microbes
Skim Milk Powder	Dry	Added to product the virus during the spray drying process
Montmorillonite Clay (K-10)	Dry	Common carrier used for wettable powder formulations for flow and mixing characteristics
Lignin	Dry	Known to encapsulate the virus to provide protection from sunlight degradation
Blankophor	Dry	Chemical brightener known to enhance insecticidal activity of some other baculovirus

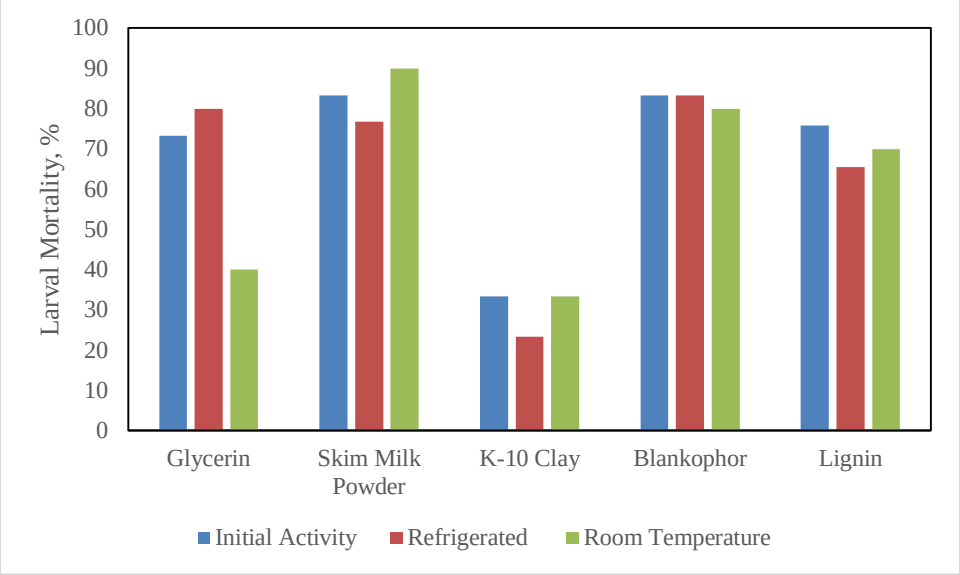

Figure 1. Stability of *Agip*MNPV formulations stored for four months under refrigeration and at room temperature. All treatments expected to cause 80% larval mortality.

Figure 2. Infesting cages with black cutworm larvae after the turf was treated with formulations of *Agip*MNPV, 2015.

Table 2. Mean (±SE) number and percent control of black cutworm larvae in plots of creeping bentgrass treated with different formulations and rates of baculovirus (*Agip*MNPV) or Talstar S (bifenthrin). Five 2nd and 3rd instar larvae were placed on plots at 0, 3 and 14 days after treatment (DAT) and number of surviving larvae (n/5) was recorded at 7, 10 and 21 DAT, respectively.

Treatment	Rate	BCW Survival 7 DAT	% Control 7 DAT	BCW Survival 10 DAT	% Control 10 DAT	BCW Survival 21 DAT	% Control 21 DAT
Untreated	---	4.5±0.3e	---	4.3±0.5d	---	4.8±0.3c	---
Talstar S	0.25 oz/M	0.0±0.0ab	100.0	1.5±0.3abc	64.7	2.5±0.3a	47.4
AgipMNPV unformulated	1×10^{12}	1.8±0.5b	61.1	1.5±0.6abc	64.7	4.3±0.9bc	10.5
AgipMNPV powder milk	1×10^{12}	1.0±0.4ab	77.8	1.5±0.6abc	64.7	4.3±0.5bc	10.5
AgipMNPV k10 clay	1×10^{12}	1.8±0.3b	61.1	2.5±0.6abcd	41.2	4.5±0.3c	5.3
AgipMNPV blankophore	1×10^{12}	2.0±0.4bc	55.6	2.0±0.4abc	52.9	4.0±0.4abc	15.8
AgipMNPV glycerin	2×10^{11}	3.0±0.6cd	33.3	2.8±0.8bcd	35.3	4.5±0.3c	5.3
AgipMNPV glycerin	1×10^{12}	0.3±0.3a	94.4	1.3±0.5ab	70.6	3.3±0.5abc	31.6
AgipMNPV glycerin	5×10^{12}	0.3±0.3a	94.4	1.0±0.7ab	76.5	4.0±0.7abc	15.8
AgipMNPV lignin	2×10^{11}	3.8±0.5de	16.7	2.8±0.9bcd	35.3	2.8±0.9ab	42.1
AgipMNPV lignin	1×10^{12}	1.8±0.8b	61.1	2.0±0.7abc	52.9	4.0±0.4abc	15.8
AgipMNPV lignin	5×10^{12}	0.0±0.0a	100.0	0.8±0.5a	82.4	2.8±0.3ab	42.1

*Numbers in same column followed by different letters are significantly different at α=0.05. Virus application rate reported as OBs/A, 1×10^{12} OB/A = 2.3×10^{10} OB/1000ft^2.

Table 3. Mean (±SE) % damage resulting from black cutworm larvae in plots of creeping bentgrass treated with different formulations and rates of baculovirus (AgipMNPV) or Talstar S (bifenthrin). Five 2nd and 3rd instar larvae were placed on plots at 0, 3 and 14 days after treatment (DAT) and damage was assessed at 7, 10 and 21 DAT, respectively.

Treatment	Rate	% Damage 7 DAT	% Damage 10 DAT	% Damage 21 DAT
Untreated	---	55.0±5.0f	28.8±6.6bc	33.8±4.7cd
Talstar S	0.25 oz/M	0.0±0.0a	5.0±0.0a	3.5±0.9a
AgipMNPV unformulated	1 x 10^{12}	32.5±10.3bcde	28.8±5.2bc	35.0±2.9d
AgipMNPV powder milk	1 x 10^{12}	15.0±2.9abc	25.0±3.5bc	33.8±2.4cd
AgipMNPV k10 clay	1 x 10^{12}	32.5±2.5bcde	41.3±5.9c	33.8±2.4cd
AgipMNPV blankophore	1 x 10^{12}	35.0±10.4cdef	23.8±3.1b	33.8±3.8cd
AgipMNPV glycerin	2 x 10^{11}	45.0±8.7ef	32.5±4.8bc	25.0±2.9bcd
AgipMNPV glycerin	1 x 10^{12}	22.5±7.5bcd	33.8±9.9bc	23.8±3.1bc
AgipMNPV glycerin	5 x 10^{12}	22.5±7.5bcd	17.5±4.3ab	30.0±7.1cd
AgipMNPV lignin	2 x 10^{11}	55.0±8.7f	30.0±3.5bc	18.8±4.3b
AgipMNPV lignin	1 x 10^{12}	40.0±12.2def	31.3±9.7bc	28.8±4.3bcd
AgipMNPV lignin	5 x 10^{12}	12.5±2.5ab	22.5±3.2b	27.5±2.5bcd

*Numbers in same column followed by different letters are significantly different at α=0.05. Virus application rates reported as OBs/A, 1 × 10^{12} OB/A = 2.3× 10^{10} OB/1000ft^2.