NYSTA Grants – 2017-18 Final Report

Project Title:	Identifying the mechanisms behind imidacloprid failures in controlling white grubs in turfgrass
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Introduction:

White grubs, or larvae of scarab beetles (Coleoptera: Scarabaeidae), are the most widely distributed and destructive turf insect pests of both warm- and cool-season turfgrasses (Vittum et al. 1999). If left uncontrolled, root-feeding larvae can cause extensive damage to a turfgrass stand, impacting the plant's ability to absorb water and nutrients, decreasing surface stability, and in the case of sports turf, increasing the probability of footing-related injuries. While cultural and biological control methods exist, chemical insecticides are favored by most turfgrass managers due to the relative effectiveness, reliability, and ease of application. Since the mid-1990s, the neonicotinoids (particularly products containing imidacloprid (e.g. Merit ®)) have been the most widely used white grub control products in turfgrass. However, reports of imidacloprid failing to control grubs in the northeastern United States turfgrass sites have been steadily increasing in recent years. Currently it remains unknown as to the cause of the failures, yet all of the affected sites have applied imidacloprid annually for a minimum of 10 years, applied controls within consistent time periods between years, and have not used other preventive insecticides for white grub control.

We conducted studies aimed at determining the underlying mechanisms in the inability for imidacloprid to control white grubs at select sites within the region. Five golf courses which have used imidacloprid exclusively (i.e. have not rotated with other active ingredients for preventive white grub control) for over 10 years were selected for the first year of the study. Each course applied imidacloprid in the previous year (2016) and experienced extensive damage in the treated areas in the fall and/or spring (2017). One severely damaged area at each course (15 m × 15 m) consisting of equal portions fairway-height (~ 1.25 cm) and rough-height turf (>5 cm) was selected for study. The study area consisted of 400- 75 cm × 75 cm plots. A golf course cup cutter was used to remove a soil/turf core (10 cm diameter × 15 cm depth) from each sample unit. Turfgrass species and thatch thickness were measured prior to manual examination of the soil for white grub larvae. Twenty soil samples (10 from the rough-, 10 fairway-height turf) were collected from plots containing the highest amount of white grubs and taken to the laboratory for further analysis. The soils were divided by area, and subdivided into sterilized and non-sterilized soil treatments. After treatment, the soils were seeded with *Lolium perenne*, grown to maturity in the greenhouse, and treated with field rates of imidacloprid. Imidacloprid concentration in the leaf tissue was observed over a two-month period to determine whether microbial populations are prematurely degrading the active ingredient.

Objective 1: *Determine spatial distribution of white grub populations, turfgrasses, and soil edaphic conditions in imidacloprid-treated turf on sites experiencing product failure*

All of the courses, with the exception of Stadium Golf Course (SGC) for white grubs and Lebanon Country Club (LCC) for turfgrasses, consisted of mixed species (Table 1). In general, total-plot grub densities were far below commonly accepted thresholds (≤ 1.68 m^2), though several courses had areas within the plot that exceeded annual white grub damage thresholds (110 m^{-2} - 636 m^{-2})(Table 2). Total grub densities, averaged over 200 plots by area, were significantly higher in rough (0.12 – 2.06 grubs m^{-2}) compared to fairways (0 – 0.6 grubs m^{-2}) ($T \geq 1.12$; $df = 398$; $P \leq 0.02$) at four of five courses. Grub counts were not high enough to perform statistical analyses at Greensburg Country Club (GCC), but all were detected in the rough-mown turf at this site.

All sites, with the exception of LCC which was 100% perennial ryegrass (*L. perenne*), consisted of mixtures of turfgrasses, ranging between two (Penn State University Golf Course (PSU)) and five species (Youghiogheny Country Club (YCC)), (Table 1). Turfgrass spatial patterns within the four mixed-species sites were significantly aggregated or patchily distributed (Figure 1). Thatch thickness was also significantly aggregated at each site ($I_a \geq 2.6$; $P_a \leq 0.0013$), and rangeed between averages of 6.7 (YCC) to 26.25 mm (LCC) (Table 2, Figure 1). Thatch thicknesses in the fairway were also significantly lower than in the rough ($T \geq 7.93$; $df = 398$; $P \leq 0.0001$) at four of the five courses.

Larval distribution was significantly aggregated at four of five sites ($I_a \geq 1.56$; $P \leq 0.004$)(Table 2). In general, aggregations of larvae tended to be in three or four sample unit groupings in both fairway- and rough-cut areas. SGC was the only population that one grub species was detected (*Anomala orientalis*, Oriental beetle), and was also the most aggregated of all grub datasets.

Strong spatial associations were detected between the spatial patterns of white grubs and turfgrass species (*L. perenne* and *Poa pratensis*) at three of four sites (Table 3). Larval spatial patterns were strongly dissociated with *Agrostis stolonifera* at all three sites ($X \leq - 0.145$; $P \geq 0.999$) where the turfgrass was detected. White grub spatial patterns were significantly associated with thatch thickness at three of five study sites ($X \geq 0.1351$; $P \leq 0.01$). A Pearson's correlation test of combined data revealed no positive correlation or level at which thatch thickness yielded higher white grub densities. When analyzed individually, only one of the five courses (SGC) had a positive correlation between white grubs and thatch thickness ($X = 0.47$; $P = 0.001$).

Objective 2*: Determine the potential for soil microbial communities to prematurely degrade imidacloprid*

Imidacloprid concentrations were measured in plants grown in sterilized and non-sterilized soils from grub-infested plots from each study site. Plants were treated with a foliar application of 59.2 mg imidacloprid/m^2 (Merit ® 75 WSP, Bayer CropScience, Research Triangle Park, NC) in a 0.47 L/ha (2 gallons/M) carrier, followed by 41.6 mL (0.5 cm) irrigation to each sample (10 cm diameter soil core). Imidacloprid concentrations in the leaves were quantified between 7 and 56 days after treatment (DAT) through the use of enzyme-linked immunosorbent assay (ELISA). Imidacloprid concentration declined rapidly in both treatments, independent of soil treatment (Figure 2). Levels ranged between 7.2 – 17.6 parts per billion (ppb per 0.5 g plant tissue) at 14 DAT and 4.8 – 11.2 at 28 DAT, representing a decline of 58 and 88

percent of 7 DAT levels. Small numerical, but statistically significant differences, were observed between sterilized and non-sterilized soil treatments at 28 and 56 DAT (Figure 2). Higher imidacloprid concentrations were found in plants grown in sterilized soils from four of five sites, which indicates soil microbes contribute to the degradation of the product. More studies are needed to determine whether these differences translate to observable effects on insect control in the field.

Objective 3: *Assess the prevalence and/or potential for the development of insecticide resistance in white grub populations*

White grubs were collected in October 2017 from three of five study sites. The larvae were subjected to serial dilutions of imidacloprid (0, 0.5x, 1x, 3x and 30x field rates = 59.2 mg imidacloprid/m^2) to measure enzymatic activity and assess the potential for imidacloprid resistance. Larvae from three sites were topically treated with imidacloprid in Petri dishes lined with filter paper and mortality was observed at 24 hrs. Grubs that remained alive (Table 4) after the holding period were frozen (- 80^o C) to stop all enzymatic activity. Biochemical assays are currently being conducted to measure activation of the three major enzyme detoxification systems, or a measure of the potential development of resistance to imidacloprid.

Summary of results:

1. Grub densities were patchily distributed (aggregated) at four of five sites that had been treated with imidacloprid in the previous year. Significantly more grubs were found in rough-mown turf than fairways. Significant aggregations were detected in both fairway- and rough-cut areas in three to four individual sample units (1 unit = 2.3m^2).
2. Three sites demonstrated significant associations between the spatial patterns of white grubs and *L. perenne* and two with *P. pratensis*. Spatial patterns were strongly dissociated with *A. stolonifera* at three sites.
3. Average thatch thickness ranged between 6.7 and 26.25 mm, with significantly higher thatch levels detected in roughs at four of five courses. Grub spatial patterns were significantly associated with thatch thickness at the same four courses. However, thatch thickness was correlated with grub density on only one course.
4. Rapid degradation of imidacloprid was observed in plants grown in soils collected from all five sites, regardless of sterilization treatment. Less than 87 and 92% of 7 DAT levels were detected in plant tissue at 28 and 56 DAT, respectively.
5. Small, but statistically significantly differences were detected in imidacloprid concentration of plants grown in sterilized and non-sterilized soils. Imidacloprid concentration was significantly higher in sterilized soils at 28 and 56 DAT, indicating that microbes contribute to the breakdown of the product.

Future Research

The findings from this study will assist turfgrass managers in understanding the post-application fate of imidacloprid, the importance of rotating products, and the value of continued use of the imidacloprid in areas with similar patterns of use. However, more research is needed to validate the findings presented here, to determine the implications of rapid loss of imidacloprid from the plant, and to determine optimal thatch thickness levels. It is possible that the rapid decline of imidacloprid concentration in the plant is an artifact of the experimental set-up as plants grown in the greenhouse had not developed a thick enough thatch-mat, and thus the active ingredient may have been flushed through the containers. Despite this, differences in imidacloprid concentration was detected between sterilized and unsterilized soils, indicating that microbial degradation is occurring. We are currently adjusting our experimental procedures and validating the ELISA assays with more sensitive analytical tests (high-performance liquid chromatography (HPLC)), including measurements in different portions of the plant (leaf, root) and soil. Additionally, more field sites will be included in future work, and controlled field trials will be initiated to assess the residual activity and efficacy against larvae in sterilized and non-sterilized soils.

Table 1. Soil texture, turf, and white grub species at turfgrass sites experiencing imidacloprid failure.

Site[1]	Soil Textural Class	Turfgrass spp.	White grub spp[2]
PSU	Loam	83 % *Lolium perenne* 16% *Poa annua* 1% Other	65% JB 35% NMC
GCC	Loam	43% *Poa annua* 31% *Poa pratensis* 24% *Agrostis stolonifera* 2% Other	7% JB 93% NMC
SGC	Sandy Loam	60% *Agrostis stolonifera* 30% *Lolium perenne* 7% *Poa pratensis* 3% *Poa annua*	100% OB
YCC	Sandy Loam	34% *Poa annua* 31% *Lolium perenne* 31% *Agrostis stolonifera* 4% *Poa pratensis* 1% Other	63% JB 37% NMC
LCC	Silt Loam	100% *Lolium perenne*	96% JB 4% EC

[1] Study sites: Pennsylvania State University (PSU); Greensburg CC (GCC); Stadium Golf Club (SGC); Youghiogheny CC (YCC); Lebanon CC (LCC)

[2] White grubs species in study areas (2017): JB = Japanese beetle (*Popilla japonica*); NMC = Northern masked chafer (*Cyclocephala borealis*); OB = Oriental beetle (*Anomala orientalis*); EC = European chafer (*Rhizotrogis majalis*)

Table 2. Spatial distribution of white grubs in study sites with perceived imidacloprid failure. I_a and P_a values indicate the aggregation value of the overall spatial pattern and the associated significance test of the spatial pattern's departure from randomness. I_a values indicate an aggregated (>1), uniform (<1), and random distribution (close to 1). Significance of the spatial aggregation is assumed at $P<0.025$.

Site[1]	**Area**[2]	**Thatch (mm)**[3]	**Larvae density/grid**[4]	I_a	P_a
PSU	Combined	11.47 +/- 0.21	0.30 +/- 0.03	**1.681**	**0.0007**
	FW	11.16 +/- 0.54	0.34 +/- 0.04		
	R	12.08 +/- 0.47	0.25 +/- 0.04		
GCC	Combined	8.83 +/- 0.25	0.04 +/- 0.01	**1.56**	**0.004**
	FW	5.22 +/- 0.14	0 +/- 0.00		
	R	12.52 +/- 0.59	0.07 +/- 0.02		
SGC	Combined	7.27 +/- 0.15	0.73 +/- 0.05	**2.844**	**0.0002**
	FW	5.52 +/- 0.15	0.26 +/- 0.04		
	R	9.32 +/- 0.35	1.12 +/- 0.09		
YCC	Combined	6.7 +/- 0.10	0.14 +/- 0.02	**2.629**	**0.0002**
	FW	5.74 +/- 0.16	0.02 +/- 0.01		
	R	7.92 +/- 0.18	0.26 +/- 0.04		
LCC	Combined	26.25 +/- 0.28	0.31 +/- 0.03	1.151	0.1314
	FW	24.3 +/- 0.32	0.27 +/- 0.04		
	R	28.42 +/- 0.86	0.34 +/- 0.05		

[1] Study sites: Pennsylvania State University (PSU), Greensburg CC (GCC), Stadium Golf Club (SGC), Youghiogheny CC (YCC) Lebanon CC (LCC)

[2] Areas sampled by turfgrass height. FW = fairway height (1.25 cm); R = rough height (5 cm)

[3] The asterix (*) indicates significantly greater ($P \leq 0.05$) thatch thickness by area (fairway vs. rough).

[4] Average grub density within an individual sample unit (grid = 2.3m^2). The asterix (*) indicates significantly greater ($P \leq 0.05$) grub counts by area (fairway vs. rough).

Figure 1. The spatial distributions of white grubs (all species combined), thatch thickness, and dominant turfgrass species as determined by SADIE analyses. Each contour map was generated from 400 regular sample points by plotting local clustering values from SADIE analyses. Areas in red (cluster values > 1.5) contribute to local aggregations or patches of large counts in close proximity, whereas blue areas (cluster values < -1.5) indicate gaps (zeroes or low counts close to one another). I_a and P_a values beneath each map indicate the aggregation value of the overall spatial pattern and the associated significance test of the spatial pattern's departure from randomness. I_a values indicate an aggregated (> 1), uniform (< 1) and random distribution (1). Significance of the spatial aggregation is assumed at the $P < 0.025$ level.

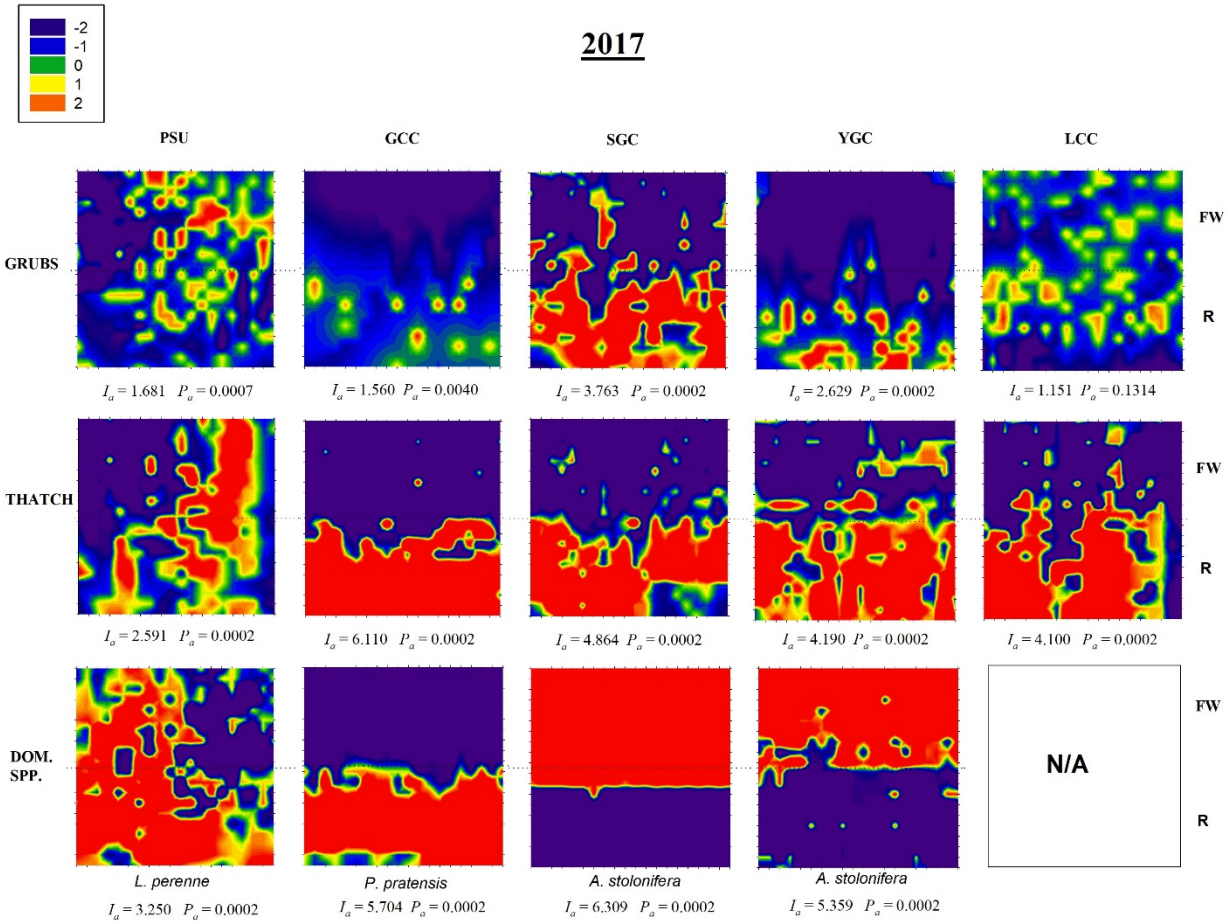

Table 3. Spatial relatedness (*X*) between white grub larvae and site characteristics and the associated probability level (P_a). *X* values greater than 0 are considered significantly associated when $P < 0.025$ and significanly dissociated with negative *X* values and when $P > 0.975$. Bolded figures represent significant spatial patterns.

Site[1]	**Relationship**	***X***	***P***
PSU	Thatch	0.013	0.396
	L. perenne	-0.099	0.970
	A. stolonifera	0.063	0.245
	P. annua	0.083	0.054
	P. pratensis	-	-
GCC	Thatch	**0.135**	**0.005**
	L. perenne	-	-
	A. stolonifera	**-0.145**	**0.999**
	P. annua	-0.069	0.955
	P. pratensis	**0.137**	**0.005**
SGC	Thatch	**0.401**	**0.001**
	L. perenne	**0.393**	**0.001**
	A. stolonifera	**-0.495**	**0.999**
	P. annua	0.088	-
	P. pratensis	**0.327**	**0.001**
YCC	Thatch	**0.171**	**0.001**
	L. perenne	**0.208**	**0.001**
	A. stolonifera	**-0.227**	**0.999**
	P. annua	-0.072	0.924
	P. pratensis	0.082	0.069
LCC	Thatch	-0.212	0.666
	L. perenne	-	-
	A. stolonifera	-	-
	P. annua	-	-
	P. pratensis	-	-

[1] Study sites: Pennsylvania State University (PSU), Greensburg CC (GCC), Stadium Golf Club (SGC), Youghiogheny CC (YCC) Lebanon CC (LCC)

Figure 2. Concentrations of imidacloprid measured in leaf tissue of plants grown in sterilized and non-sterilized soil treatments. Statistical significance is expressed with an asterisk (*) above the measurement date.

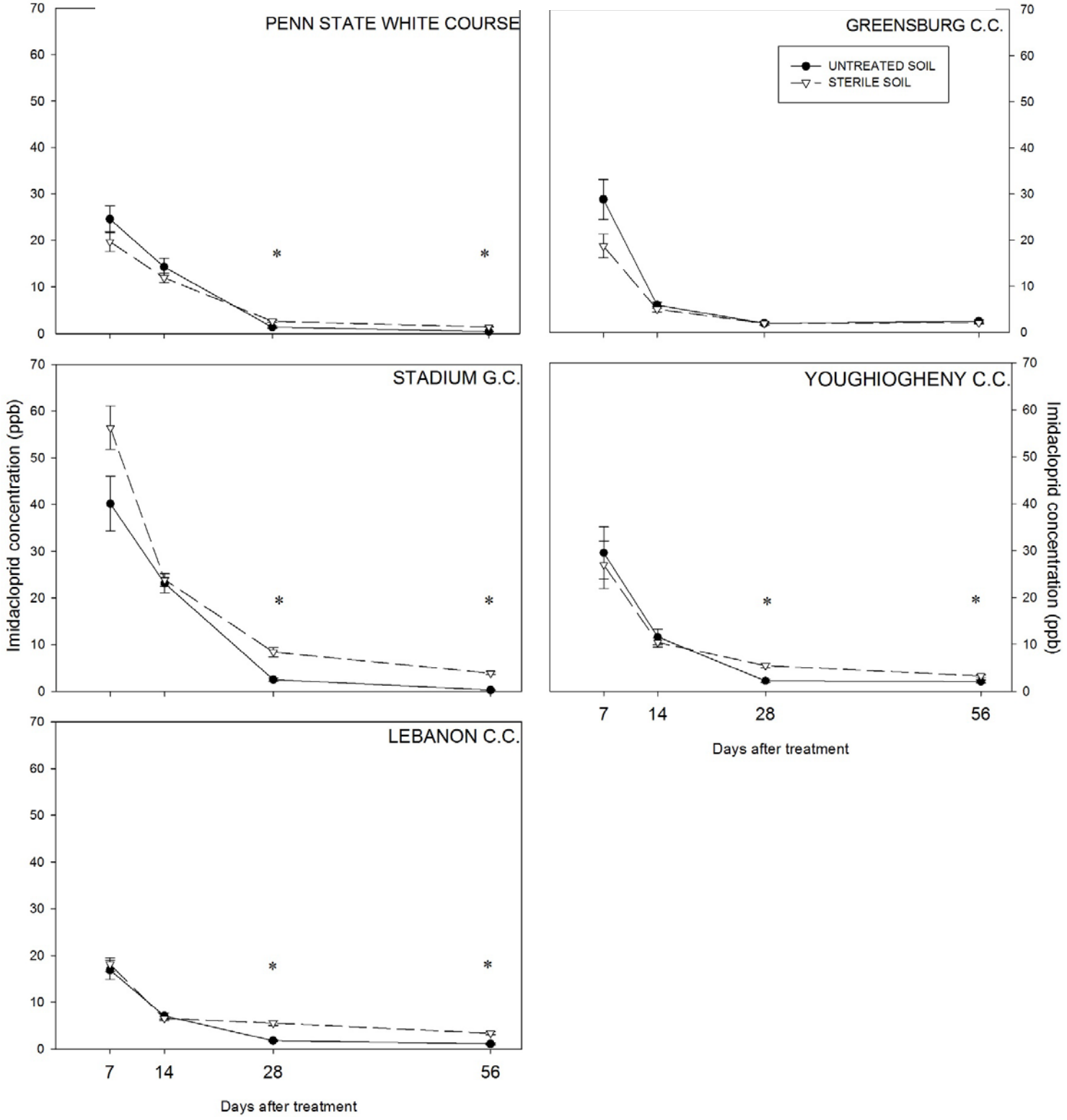

Table 4. Mortality of white grubs from three sites after 24 hour exposure to topically applied insecticide. Values are calculated by dividing number of white grubs that remained alive after 24 hours by total number of white grubs exposed.

Site[1]	White Grub spp.[2]	Concentration[3]	Percent mortality[4]
PSU	NMC	-	-
		30x	0/15 (100%)
		3x	14/15 (93%)
		1x	13/15 (87%)
		0.5x	12/15 (80%)
		0x	2/15 (13%)
	JB	-	-
		30x	13/15 (87%)
		3x	5/15 (33%)
		1x	4/15 (27%)
		0.5x	11/15 (73%)
		0x	0/15 (0%)
SGC	OB	-	-
		30x	16/20 (80%)
		3x	14/20 (70%)
		1x	15/20 (75%)
		0.5x	12/20 (60%)
		0x	7/20 (35%)
LCC	NMC	-	-
		30x	3/6 (50%)
		3x	1/6 (17%)
		1x	0/6 (0%)
		0.5x	2/6 (33%)
		0x	0/6 (0%)
	JB	-	-
		30x	7/7 (100%)
		3x	7/7 (100%)
		1x	4/7 (57%)
		0.5x	4/7 (57%)
		0x	0/7 (0%)

[1] Study sites: Pennsylvania State University (PSU), Greensburg CC (GCC), Stadium Golf Club (SGC), Youghiogheny CC (YCC) Lebanon CC (LCC)

[2] White grubs species in study areas (2017): JB = Japanese beetle (*Popilla japonica*); NMC = Northern masked chafer (*Cyclocephala borealis*); OB = Oriental beetle (*Anomala orientalis*)

[3] Adjusted topical treatment of Merit 75 WSP at label rate (1x = 1.6 oz. / 8,250ft^2)

[4] White grub mortality percentages at each concentration after 24 hour exposure to imidacloprid