New York Greengrass Association / Turfgrass Environmental Stewardship Fund

Insecticide Resistant Annual Bluegrass Weevil: Understanding, Managing, and Preventing a Superintendent's Nightmare

PIs: **Albrecht M. Koppenhöfer, Olga S. Kostromytska, Shaohui Wu**

Rutgers University, Dept. of Entomology, Thompson Hall, 96 Lipman Dr., New Brunswick, NJ 08901-8525

Summary: The annual bluegrass weevil is a serious and expanding golf course pest in the Northeast. Due to excessive insecticide use against this pest, the number of golf courses with insecticide resistant ABW populations is on the rise. Our studies show that insecticide resistance is wide spread, can reach very high levels to pyrethroids, and affects most other insecticide classes albeit at much lower levels. We have refined a simple Petri dish test that should be a viable option for resistance diagnostics for adulticides with the common active ingredients bifenthrin and chlorpyrifos, and could be used by scientists, consultants, and plant diagnostics labs to monitor resistance development and levels.

INTRODUCTION

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, has become the most difficult-to-manage golf turf insect pest throughout the Northeast. Multiple insecticide applications per season, mostly of pyrethroids, has led to the development of pesticide-resistant populations on an increasing number of golf courses. It is likely that any golf course with intensive insecticide use for several years vs. ABW already has some level of resistance that can only get worse with continued insecticide use.

Studies from New England ABW populations suggest that enhanced enzymatic detoxification, a non-specific mechanism likely to entail cross-resistance, plays a major role in ABW insecticide resistance. In highly resistant populations up to 3 detoxifying enzyme systems were involved. As a result, the efficacy of most presently used insecticides can be compromised. In highly resistant populations, only Conserve (AI: spinosad) is still effective, and only against larvae. But Conserve overuse will likely lead to resistance as well.

Further spread and intensification of insecticide resistance can only be prevented with a broad approach that involves studying resistance mechanism as a foundation for the development of effective resistance management strategies, exploring alternatives to synthetic insecticides (cultural, biological), developing a better understanding of key biological features of the pest and more effective and feasible monitoring methods. We are examining these poorly studied areas in an interconnected approach. Here we present some of our recent findings on insecticide resistance and the development of tools for monitoring resistance.

Toxicity of select insecticides to ABW adults in topical bioassays.

Detailed studies on resistance have thus far been conducted only with pyrethroid insecticides. To expand on previous studies, we conducted topical bioassays to determine ABW susceptibility and/or level of resistance to all the major insecticide classes (Table 1) used for ABW control. Different rates of technical grade insecticide active ingredient (AI) were applied to adult ABW in

1 µl between the prothorax and the wing covers with a microapplicator (Fig. 1). Mortality was evaluated at 24, 48 and 72 h after treatment. Dose-response curves with lethal doses that kill 50% of population (LD_{50}) and Resistance Ratios (RR_{50} = LD_{50} of resistant / LD_{50} of susceptible population) were calculated.

Significant variation in susceptibility to bifenthrin was observed among the 9 populations tested (Table 2). The population from the Rutgers Horticultural Farm II (HF, North Brunswick, NJ) had the lowest LD_{50} value and was used as the susceptible baseline. The most susceptible golf course population (PB, Manalapan, NJ) was significantly more tolerant to bifenthrin compared to HF (RR_{50} = 2.2). The other populations had RR_{50}s compared to the most susceptible population ranging from 18× to 343× for bifenthrin (AI of Talstar) and 18× to 324× for λ-cyhalothrin (AI of Scimitar). Pyrethroid resistant populations also demonstrated elevated tolerance to chlorpyrifos (3× to 15×) (AI of Dursban), clothianidin (3× to 10×) (AI in Arena), and spinosad (3× to 5×) (AI in Conserve). Toxicity assays with indoxacarb and chlorantraniliprole yielded only up to 20% mortality even for susceptible populations. To determine resistance/tolerance with these AIs, other bioassay types with different exposure types (feeding, injection) or other ABW stages (larval) will have to be used.

Toxicity of insecticides against adults in greenhouse pot assays.

Four to five different concentrations of commercial formulations of bifenthrin (Talstar Pro, range 0.03× to 600× labeled rate) and chlorpyrifos (Dursban, range 0.006× to 3× labeled rate) were tested in greenhouse experiments against ABW adults from two susceptible (PB and HP) and 5 resistant (GB, CN, EW, JC, LI) populations. Ten unsexed adults were introduced and caged in pots with seeded *Poa annua* 2 hours before treatments. Treatments were applied using a Generation III Research sprayer (Devries Manufacturing, Hollandale, MN) with a spray volume of 80 GPA. Treatments were watered in with 10 ml of water immediately after application. Pots were covered with ventilated lids, placed on plastic plates and left in the greenhouse. After 72 h, pots were submerged in lukewarm water, all adults recovered, and dead and surviving adults counted.

The RR_{50}s for bifenthrin and chlorpyrifos calculated from the greenhouse experiments followed a similar pattern as those from the topical assays in the laboratory (Table 3). RR_{50}s for most populations were somewhat lower than in the laboratory because we used the PB population rather than the HF population as the baseline susceptible population. However, the RR_{50}s of the LI population for both AIs were even higher than in the topical laboratory test.

Developing assays for resistance diagnostics and monitoring.

According to the above presented data, the efficacy of bifenthrin and chlorpyrifos was most affected by ABW resistance among all tested AIs. Thus, it is crucial to monitor and diagnose resistance and its levels for these two compounds. We conducted several experiments to develop practical, easy to conduct diagnostic assays with high discriminative power and to determine corresponding diagnostic doses.

Four to five concentrations of formulated bifenthrin (Talstar Pro) (range 0.01-600× of labeled rate) and chlorpyrifos (Dursban) (range 0.001-3×) were tested against five ABW populations in Petri dish assays (Fig. 2A), and corresponding insecticide AIs (technical grade) in various concentrations were tested in vial assays (Fig. 2BC). Ten adults were introduced per dish or vial. Mortality was evaluated at 24, 48, and 72 hours after treatment and lethal concentrations (LCs) determined.

In the Petri dish assay, filter paper was fitted in the Petri dishes (9 cm diameter) and treated with aqueous solution (1 ml) of insecticides using a pipette. Vial assays were conducted in 16 ml glass vials with an internal surface area of 35.9 cm^2. Vials were treated with 1 ml of AI in acetone and were manually rolled until most of the acetone had evaporated (~60 s). Vials were then rolled horizontally on a hot dog roller until completely dry (~1 h). Vials with insects were plugged with cotton and held horizontally.

Both Petri dish assay and vial assay showed similar resistance level for all tested populations and effectively separated resistant and susceptible ABW populations. However, the Petri dish assay with formulated products is easier to set up and less time/labor intensive than the vial assay. While we still have to validate our observations, the Petri dish assay seems to be a viable option for resistance diagnostics for both active ingredients (bifenthrin and chlorpyrifos) that could be used by scientists, consultants, plant diagnostics labs, and even trained practitioners.

Implications

Our study shows that ABW resistance to pyrethroids is widely spread and that the resistance levels can be very high, which are likely the cause of reported control failures. Furthermore, we found that other insecticide classes are also affected by the resistance, albeit not nearly at the resistance levels observed for pyrethroids. The observed cross resistance patterns indicate that enzymatic detoxification might be involved in ABW resistance, suggesting a risk for development of high resistance levels to other chemical classes as well. Ongoing studies on the involved resistance mechanisms are also pointing at the involvement of enhanced enzymatic detoxification as at least one resistance mechanism. The similarity of our results from laboratory and greenhouse pot studies suggests that level of resistance measured in our laboratory assays correctly reflects the present resistance in the field. Preliminary observations from field studies seem to confirm this.

The involvement of the not very specific enzymatic detoxification in resistance is particularly troublesome. Very few new materials are in development for ABW, none of which is different enough in mode-of-action from already compromised compounds to be safe from resistance development. Careful measures need to be taken to prevent resistance development in new populations and to new chemistries and to effectively manage resistant populations. These will have to involve the whole tool box of integrated turf management from spraying only when and where necessary based on careful and diligent monitoring, including biorational products in the arsenal, to replacing the more susceptible and preferred ABW host *P. annua* with new creeping bentgrass varieties.

Table 1. Active ingredients and products of insecticides tested against ABW

Insecticide class	Active ingredient	Trade name	Company/ manufacturer
Pyrethroid	Bifenthrin	Talstar	FMC, Princeton, NJ
	λ-cyhalothrin	Scimitar	Syngenta Crop Prot., Greensboro, NC
Organophosphate	Chlorpyrifos	Dursban	Dow AgroSciences Indianapolis, IN
Spinosyn	Spinosad	Conserve	Dow AgroSciences Indianapolis, IN
Oxadiazine	Indoxacarb	Provaunt	DuPont, Wilmington, DE
Anthranilic diamide	Chlorantraniliprole	Acelepryn	DuPont, Wilmington, DE
Neonicotinoid	Clothianidin	Arena	Valent, Walnut Creek, CA

Table 2. LD_{50}- (in ng/insect) and RR_{50}-values (LD_{50} resistant/ LD_{50} susceptible) for 9 different ABW populations determined in topical biossays.

ABW populations and collection sites		Bifenthrin		λ-Cyhalothrin		Chlorpyrifos		Spinosad		Clothianidin	
		LD_{50}	RR_{50}[1]	LD_{50}	RR_{50}	LD_{50}	RR_{50}	LD_{50}	RR_{50}	LD_{50}	RR_{50}
HF	North Brunswick, NJ	2.4 a	NC	1.1 a	NC	210 a	NC	427 a	NC	NC	NC
PB	Manalapan, NJ	5.1 b	2.2*	2.6 b	2.3*	299 a	1.4	655 a	1.5	696 a	NC
GB	Somers Point, NJ	72.7 c	30.5*	20.3 c	18.1*	852 c	4.1*	1949 b	4.6*	5894 c	8.5*
CN	Easton, CT	123.1 d	51.6*	85.8 d	76.6*	1118 c	5.3*	1437 b	3.4*	6777 d	9.7*
RW	Paramus, NJ	155.9 de	65.3*	72.2 d	64.4*	841 bc	4.0*	1988 bc	4.7*	4727 d	6.8*
PF	Edison, NJ	181.7 de	76.1*	137.9 de	123.1*	806 bc	3.8*	2065 bc	4.8*	2065 b	2.9*
EW	River Vale, NJ	225.8 de	94.6*	131.2 de	117.0*	688 bc	3.3*	1963 bc	4.6*	4532 cd	6.5*
JC	Cheltenham, PA	326.9 e	136.6*	194.6 e	173.6*	683 b	3.3*	1041 b	2.4*	5537 d	7.9*
LI	Glen Cove, NY	819.1 f	343.1*	362.7 f	323.6*	3203d	15.3*	3305 c	7.7*	3212 bc	4.6*

[1] RR_{50} were calculated with HF population as susceptible except for clothianidin for which the PB population was used.
[2] LD_{50} marked with the same letters do not differ significantly at $\alpha = 0.05$.
[3] RR_{50} marked with an asterisk differ significantly from the susceptible population.

Table 3. LC_{50}s and RR_{50}s for commercial formulations of bifenthrin (Talstar Pro) and chlorpyrifos (Dursban 50W) in greenhouse assays at 72 hours after treatment.

ABW population	Talstar		Dursban	
	LD_{50}	RR_{50}[1]	LD_{50}	RR_{50}[1]
LI	47.3	525.6*[2]	1.09	27.3*
EW	3.9	43.3*	0.49	12.3*
JC	5.9	65.6*	0.15	3.8*
CN	4.8	53.3*	0.48	12.0*
GB	0.7	7.8*	0.28	7.0
HP	0.1	1.1	0.04	1.0
PB	0.1	1.0	0.05	1.3

[1] RR_{50}s were calculated with PB population for Talstar and HP population for Dursban.
[2] RR_{50}s marked with an asterisk differ significantly from the susceptible population within the column.

Fig. 1. Microapplicator (Hamilton repeating dispenser) used for insecticide AIs application in topical bioassays.

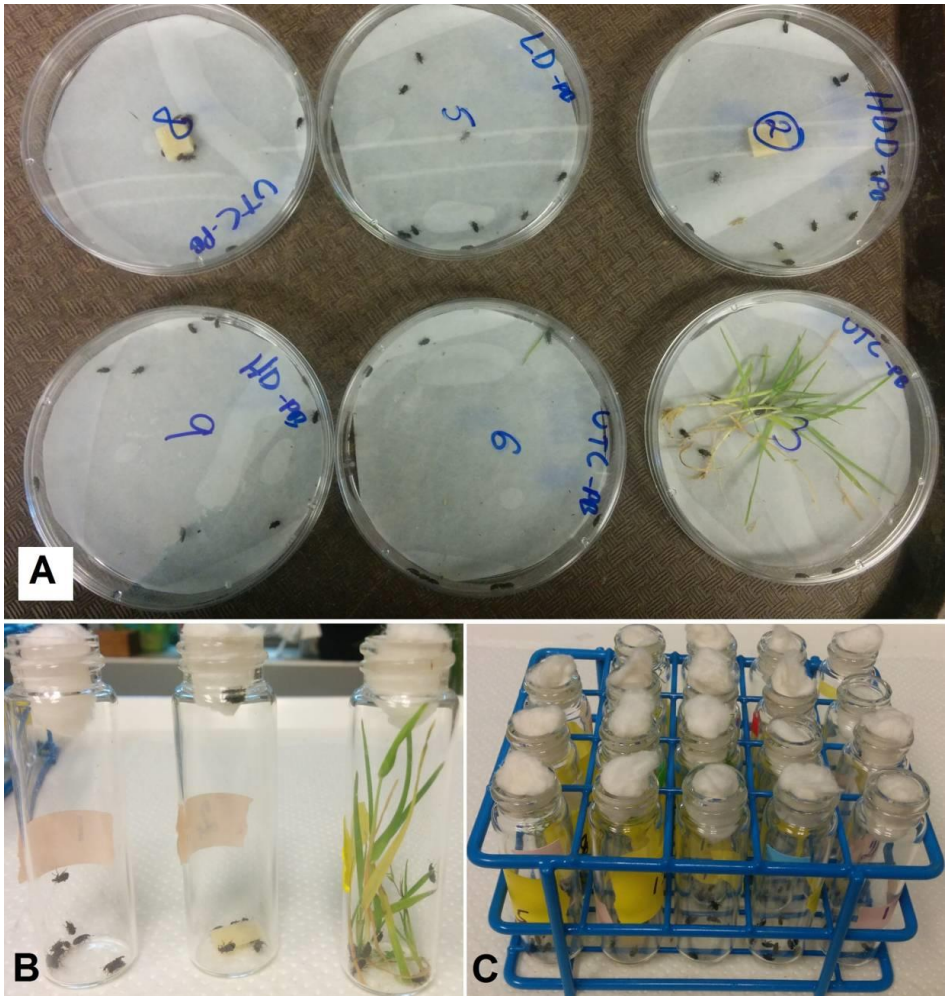

Figure 2. Petri dish (A) and vial bioassays (B, C) tested as potential diagnostic assays.