

# Insecticide resistant annual bluegrass weevil: Understanding, managing, alleviating, and preventing a superintendent's nightmare

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## Objectives:

*The overall goal is to develop a better understanding of the degree and scope of insecticide resistance in ABW populations as a basis for the development of recommendations on resistance management. This will be achieved through the following objectives:*

1. Establish baseline susceptibility of ABW to selected insecticides.
2. Determine resistance and cross resistance patterns and possible mechanisms.
3. Compare efficacy of selected insecticides against ABW adults and larvae of susceptible and resistant populations.

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a serious and expanding golf course pest in the Northeast. Recently, decreased efficacy of pyrethroid adulticide applications and pesticide-resistant populations are reported from an increasing number of golf courses (Ramoutar et al. 2009a). The efficacy of most insecticides against pyrethroid-resistant ABW populations seems to be reduced on average by 15-57%, but for pyrethroid resistant ABW populations is extremely limited (Koppenhöfer et al. 2012). To develop good recommendations for the mitigation of ABW resistance it is essential to better understand the degree and scope of resistance (different ABW stages, different insecticide modes of action) and the resistance mechanisms involved. Practical assays for resistance detection and monitoring also need to be developed.

Topical bioassays were conducted to determine resistance levels and cross resistance patterns to the major insecticide modes of actions in adult ABW (Table 1). Nine different populations were collected from golf courses with different histories of insecticide use and ABW infestation (Table 2). Six concentrations of the insecticide active ingredients (AI, technical grade) were applied topically (1  $\mu$ l/adult) using microapplicators (Figure 1). Treated ABW were placed in Petri dishes (10 per dish) lined with moist filter paper with food provided



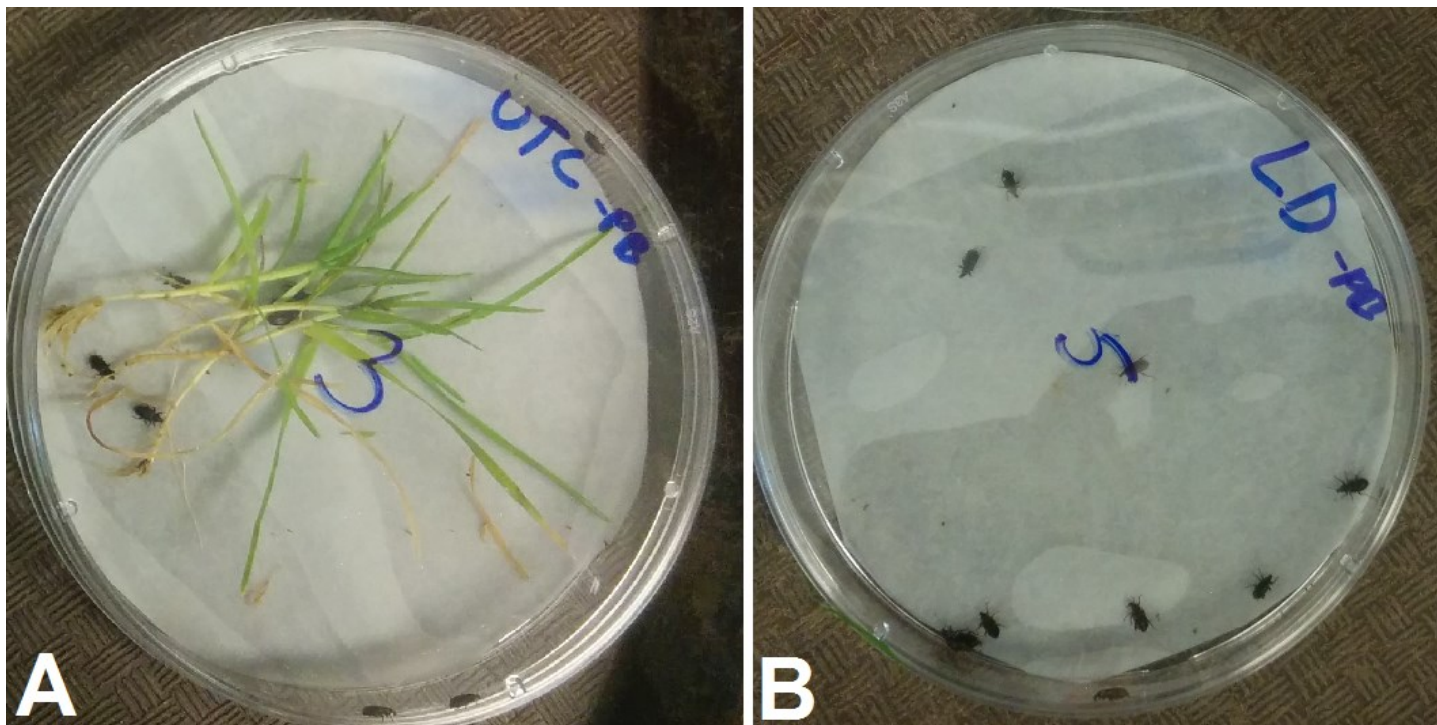
**Figure 1. Hamilton microapplicator used for topical application of the insecticide active ingredients (AI).**

(Figure 3A). Each dose of each AI was replicated four times. Mortality was evaluated 72 h after treatment and the lethal dose killing 50% of tested individuals ( $LD_{50}$ ) was determined. Resistance ratios were calculated ( $RR_{50} = LD_{50}$  of resistant /  $LD_{50}$  of susceptible population) and their significance determined (Robertson et al. 2007).

The populations collected at Rutgers Horticultural Farm 2 (HF), North Brunswick, NJ and at Pine Brook GC, Manapalan, NJ (PB) were relatively pyrethroid-susceptible and were considered susceptible. The other

**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
	$\lambda$ -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

**Figure 2. Petri dishes used as experimental arenas for topical assays (A) and diagnostic assays (B).****Table 2. LD<sub>50</sub>- (in ng/insect) and RR<sub>50</sub>-values (LD<sub>50</sub> resistant/ LD<sub>50</sub> susceptible) for 9 different ABW populations determined in topical bioassays.**

ABW populations and collection sites		Bifenthrin		$\lambda$ -Cyhalothrin		Chlorpyrifos		Spinosad		Clothianidin	
		LD <sub>50</sub>	RR <sub>50</sub> <sup>1</sup>	LD <sub>50</sub>	RR <sub>50</sub>	LD <sub>50</sub>	RR <sub>50</sub>	LD <sub>50</sub>	RR <sub>50</sub>	LD <sub>50</sub>	RR <sub>50</sub>
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*

<sup>1</sup>RR<sub>50</sub> were calculated with HF population as susceptible except for clothianidin for which the PB population was used.

<sup>2</sup>LD<sub>50</sub> marked with the same letters do not differ significantly at  $\alpha=0.05$ .

<sup>3</sup>RR<sub>50</sub> marked with an asterisk differ significantly from the susceptible population.

**Table 3. RR<sub>50</sub>-values (LD<sub>50</sub> resistant/ LD<sub>50</sub> susceptible) obtained in the different types of bioassays: Petri dishes assay (PDA), greenhouse assay (GHA) and topical assay (TA).**

ABW population	Bifenthrin			Chlorpyrifos	
	PDA	GHA	TA	PDA	TA
LI	3313.9*	384.8*	159.6*	61.3*	10.7*
EW	489.6*	84.7*	44.0*	17.6*	2.4*
JC	482.4*	ND	63.6*	16.2*	2.3*
HP	1.2	0.9		1.4	

RR<sub>50</sub> marked with an asterisk differ significantly from the susceptible population (PB).

populations had various levels of pyrethroids resistance/ tolerance (Table 2, Figure 2), with RR<sub>50</sub> ranging 14.2-343.1 (bifenthrin) and 7.8-323.6 ( $\lambda$ -cyhalothrin) (Figure 2). Pyrethroid resistant populations also demonstrated elevated tolerance to chlorpyrifos (RR<sub>50</sub> 3.3-15.5), clothianidin (RR<sub>50</sub> 2.9-9.7), and spinosad (RR<sub>50</sub> 3.0-5.1) (Figure 2). Topical assays with indoxacarb and chlorantraniliprole did not yield meaningful dose-response curves due to low mortality for the resistant populations.

To develop diagnostic assays for resistance monitoring and detection, greenhouse and laboratory assays were conducted using one susceptible and four resistant populations. Four to five concentrations of formulated bifenthrin (Talstar Pro) (range 0.01-600x of labeled rate) and chlorpyrifos (Dursban) (range 0.001-3x) were tested against five populations in Petri dish assays (Figure 3B) and corresponding insecticide AIs (technical grade) concentrations in vial assays (Figure 4). Ten adults were introduced per dish/vial. Mortality was evaluated 72 h after treatment and lethal concentrations (LCs) determined. A greenhouse test was conducted to validate results of the lab assays. Four to five concentrations of the formulated insecticides were sprayed on pots with established grass. Ten adults were introduced per pots immediately after treatment application. Adults were extracted 72 h later and survival rates recorded for LC determination. Our preliminary data suggest that RRs obtained from different assays types, except vial assays, were proportionally similar (Table 3). We did not observe significant differences among populations in vial assays. Resistance level could be determined in any of the conducted assays. The petri dish assay was the simplest and the least labor intensive assay. More replications are needed to validate our findings and determining diagnostic doses for practical use.

#### Summary Points:

- Pyrethroids resistance is widely spread among ABW populations. Most of the tested population had moderate to high level of resistance (RR<sub>50</sub> > 20).
- Populations resistant to pyrethroids have an elevated tolerance to insecticides of other chemical classes (RR<sub>50</sub> range 3-15)
- A petri dish assay with formulated products is likely the best option for resistance diagnostics and monitoring due to the assay's simplicity, practicality and discriminating power. More research is needed to validate our findings and to determine diagnostic doses for this assay type.

#### Literature cited

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