Annual Report for 2008

Title: Best Management Practices to Combat Pyrethroid Resistant Annual Bluegrass Weevils on Golf Course Turf

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In 2007 the development of pyrethroid resistance was confirmed for adults of several "annual bluegrass weevil" *Listronotus maculicollis* Kirby (Coleoptera, Curculionidae) populations in southern New England. The mechanisms responsible for conferring this resistance were unknown. In most cases, pyrethroid resistance can be linked to insecticide metabolic detoxification through interaction with endogenous enzymes. The best known of these enzymes, the cytochrome P450 monooxgenases (P450s or mixed function oxidases), the glutathione S-transferases (GSTs) and the carboxyl-esterases (COEs) render insecticides water soluble.

The involvement of the P450s, GSTs and COEs in insecticide resistance can be demonstrated with inhibitors of these enzymes, also known as insecticide synergists. The most widely utilized P450, GST and COE inhibitors are piperonyl butoxide (PBO) diethyl maleate (DEM) and *S*,*S*,*S*-tributyl phosphorotrithioate (DEF) respectively. In 2008, topical application bioassays with bifenthrin and bifenthrin combined with the synergists PBO, DEM and DEF were conducted on four field-collected populations of adult annual bluegrass weevils from Connecticut (New Haven, Norwich, Stamford and Hartford) to determine if P450s, GSTs and COEs mediated metabolic detoxification. Since pyrethroid resistance has emerged as a serious hurdle to adequately managing *L. maculicollis*, gaining a better understating of the mechanisms involved in this phenomenon may lead to improved management.

The New Haven population, which was the most susceptible to bifenthrin, exhibited no evidence of metabolic detoxification. Reduction in the LD_{50} of bifenthrin alone (from 3.1 to 2.2 µg/insect, yielding a synergist ratio [SR] of 1.4) was seen when this population was exposed to DEF, but not from exposure

to PBO or DEM (LD_{50}s of 3.9 and 5.7 µg/insect, respectively) (Table 1). The LD_{50}s for this population, with and without synergists, did not differ statistically, based on overlap of their 95% confidence limits.

The Norwich population exhibited a low level of resistance to bifenthrin (resistance ratio [RR] = 8.2) (Table 1). A reduction in the LD_{50} of bifenthrin was seen when this population was exposed to PBO (SR = 5.2). The LD_{50}s of bifenthrin, bifenthrin/DEM, and bifenthrin/DEF did not significantly differ. The Stamford population exhibited the second highest level of resistance to bifenthrin (RR = 28.1). A reduction in the LD_{50} of bifenthrin was seen when this population was exposed to bifenthrin/PBO (SR = 5.9) and bifenthrin/DEM (SR = 3.2). The LD_{50}s of bifenthrin and bifenthrin/DEF did not significantly differ. The Hartford population exhibited the highest level of resistance to bifenthrin (RR = 206). A reduction in the LD_{50} of bifenthrin was seen when this population was exposed to bifenthrin/PBO (SR = 4.5), bifenthrin/DEM (SR = 2.5) and bifenthrin/DEF (SR = 3.2).

Response of the bifenthrin-susceptible population (New Haven) provided a baseline against which all other populations were compared. In the population with a low level of resistance (Norwich), only detoxification by P450s was significant. The population with the second highest level of resistance (Stamford), involved both P450s and GSTs. The population with the highest level of resistance (Hartford) involved P450s, GSTs and COEs. This study suggests that enzyme-mediated metabolic detoxification plays an important role in annual bluegrass weevil pyrethroid resistance and may need to be overcome, for example by synergists, for proper control.

Future experiments will further define which chemicals are most effective against weevils and larvae.

Table 1. LD_{50}s for adult annual bluegrass weevils for bifenthrin and bifenthrin/synergist combinations.

Population	Treatment	N	Slope (SE)	LD_{50} (µg/insect)[a]	χ^2 (df)[b]	95% FL	RR[c]	SR[d]
New Haven								
	Bifenthrin	180	3.3 (0.6)	3.1 a	4.7 (3)	2.4-3.8	---	---
	Bifenthrin+PBO	140	2.4 (0.5)	3.9	1.2 (4)	2.7-5.6		---
	Bifenthrin+DEM	140	1.7 (0.3)	5.7	3.7 (4)	3.7-9.0		---
	Bifenthrin+DEF	140	1.7 (0.3)	2.2	1.9 (4)	1.4-3.3		1.4
Norwich								
	Bifenthrin	140	1.7 (0.3)	25.3 b	5.7 (4)	14.9-48.5	8.2	---
	Bifenthrin+PBO	140	1.1 (0.3)	4.9	2.8 (4)	1.7-8.8		5.2[e]
	Bifenthrin+DEM	140	0.9 (0.2)	8.7	5.1 (4)	3.1-16.8		2.8
	Bifenthrin+DEF	210	1.4 (0.2)	26.9	4.3 (4)	18.6-40.5		---
Stamford								
	Bifenthrin	216	3.5 (0.9)	87.1 c	3.2 (6)	66.3-126	28.1	---
	Bifenthrin+PBO	216	2.3 (0.3)	14.6	8.3 (5)	10.6-19.4		5.9[e]
	Bifenthrin+DEM	210	2.1 (0.4)	27.4	3.7 (5)	18.8-37.9		3.2[e]
	Bifenthrin+DEF	216	1.7 (0.3)	56.6	10.5 (5)	38.9-85.3		1.5
Hartford								
	Bifenthrin	320	1.9 (0.3)	638 d	9.7 (13)	468-824	206	---
	Bifenthrin+PBO	220	1.4 (0.2)	143	4.4 (8)	84.6-224		4.5[e]
	Bifenthrin+DEM	220	1.0 (0.2)	260	6.7 (8)	144-457		2.5[e]
	Bifenthrin+DEF	220	1.5 (0.3)	199	6.1 (8)	109-309		3.2[e]

[a] LD_{50} followed by different letters are significantly different ($P = 0.05$) for unsynergized bifenthrin treatment, compared among the four populations; significant difference is based on a failure of 95% fiducial limit overlap.
[b] L.R. chi-square goodness-of-fit values. Tabular values at $P = 0.05$: 3 df, 7.82; 4 df, 9.49; 5 df, 11.07; 6 df, 12.59; 8 df, 15.51; and 13 df, 22.36
[c] Resistance Ratio (RR): LD_{50} resistant population ÷ LD_{50} most susceptible population.
[d] Synergism Ratio (SR): LD_{50} bifenthrin alone ÷ LD_{50} bifenthrin + synergist, either PBO, DEM or DEF.
[e] Synergism ratio is significant, $P = 0.05$ (LD_{50} within a population for bifenthrin + synergist is significantly lower than the LD_{50} for bifenthrin; significant difference is based on lack of 95% fiducial limit overlap).

Field experiments showed that Aloft and Dylox were very effective for curative control of 4th and 5th instar larvae (Table 2). If these results can be repeated for Aloft, this adds another control timing strategy for a pyrethroid/neonicotinoid combination (bifenthrin/clothianidin). Conserve did provide some control but was not as effective as either Aloft or Dylox.

Another field experiment versus second generation larvae was applied August 8, 2008 (Table 3). Both Dylox and Provaunt provided 56% control which was significant but based on the number of pupae collected, the application was probably a week to 10 days later than optimal for this population. This emphasizes the importance of monitoring for this pest for optimal control timing.

Table 2. Efficacy of Conserve, Aloft, and Dylox for curative control of annual bluegrass weevil larvae in a golf course fairway, New Haven, Conn., 2008.

Treatment	Rate (lbs. ai/acre)	Timing	$\overline{X} \pm$ SEM live larvae / 0.5 ft^2 13 June	Percent Control
Conserve SC	0.4	6 June	29.5 ± 8.7b	62
Aloft SC	2.5lbs clothianidin/ 1.26lbs bifenthrin	6 June	10.3 ± 2.1c	87
Dylox 80S	8.1	6 June	3.3 ± 0.9c	96
Control	---		77.0 ± 2.1a	--

$F = 43.61$, df = 3,9 $P < 0.01$

[a]Means in the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD).

DATE OF APPLICATION: 6 June 2008. Rainfall post treatment to evaluation date = 0.34”; randomized complete block design, four replicates, plot size = 32.3 ft^2; application water was equivalent to 2 gal./1,000 ft^2, the turf consisted of annual bluegrass; thatch = 0.25"; texture = sandy loam. Treatments were evaluated by taking five 4.25” cores per plot 8 days after treatment (DAT) and extracting live larvae with forceps by hand.

Table 3. Efficacy of Dylox and Provaunt for curative control of annual bluegrass weevil larvae and pupae in a golf course fairway, Pawtucket, RI, August 2008.

Treatment	Rate (lbs. ai/acre)	Timing	$\overline{X} \pm$ SEM live larvae and pupae / 0.5 ft^2 13 August (5 DAT)	Percent Control
Dylox 80S	8.1	8 August	39.8 ± 6.5b	56
Provaunt 30WDG	0.15	8 August	39.5 ± 5.6b	56
Provaunt 30WDG	0.225	8 August	39.3 ± 4.6b	56
Control	---		89.5 ± 19.4a	--

$F = 4.33$, df = 3,9 $P = 0.03$

[a]Means in the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD).

DATES OF APPLICATION: 8 August 2008. Rainfall post treatment to evaluation date = 0.36"; randomized complete block design, four replicates, plot size = 32.3 ft^2; application water was equivalent to 2 gal./1,000 ft^2, the turf consisted of annual bluegrass; thatch = 0.25"; texture = sandy loam. Treatments were evaluated by taking five 4.25" cores per plot 5 days after treatment (DAT) and extracting live larvae with forceps by hand.