Title: 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 and determine possible diagnostic doses to detect resistant populations in the field.
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.

Start Date 2014

Project Duration 2 years

Total Funding *$19,642*

Summary text

Pyrethroid resistance of annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a growing problem on golf courses in the Northeast (Ramoutar et al. 2009, Koppenhöfer et al. 2012). Our previous findings demonstrated that pyrethroid resistance is widely spread among ABW populations. Moreover, populations resistant to pyrethroids have an elevated tolerance to insecticides of other chemical classes (RR_{50} range 3-15). The broad nature of the resistance strongly suggest involvement of detoxification enzymes as a resistance mechanism.

To determine involvement of enzymatic detoxification in ABW resistance to pyrethroids, combinations of synergists (oxidase inhibitor PBO, glutathione transferase inhibitor DEM, esterase inhibitor DEF) and bifenthrin or chlorpyrifos were tested in laboratory bioassays against adults from seven ABW populations. Bifenthrin toxicity was significantly increased in presence of PBO (8-20 fold) and DEF (9-39 fold) which indicates involvement of oxidase and esterase systems as possible resistance mechanisms (Table 1). DEM had a weak effect on bifenthrin toxicity for most populations. Synergists did not significantly affect chlorpyrifos toxicity in our study (Table 1).

To determine and compare level of adult and larval resistance selected insecticides of different chemical classes (Table 2) were tested against susceptible and resistant ABW populations in the greenhouse experiments. Ten adults were caged in the *P. annua* pots 2 h before treatments (Fig. 1A). Treatments were applied using a Generation III Research sprayer

(Fig. 1D,E). For larval assays, adults (3 pairs) were caged in containers with established *P. annua* (Fig. B,C) for 1 week. Treatments were applied 10 days after adult removal (average larval stage ~3-3.5 instar), and mortality evaluated 10 days after application. Results of our greenhouse adult bioassays corresponded to results obtained in other assays types (Table 3,4). The LI population had the highest RR_{50} for bifenthrin and chlorpyrifos. The HP and PB populations were most susceptible for both tested insecticides.

Larvae of the resistant populations were less susceptible to chlorantraniliprole, bifenthrin, chlorpyrifos compared to susceptible populations. These insecticides provided higher percent reduction in susceptible populations (80-90%) compared to resistant populations (up to 57% reduction) (Fig. 2,3). Percent reduction provided by spinosad and indoxacarb differed only between the most resistant LI population and susceptible populations.

Petri dish and vial bioassays were further evaluated as possible diagnostic assays for resistance detection and monitoring. Five concentrations of formulated bifenthrin (Talstar Pro) and chlorpyrifos (Dursban) were tested against susceptible and resistant populations in Petri dish assays and corresponding AI concentrations in vial assays (Fig. 4). Resistance ratios obtained from different assays types were proportionally similar (Table 3,4). The population with the highest resistance level (LI) in the topical assays was also the most resistant in the Petri dish and vial assays. Lowest LD_{50} were observed in the population previously considered susceptible (PB). Vial assays were consistent with other assays and effectively separated resistant and susceptible populations (Tables 3, 4).

- Moderate to high levels of resistance ($RR_{50} > 20$) were repeatedly observed among ABW populations which did not change significantly over the 2 years of our study.
- Resistance levels of tested ABW populations were significantly reduced in presence of the enzyme inhibitors PBO and DEF, suggesting that enzymatic detoxification plays important role in ABW resistance to pyrethroids.
- Larvae of the resistant population were less susceptible to most insecticide compared to susceptible populations.
- Any of the tested diagnostic assay could be used for resistance diagnostic. 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.

Figure and table captions:

Table 1. Effect of synergists on toxicity of bifenthrin and chlorpyrifos to susceptible and resistant ABW populations.

Table 2. Active ingredients and products of insecticides tested against ABW population in a greenhouse assay.

Table 3. LD_{50} and RR_{50}-values (LD_{50} resistant / LD_{50} susceptible) for bifenthrin obtained in the different types of bioassays in 2014-2015.

Table 4. LD_{50} and RR_{50}-values (LD_{50} resistant / LD_{50} susceptible) for chlorpyrifos obtained in the different types of bioassays in 2014-2015.

Figure 1. Experimental set up for the greenhouse assays: cages for adult greenhouse assays (A), cages for adult oviposition for larval assay (B, C), spray system used in the greenhouse assays in 2015 (D, E).

Figure 2. Comparative efficacy of selected insecticides against susceptible and resistant ABW populations. Means with the same letter are not significantly different within insecticides.

Figure 3. Efficacy of selected insecticide against larvae of four ABW populations (resistant and susceptible).
[1] Means with the same letter are not significantly different within populations.
[2] Means marked with an asterisk differ significantly from the untreated control.

Literature cited

1. Koppenhöfer A.M., Alm S.R., Cowles R.A., McGraw B.A., Swier S., Vittum P.J. 2012. Controlling annual bluegrass weevil: optimal timing and rates. Golf Course Manag., March 2012, 98-104.

2. Ramoutar D., S.R. Alm, R.S. Cowles. 2009. Pyrethroid resistance in populations of *Listronotus maculicollis* (Col.: Curculionidae) from south. New England golf courses. J. Econ. Ent. 102, 388–392.

Table 1. Effect of synergists on toxicity of bifenthrin and chlorpyrifos to susceptible and resistant ABW populations.

	LD_{50}	**SR^{2}_{50}**	**LD_{50}**	**SR_{50}**	**LD_{50}**	**SR_{50}**	**LD_{50}**	**SR_{50}**	**LD_{50}**	**SR_{50}**	**LD_{50}**	**SR_{50}**	**LD_{50}**	**SR_{50}**
ABW populations	**PB**		**HP (1.3)[1]**		**GB (8.1*)**		**CN (10.9*)**		**EW (24.3*)**		**JC (37.2*)**		**LI (222.7*)**	
Bifenthrin	**8.9**	NC	**11.4**		**71.6**		**96.6**		**215.9**		**215.9**		**1982**	
Bifenthrin+PBO	1.1	7.9*3	0.7	16.3*	3.8	18.8*	9.4	10.3*	19.3	11.2*	19.3	7.9*	216.9	9.1*
Bifenthrin+DEM	10.5	0.8	3.5	3.3*	29.5	2.4	61.7	1.6	113.8	1.9	113.8	3.1*	1611.0	1.2
Bifenthrin+DEF	0.9	9.9*	1.1	10.4*	5.1	14.2*	6.6	4.6*	5.6	8.6*	5.6	13.4*	127.5	15.5*
ABW populations	**PB**		**HP (2.08)**		**GB (1.66)**		**CN (4.2*)**		**EW (3.8*)**		**JC (1.4*)**		**LI (12.9*)**	
Chlorpyrifos	**214.5**		**102.9**		**357.5**		**894.9**		**823.2**		**308.4**		**2783**	
Chlorpyrifos+PBO	242.2	0.81	63.9	1.6	310.3	1.15	442.4	2.02	899.9	0.91	239.9	1.29	2006	1.39
Chlorpyrifos+DEM	211.5	1.01	108.5	0.9	176.7	2.02	899.9	0.99	735.7	1.12	255.3	1.21	2568	1.08
Chlorpyrifos+DEF	192.9	1.11	136.6	0.8	172.4	2.07	525.7	1.7	836.6	0.98	137.2	2.25	2662	1.05

[1] Population tested followed by resistance ratios in parenthesis. RR_{50} (resistance ratios) were calculated with PB population as susceptible (LD_{50} of susceptible/LD_{50} resistant). RR $\geq$ 20 reflect moderate to high level of resistance.

[2] SR_{50} (synergist ratios) were calculated using following formula LD_{50} of bifenthrin (or chlorpyrifos) alone /LD_{50} of bifenthrin (or chlorpyrifos)+synergist (PBO, DEF or DEM)

[3] RR_{50} marked with an asterisk differ significantly from the susceptible population. SRs marked with asterisks represent significant reduction of resistance level in presence of the synergist.

Table 2. Active ingredients and products of insecticides tested against ABW populations in a greenhouse assay

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
	Trichlorfon	Dylox	Bayer, Research Triangle Park, NC
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

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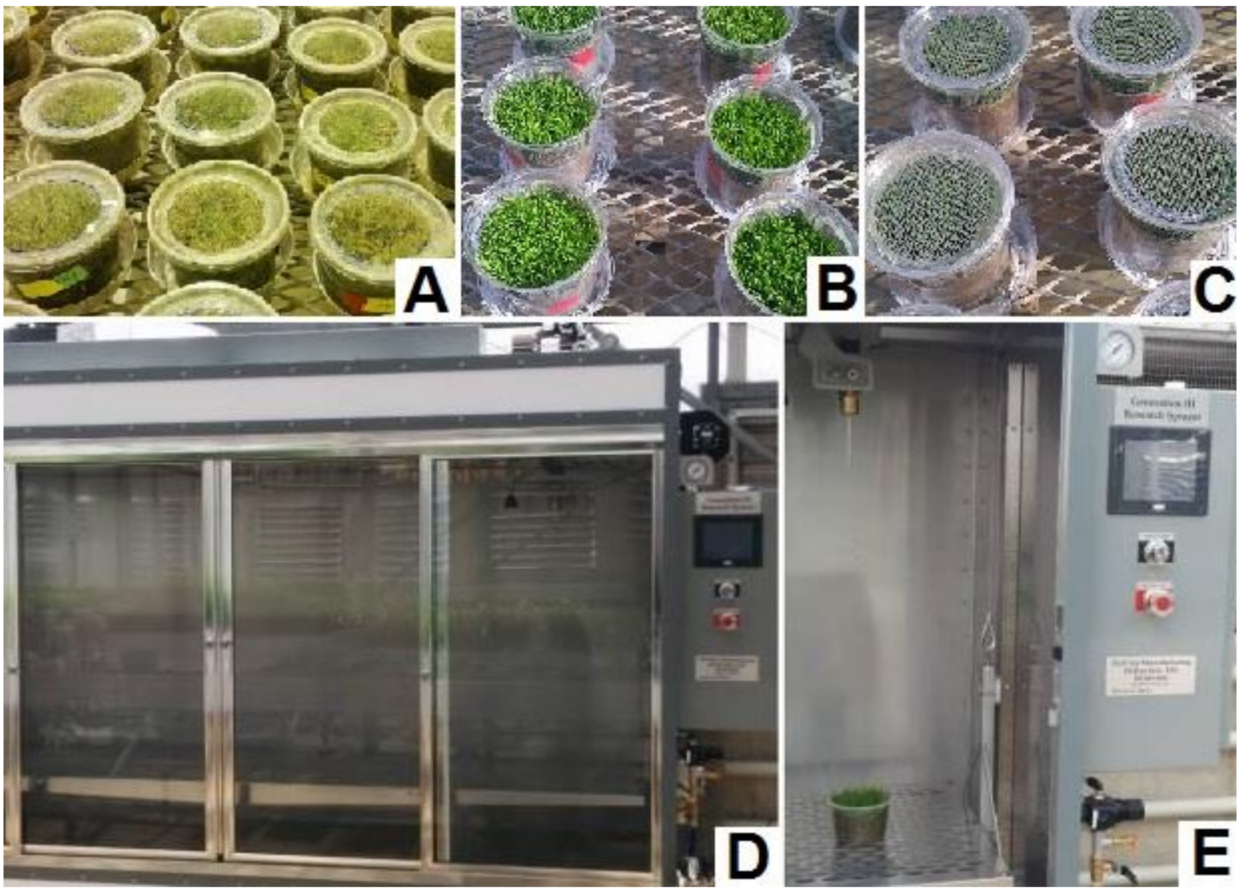

Figure 1. Experimental set up for the greenhouse assays: cages for adult greenhouse assays (A), cages for adult oviposition for larval assay (B, C), spray system used in the greenhouse assays in 2015 (D, E).

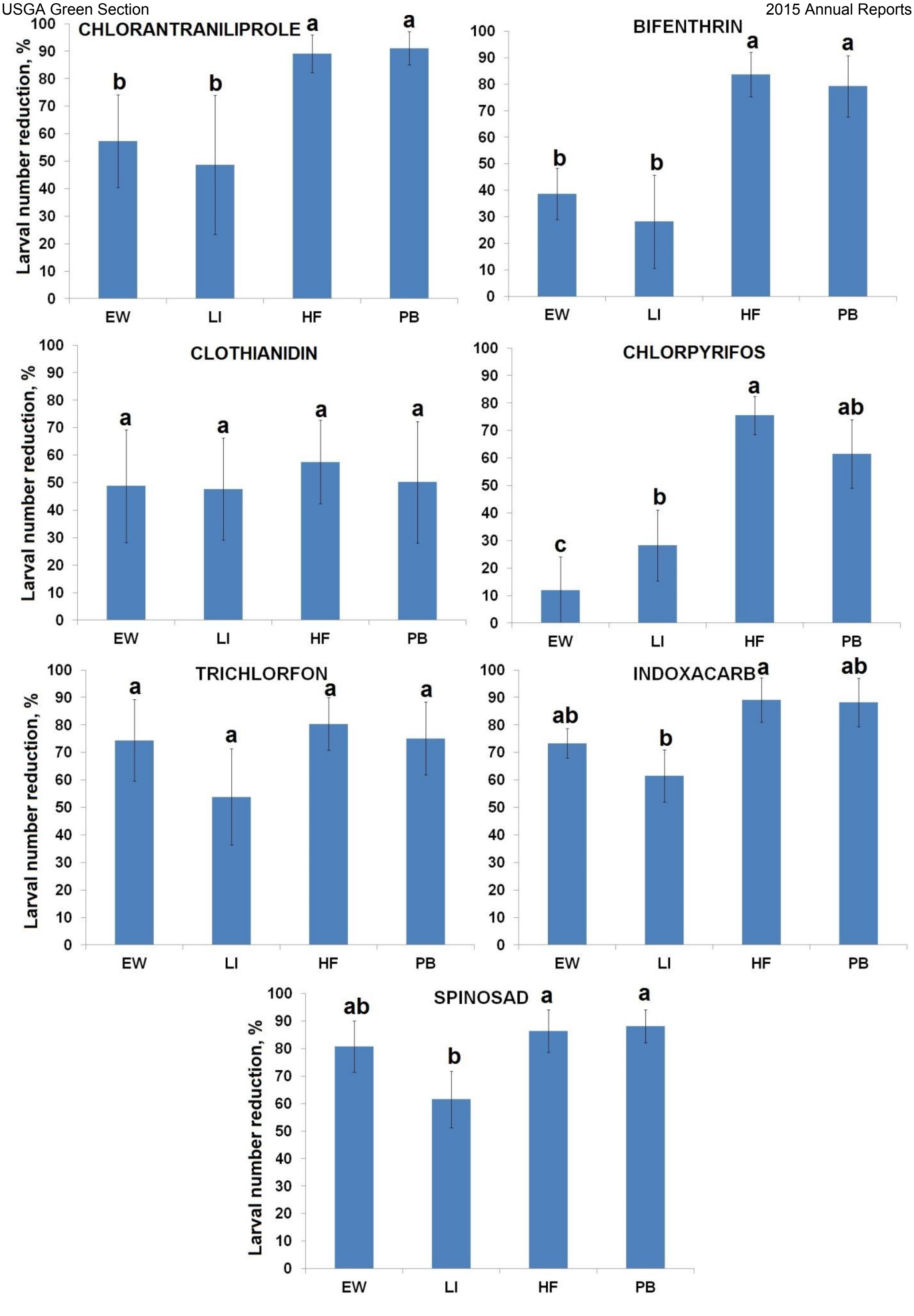

Figure 2. Comparative efficacy of selected insecticides against susceptible and resistant ABW populations. Means with the same letter are not significantly different within insecticides.

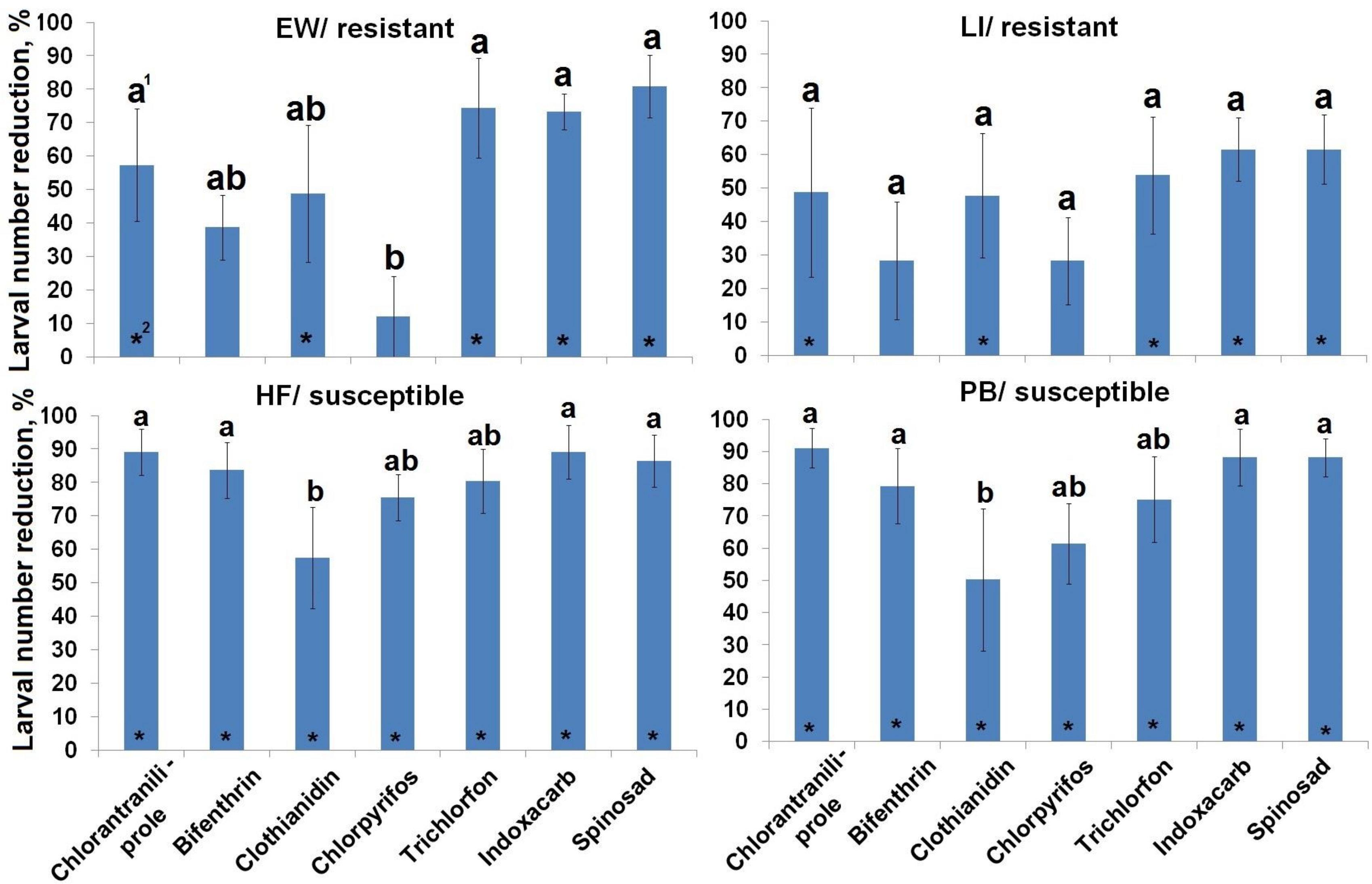

Figure 3. Efficacy of selected insecticide against larvae of four ABW populations (resistant and susceptible).
1 Means with the same letter are not significantly different within populations.
2 Means marked with an asterisk differ significantly from the untreated control.

Table 3. LD_{50} and RR_{50}-values (LD_{50} resistant / LD_{50} susceptible) for bifenthrin obtained in the different types of bioassays in 2014-2015.

	Vial (24h)		**Petri dish (24h)**		**Greenhouse (72h)**		**Topical (72h)**			
							2014		**2015**	
	LC_{50}	**RR_{50}[1]**	**LC_{50}**	**RR_{50}**	**LD_{50}**	**RR_{50}**	**LD_{50}**	**RR_{50}**	**LD_{50}**	**RR_{50}**
PB	3.2	**2.8**	0.4	**3.7**	0.1	**1.1**	5.1	**NC**	8.9	**NC**
HP	1.1	**NC**	0.1	**NC**	0.1	NC	NT	**NT**	11.4	**1.3**
GB	28.2	**25*[2]**	3.4	**31.4*[2]**	0.7	**7.8***	72.7	**14.3***	71.6	**8.1***
CN	51.8	**45.9***	3.3	**30.2***	3.9	**43.3***	123.1	**24.1***	96.6	**10.9***
JC	NT	**NT**	NT	**NT**	4.8	**53.3***	225.8	**44.3***	215.9	**24.3***
EW	7.0	**62.9***	5.9	**54.2***	5.9	**65.6***	326.9	**64.1***	331.1	**37.2***
LI	294.6	**261.3***	43.1	**392.0***	47.3	**525.6***	819.1	**160.6***	1982.0	**222.7***

RR_{50} were calculated using population with the lowest LC 50 as most susceptible.
[2] RR_{50} marked with an asterisk differ significantly from the most susceptible population.

Table 4. LD_{50} and RR_{50}-values (LD_{50} resistant / LD_{50} susceptible) for chlorpyrifos obtained in the different types of bioassays in 2014-2015.

	Vial (24 h)		**Petri dish (24 h)**		**Greenhouse (72 h)**		**Topical (72h)**			
							2014		**2015**	
	LC_{50}	**RR_{50}[1]**	**LC_{50}**	**RR_{50}**	**LD_{50}**	**RR_{50}**	**LD_{50}**	**RR_{50}**	**LD_{50}**	**RR_{50}**
PB	0.000		0.001	**NC**	0.04	**NC**	299	**NC**	214.5	**2.1**
HP	0.001	**2**	0.002	**2**	0.05	**1.3**	NT	**NT**	102.9	**NC**
GB	0.003	**6*[2]**	0.005	**5***	0.28	**3.8***	852	**2.8**	357.5	**1.7**
CN	0.003	**6***	0.010	**10***	0.48	**12.0***	1118	**3.7**	894.9	**4.2***
JC	NT	**NT**	NT	**NT**	0.15	**7.0**	688	**2.3**	823.2	**3.8***
EW	0.002	**4***	0.006	**6***	0.49	**12.3***	683	**2.3**	308.4	**1.4**
LI	0.010	**20***	0.182	**182***	1.09	**27.3***	3203	**10.7**	2783	**12.9**

[1] RR_{50} were calculated using population with the lowest LC 50 as most susceptible.
[2] RR_{50} marked with an asterisk differ significantly from the most susceptible population.