CONTACT TOXICITY OF DIACYLHYDRAZINE AND DIPHENYL BENZOYL UREA INSECT GROWTH REGULATORS AGAINST BEET ARMYWORM, BOLLWORM AND TOBACCO BUDWORM

D. A. Wolfenbarger and D. J. Wolfenbarger
D2 Consultants
Brownsville, TX

Abstract

Contact toxicity was shown by diacylhydrazine insect growth regulators (IGRs) against strains of beet armyworm, bollworm and tobacco budworm when topically applied to different ages of their larvae. Methoxyfenozide (Intrepid) was the most toxic most of the time against strains of all three pests but 7 days were required for maximum toxicity. Of the five diphenyl benzoyl urea IGRs tested for contact toxicity only lufenuron showed an LD_{50} against a reference strain of the beet armyworm. Great differences in toxicity between methoxyfenozide and tebufenozide (Confirm) were shown between five field collected strains of beet armyworm from the Lower Rio Grande Valley of Texas, U. S. A. and Tamaulipas, Mexico. There was an 891 fold difference in LD_{50}s for methoxyfenozide of the reference strain of beet armyworm and one of the field collected strains. LD_{50}'s for two of the field collected strains showed no significant differences. LD_{50} 's of methoxyfenozide by the other three strains were significantly different from those shown for tebufenozide. A trend toward greater contact toxicity at all ages to bollworm than to tobacco budworm was shown for halofenozide, methoxyfenozide and tebufenozide.

Introduction

The diacylhydrazine and the diphenyl benzoyl urea classes of chemistry have compounds which were evaluated as insect growth regulators (IGRs) against three lepidopteran pests of cotton. They are considered to be more toxic to these pests when ingested but we wanted to determine the magnitude of their contact toxicity because this is an important route of administration to larvae of the beet armyworm, *Spodoptera exigua* (Hubner), bollworm, *Heliocoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.). Compounds could contact larvae in spray particles at the time of application or when larvae walk over the plant surface. IGRs which are effective against lepidopteran pests of cotton and are not toxic to mammals are needed. All compounds we tested are considered to be safe to mammals.

Recently a series of diacylhydrazine compounds which are ecdysone agonists became available for testing. The first reports on this mode of action to insects were shown for the first diacylhydrazine ecdysone agonist, RH 5849 (1,2-dibenzoyl-1-tert-butylhydrazine), by Wing (1988) and Wing et al.(1988). Wing and Aller (1990) and Darvas et al (1992) showed widespread toxicity among various insect orders by RH 5849. In 1992 and 1995 tebufenozide showed excellent activity in the field against the beet armyworm in Guatemala, Heller et al (1992) and the Lower Rio Grande Valley of Texas, Sparks et al (1996). In laboratory experiments tebufenozide affected mortality and longevity of bollworm larvae when doses were placed on diet surface, Chandler et al (1992ab).

Various diphenyl benzoyl urea IGRs have been tested on cotton for a decade against the bollworm/tobacco budworm. Diflubenzuron (Dimilin), the only registered compound of the class in the United States, was shown to be effective against the beet armyworm in field tests by Sparks et al (1996). In laboratory experiments diflubenzuron was toxic to neonate beet armyworm after 7 d following sprays to cotton leaf surface Weiland et al (1995).

Here, contact toxicity of 9 diacylhydrazine and diphenyl benzoyl urea IGRs was tested against larvae of a laboratory reference strain of the beet armyworm. Four of these diacylhydrazine IGRs and diflubenzuron were tested against larvae of 5 field collected strains of the beet armyworm. Then two of these diacylhydrazines were tested against different ages of larvae of beet armyworm. Contact toxicity of three diacylhydrazine IGRs to laboratory reference strains of bollworm and tobacco budworm at different ages of larvae were determined. Objective was to show doses which indicate the magnitude of contact toxicity and when this toxicity will occur against strains of these three lepidopteran pests of cotton.

Materials and Methods

Technical halofenozide, methoxyfenozide, Rohm & Haas (RH) 5849, and tebufenozide were obtained from Rohm & Haas, Philadelphia, PA, diflubenzuron was obtained from Duphar, Inc., Amsterdam, Netherlands, tefluron was obtained from EM Industries, Inc., White Plains, NY, chlorflurazum and lufenuron were obtained from Novartis, Inc., Greensboro, NC and triflumuron was obtained from Bayer, Inc., Kansas City, KS.

A reference strain of beet armyworm from DOW-Zeneca was tested in 1989 and 1995. In 1995 five field strains were collected from cotton near Donna, Lyford, Mercedes and Weslaco, Texas and Rio Bravo, Tamaulipas, Mexico and treated in generation one. Larvae of the Weslaco strain were tested at different ages in generation two. IGRs were tested against reference strains of bollworm and tobacco budworm maintained in the USDA-ARS laboratory at Weslaco, TX. Beet armyworm, bollworm and tobacco budworm of all strains were reared on the same artificial diet for all tests.

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Technical of all compounds was diluted in acetone and μg doses were applied in one μl. Doses were topically applied to dorsum of thorax of larvae at indicated d of age.

Doses, as μg/larva, of diacylhydrazine methoxyfenozide and tebufenozide were 0.78 to 50, RH 5849 were 1.56 to 100 and halofenozide were 3.125 to 50 and diphenyl benzoyl urea chlorfluazum, diflubenzuron, lufenuron, tefluron and triflumuron were tested at 0.5 to 12.5.

Reference strain and field collected strains of beet armyworm larvae were treated at 5 or 6 days when they weighed 15 ± 6 mg. In addition, larvae of one field collected strain were treated at 8, 10, 11, and 18 d of age. Larvae of bollworm and tobacco budworm were treated at 5, 7, 9 and 12 d. Weights were not determined. Following treatment mortalities were determined at 72, 96 or 168 h.

Probit analysis (SAS 1988) was conducted to determine LD_{50}, slope ± standard error (S.E.) and 95% Confidence Interval (C.I.) for each insecticide, insect or age at time of treatment. When confidence intervals did not overlap LD_{50} values were considered to be significantly different. When ratio of slope/SE was <1.96 $t_{0.05}$ for infinity, regression did not differ from zero. Slope ± S.E.. and % mortality at indicated dose was shown for each of these non-significant regressions.

Results and Discussion

Beet Armyworm

Great variation in toxicity was determined for four ecdysone agonists and five diphenyl benzoyl urea compounds against a reference strain (data not shown in table). Hr posttreatment, number larvae tested, slope ± SE, LD_{50} as μg/larvae and (95% C. I.) for methoxyfenozide and lufenuron were 168, 259, 0.46 ± 0.14, 0.0016 (4.0 x 10-105- 0.22) and 96, 137, 0.2± 0.1, 13.78 (∞-∞), respectively. Methoxyfenozide was 8,613 fold more toxic than lufenuron. LD_{50} for methoxyfenozide was shown by Sparks et al (1995). Non-significant regressions were shown for halofenozide, tebufenozide, RH 5849, diflubenzuron, chlorfluazum, tefluron and triflumuron as 0.38 ± 0.24 (42% at 50), 0.19 ± 0.18 (82% at 0.0475), 0.56 ± 0.39 (33% at 100), 0.032 ± 0.67 (19% at 0.5), 0.43 ± 0.34 (80% at 0.5), 0.24 ± 0.23 (35% at 0.5) and 3.83 x 10 -17 ± 0.19 (13% at 0.5), respectively. Mortalities of all phenyl benzoyl urea IGRs and RH5849 were determined after 96 h. Mortality of the diacylhydrazines were determined after 168 h. Topical applications were made to 50-1350 larvae with each IGR. RH 5849, halofenozide, diflubenzuron, tefluron and triflumuron did not kill 50% of treated 5 to 6-d larvae at the greatest dose tested. Tebufenozide and chlorfuazum killed >80% at the lowest and greatest dose tested, respectively. Regressions of these seven compounds did not differ from zero, because toxicity by all the doses tested was high or low, the same toxicity was determined for all doses tested, or toxicity of all doses was variable.

LD_{50}'s of field collected strains (Table 1) for methoxyfenozide were all greater than the laboratory reference strain with one exception. LD_{50}'s for methoxyfenozide by the strain collected in Mexico and the laboratory reference strain had overlapping confidence limits. LD_{50}'s for these field collected strains ranged from <1 to >11 for methoxyfenozide and differed 151 fold. This is great variation. LD_{50}'s of these two IGRs ranged from 3 to 64, a 21-fold difference. LD_{50}'s of tebufenozide and methoxyfenozide of the strains from Mercedes and Donna were similar since the C.I. values overlapped. Diflubenzuron had an LD_{50} of 5 μg/larva against the strain from Donna.

Non-significant regressions of these field collected strains for RH 5849 and diflubenzuron from Mercedes, RH 5849, halofenozide, diflubenzuron from Lyford, RH 5849 and diflubenzuron from Rio Bravo, Tamaulipas, Mexico and RH5849, halofenozide and diflubenzuron were 0.41 ± 0.31 (20% at 100), 0.29 ± 0.22 (54% at 12.5), 0.54 ± 0.47 (19% at 3.125), 0.44 ± 0.55 (13% at 50), 0.01 ± 0.54 (14% at 0.78), 0.21 ± 0.28 (21% at 1.56), 0.097 ± 0.19 (32% at 0.78), 0.25 ± 0.28 (40% at 50), 0.22 ± 0.16 (36% at 50) and 0.037 ± 0.3 (35% at 0.78), respectively. Mortalities were taken at 168 hr except for RH 5849 from Mercedes and Weslaco and diflubenzuron from Weslaco. Mortalities were determined after 96 h for these two IGRs. Topical applications were made to 77-261 larvae with each IGR. Both RH 5849 and halofenozide showed non-significant regressions against all the field collected strains and <50% mortality at 1.56 to 50 μgs/larva. These results show great strain specificity within this species.

Comparison of toxicity by tebufenozide and methoxyfenozide to 8, 10, 11 and 18 d larvae of Weslaco strain of beet armyworm was determined (data not shown in table). Number larvae tested, slope ± SE, LD_{50} as μg/larva and (95% Confidence Interval) for tebufenozide and methoxyfenozide to 8 and 11 day larvae were 83, 1.0 ± 0.47, 0.15 (4.2 x 10-17- 0.66) and 107, 1.36 ± 0.42, 0.41 (0.023-0.93), respectively. LD_{50}'s were <1 for larvae of this strain for these IGRs indicating toxicity to different ages of older larvae.

Against this same strain nonsignificant regression for methoxyfenozide at 0.59 ± 0.56 (91% at 0.78), 0.79 ± 0.45 (84% at 1.56) and 0.86 ± 0.47 (89% at 1.56) after 8, 10, and 18 d, respectively, and for tebufenozide at 1.09 ± 0.63 (8% at 1.56), 0.39 ± 0.38 (81% at 1.56) and 0.12 ± 0.35 (63% at 1.56) after 10, 11 and 18 d, respectively, was determined. Mortalities were taken after 168 h and 73 to 211 larvae were treated. Mortalities of 84% to 91% were 0.78 and 1.56 μg/larvae, respectively, with methoxyfenozide. Mortalities of 63% to 89% were 1.56 μg/larva for tebufenozide. Greater doses caused mortalities of 95% to 100% for both IGRs. These high mortalities caused the non-significant regressions. As suggested by Darvas et al (1992) there is great variation in toxicity of larval stages for these diacylhydrazine compounds.

Bollworm

At 5 d methoxyfenozide and tebufenozide had LD_{50}s 10-fold lower than shown on any other day of treating (Table 2). At 7 d LD_{50}'s of halofenozide, methoxyfenozide and tebufenozide were <0.44 μg/larva; methoxyfenozide was the most toxic. After 12 d tebufenozide showed an LD_{50} 50-fold greater than shown at 5 and 7 d. Zero to 93% mortality was found to 9 and 12 d larvae at 0.00775 to 50 μg tebufenozide/larva, respectively. Non significant regression for bollworm was shown for halofenozide after 5 d, halofenozide, methoxyfenozide and tebufenozide after 9 d, halofenozide and methoxyfenozide after 12 d as 0.11 ± 0.11 (100 % at 0.0075), 0.089 ± 0.29 (93% at 0.049), 0.13 ± 0.15 (64% at 0.12), 0.4 ± 0.023 (50% at 0.39), 0.24 ± 0.14 (82% at 0.0075) and 0.12 ± 0.1 (0% at 50), respectively. Mortalities were determined after 168 hr except for halofenozide, which was determined after 5 d. After 9 d mortalities of halofenozide, methoxyfenozide and tebufenozide were determined after 96 h. The 72 h mortality was shown because all the larvae were killed at all doses tested in 96 h. The 96 h mortalities were shown because there was no difference between the 96 and 168 h determinations. Toxicity was determined for 54-527 larvae by each IGR. These compounds indicate great variation in toxicity for the ages tested.

Tobacco Budworm

Methoxyfenozide showed an LD_{50} <1 against 7 and 12 d larvae while halofenozide showed an LD_{50} <1 against 12 d larvae (Table 2). Tebufenozide showed an LD_{50} of 3 to 5 μg/larva to 5 and 7 d larvae, while 66 to 1,543 μg/larva were needed for LD_{50} to 9 and 12 d larvae. respectively. Non significant regression of tobacco budworm was shown for halofenozide and methoxyfenozide after 5 d and methoxyfenozide after 9 d as 0.21 ± 0.24 (45% at 100), 0.11 ± 0.21 (70% at 0.39) and 0.15 ± 0.09 (62% at 0.024), respectively. All mortalities were taken after 168 h and 269-491 larvae were treated by each IGR.

Significant and non-significant slopes epitomize the slow action of these IGR compounds by contact to the different ages, stages and strains used here for all three species of these Lepidoptera. Flat slopes of larvae treated with IGR suggest interaction with site(s) of action slowly and that increasing doses do not necessarily cause greater mortalities. Flat slopes are caused when low doses have the same final effect as high doses. Slopes of diacylhydrazines ranged from 0.19 to 0.56 for the reference strain of beet armyworm. For this same strain they ranged from 3.83 x 10-17 to 0.24 for the diphenyl benzoyl urea IGRs. Slope values >0.56 of 5-6 d (Table 1) and 8-10 d and 11-18 d (data not shown in table) of the field collected strains were shown by 19%, 100 % and 50% of the significant and non-significant regressions, respectively. Slopes for tebufenozide were 0.42 (Table 1), 1.0, 1.09, 0.39 (data not shown in table) for 5-6, 8, 10, 11 and 18 d larvae of beet armyworm from Weslaco, respectively. Slopes for the diphenyl benzoyl urea IGR diflubenzuron against this insect ranged from 0.01 to 0.45 and were extremely flat. Slopes of significant regressions for tebufenozide and methoxyfenozide were >1 for Donna strain while slopes of these IGRs of the four remaining strains were <1 (Table 1). Slopes for the four diacylhydrazine compounds tested against the five field collected strains ranged from 0.21 to 1.18.

Slopes for significant regressions of three diacylhydrazines (Table 2) and the non-significant regressions for bollworm ranged from 0.11 to 1.01 for larvae treated at 5 to 12d (data not shown in table). Slopes versus age of larvae by the three compounds after 5, 7, 9 and 12 d ranged from 0.11 to 0.64, 0.94 to 1.01, 0.089 to 0.4 and 0.12 to 0.31, respectively. Slopes were steepest at 7 d and were about equal for bollworm larvae 5, 9 and 12 d. Slopes for methoxyfenozide, the most toxic IGR, of the same ages were 0.64, 0.94, 0.13 and 0.12, respectively. Slopes for this IGR were extremely flat for larvae 9 and 12 d.

Slopes for significant regressions of the same three diacylhydrazines (Table 2) and the non-significant regressions (data not shown in table) for tobacco budworm ranged from 0.11 to 1.55 for larvae treated at 5 to 12 d. Slopes versus age of larvae by the same compounds after 5, 7, 9 and 12 d ranged from 0.11 to 0.41, 0.82 to 1.55, 0.15 to 0.72 and 0.27 to 0.36, respectively. Slopes were steepest after 7 d. This was the same trend as shown for the bollworm. Slopes for methoxyfenozide for the tobacco budworm were 0.11, 0.82, 0.15 and 0.27 and followed the same trend as shown for the bollworm. For this IGR slopes were extremely flat for larvae 5, 9 and 12 d.

LD_{50} values of halofenozide, methoxyfenozide, and tebufenozide IGRs are more toxic to larvae of the bollworm than to the tobacco budworm. Results suggest that each compound has to be tested separately for contact toxicity against each species, age and stage. Perhaps one compound will be used against one stage of one species and another compound will be used against another stage. Perhaps one IGR will be applied for ingestion and another will be applied for contact toxicity.

In this test only contact toxicity was evaluated. It is acknowledged that quantities applied to the exoskeleton may be subject to more modes of degradation than the same quantities which are ingested. Modes of degradation include biochemical factors, reduced penetration and increased excretion. Data to confirm this statement against these lepidopteran strains and species has not been found in the literature. If quantities contact the insect which can kill it then the requirement for ingestion will not be as important.

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Table 1. Contact toxicity of diacylhydrazine and dephenyl benzoyl urea insect growth regulators to field collected larvae of five strains of beet armyworm. Weslaco, TX. 1995.

Insect growth regulator	Hours post-treatment	Number larvae tested
	Donna	
tebufenozide	168	179
methoxyfenozide	168	116
diflubenzuron	168	282
Mercedes		
tebufenozide	168	233
methoxyfenozide	96	332
	Lyford	
tebufenozide	168	246
methoxyfenozide	168	189
	Rio Bravo, Tamaulipas, Mexico	
tebufenozide	168	325
methoxyfenozide	168	206
	Weslaco	
tebufenozide	96	421
methoxyfenozide	96	282

Table 1. Continued.

Insect growth regulator	Slope±SE	LD_{50} (μg/larva) or mortality (%) at μg/larva	(95% Confidence Interval)
		Donna	
tebufenozide	1.18±0.21	5.21	(3.5-7.92)
methoxyfenozide	1.09±0.5	1.57	(∞ - ∞)
diflubenzuron	0.45±0.18	5.03	(2.27-43.03)
		Mercedes	
tebufenozide	0.6±0.15	3.99	(1.7-7.64)
methoxyfenozide	0.34±0.12	11.45	(4.43-68.39)
		Lyford	
tebufenozide	0.23±0.11	64.35	(12.94-5.21x10^{19})
methoxyfenozide	0.72±0.19	1.26	(0.29-2.42)
		Rio Bravo, Tamaulipas, Mexico	
tebufenozide	0.54±0.13	2.99	(1.54-5.73)
methoxyfenozide	0.4±0.18	0.076	(1.7x10^{-18}-0.59)
		Weslaco	
tebufenozide	0.42±0.11	11.86	(6.09-35.45)
methoxyfenozide	0.77±0.14	0.83	(0.26-1.56)

Table 2. Contact toxicity of three diacylhydrazine insect growth regulators at four different days to reference strain of bollworm and tobacco budworm. Weslaco, 1991-1995.

Insect growth regulators	Hours post-treatment	Number larvae tested	Slope±SE
	Bollworm		
	5 d		
methoxyfenozide	168	374	0.64±0.14
tebufenozide	168	336	0.36±0.13
	7 d		
halofenozide	168	298	0.99±0.22
methoxyfenozide	168	353	0.94±0.16
tebufenozide	168	433	1.01±0.16
	12 d		
tebufenozide	168	308	0.31±0.092
	tobacco budworm		
	5 d		
tebufenozide	168	446	0.41±0.13
	7 d		
halofenozide	168	283	1.55±0.19
methoxyfenozide	168	339	0.82±0.13
tebufenozide	168	286	0.88±0.12
	9 d		
halofenozide	168	410	0.72±0.18
tebufenozide	168	311	0.38±0.14
	12 d		
halofenozide	168	391	0.36±0.14
methoxyfenozide	168	392	0.27±0.09
tebufenozide	168	241	0.26±0.076

Table 2. Continued.

Insect growth regulator	LD_{50}(μg/larva) or mortality (%) at μg/larva	(95% confidence interval)
	Bollworm	
	5 d	
methoxyfenozide	0.0054	(0.0004-0.021
tebufenozide	0.015	($9.95x10^{-7}$-0.22)
	7 d	
halofenozide	0.44	(0.21-1.94)
methoxyfenozide	0.068	(0.06-0.12)
tebufenozide	0.2	(0.15-0.49)
	12 d	
tebufenozide	24.58	(2.86-15737)
	tobacco budworm	
	5 d	
tebufenozide	4.41	(0.21-22.23)
	7 d	
halofenozide	2.93	(2.12-4.44)
methoxyfenozide	0.14	(0.040-0.30)
tebufenozide	3.39	(2.24-5.36)
	9 d	
halofenozide	2.89	(0.55-7.86)
tebufenozide	66.65	(11.09-$1.7x10^{7}$)
	12 d	
halofenozide	0.54	(0.00043^{-12})
methoxyfenozide	0.0021	$3.2x10^{-9}$-0.039)
tebufenozide	1543	(88.39-$2.1x10^{7}$)