RESPONSE TO INSECTICIDES BY SUSCEPTIBLE STRAINS OF THE BEET ARMYWORM (LEPIDOPTERA:NOCTUIDAE)

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Abstract

A strain of beet armyworm, *Spodoptera exigua* (Hubner), designated ICI-DOW is proposed as a standard susceptible strain to all insecticides. Strains were combined from ICI (now Syngenta) and DOW. Discriminating doses and resistance ratios can be established for each insecticide when LD50s and consistent sizes of 95% confidence intervals are determined for the susceptible strain. Range of LD50s or an LD50 was also determined for strains considered to be susceptible obtained from USDA-ARS laboratories in Phoenix, AZ, Stoneville, MS and Orange County, CA. Range of LD50s or an LD50 for ICI-DOW strain for methomyl, methyl parathion, chlorpyrifos, emamectin benzoate, fenvalerate, permethrin, cypermethrin, profenofos, bifenthrin, cyfluthrin, *lambda* cyhalothrin and deltamethrin were 0.00094 to 0.8 µg/larva, 0.12 to 0.16 µg/larva, 0.00002 to 0.48 µg/larva, 0.008 to 0.064 µg/larva, 0.037 µg/larva, 0.0086 µg/larva, 0.00021 µg/larva, 0.00029 µg/larva, 0.0013 µg/larva, 0.0044 µg/larva, 0.0091 µg/larva and 0.0034 µg/larva for this strain, respectively. Chlorpyrifos showed a 24,000 difference while methomyl showed a 5,511 difference. The strain is not homozygous for susceptibility to chlorpyrifos and methomyl because of these large differences in LD50s, but the strain was still susceptible. In 1990 the strain from Phoenix, AZ, maintained in the laboratory for >20y, showed an LD50 for methomyl of 11.23 µg/larva; in 1995 an LD50 of 0.15 µg/larva was determined, a 75 fold difference. This strain was resistant to methomyl in 1990, but susceptible in 1996. LD50s for a strain from Orange County, CA were 0.053, 9.37, 0.55 and 0.97 µg/larva for fenvalerate, methomyl, methyl parathion and permethrin, respectively. Strain was resistant to methomyl but susceptible to the other insecticides. When reared from individual egg masses 0% to 86% mortality with four doses of methomyl and 0% to 100% mortality with three doses of fenvalerate and esfenvalerate were determined. Heterozygosity was exhibited in the ICI-DOW from larvae reared from individual egg masses.

Introduction

Collections of populations of beet armyworm were brought from the field to the laboratory and treated with insecticides for LD50s. When a strain indicated a low LD50 it was deemed to be susceptible. No consideration was ever given to determine if the strain was homozygous or heterozygous for susceptibility to any insecticide. A consistent LD50 applied topically to larvae of a susceptible strain of this insect is needed to determine discriminating doses. It is desirable that any susceptible strain should be homozygous for response to every insecticide each generation y after y. Strain may be susceptible the first or the second time they are treated within a y, but two, three or 10 y later they may not be susceptible. This can happen even if no other strain(s) are included in the strain. The size of the LD50 will vary with each insecticide, but the sizes should be consistent.

In 1990, the ICI-DOW strain had LD50s of 0.037, 0.16, 0.8, 0.0086, 0.013, 0.0034, 0.0044, 0.0013 and 0.0091 µg/larva for fenvalerate, methyl parathion, methomyl, permethrin, cypermethrin, deltamethrin, cyfluthrin, bifenthrin and *lambda* cyhalothrin, respectively (Wolfenbarger and Brewer, 1993 and Wolfenbarger, et al. 1997). In 1991 LD50s for methomyl, methyl parathion, permethrin and fenvalerate were 0.42, 0.26, 0.0097 and 0.0031 µg/larva for the same strain, respectively (Wolfenbarger 2002). In 1995, LD50s of this strain were 0.064, 5.18, 0.48, 1.3 and 1.02 µg/larva for emamectin benzoate, methomyl, chlorpyrifos, spinosid and methyl parathion, respectively (Sparks et al. 1996 and Wolfenbarger et al. 1997). In 1996, LD50s for methyl parathion, profenofos, chlorpyrifos, methomyl, permethrin and cypermethrin against the ICI-DOW strain were 0.12, 0.00029, 0.00002, 0.000097, 0.00025 and 0.002 µg/larva, respectively, at Estacion Cuauhtemoc (Teran -Vargas 1997). LD50s for chlorpyrifos, methomyl permethrin and profenofos were equal.

LD50s for methomyl of strains maintained in the laboratory generation after generation for multiple y were 0.8, 9.37.0 and 11.23 µg/larva for a strain from ICI-DOW, Orange County, Riverside, CA. and the USDA-ARS, Phoenix, AZ, respectively (Wolfenbarger and Brewer 1994). The LD50s of the strain from ICI-DOW were significantly lower than the

other two strains, yet they are all considered to be susceptible. For fenvalerate, LD50s were 0.037, 0.053, 4.19 and 24.76 µg/larva for a strain from ICI-DOW, Orange County, CA and the USDA-ARS from Stoneville and Phoenix, respectively. Strains from ICI-DOW and Orange County, CA were equal in response to fenvalerate and significantly different from both USDA strains. For permethrin, the strain from ICI-DOW had a significantly lower LD50 than the USDA strain from Stoneville, MS, the Orange County CA and the USDA strain from Phoenix; the last three strains were equal in response. For methyl parathion the ICI-DOW and the Orange County, CA, strains were equal in response. The Orange County, CA and the USDA, Stoneville, MS were equal in response and all had significantly lower LD50s than shown for the USDA Phoenix, AZ strain.

From 1988 to 1993 Orange County, CA, LD50s were determined for 4 ± 1 µg third instar larvae (Brewer and Trumble 1989, 1991 and 1994). The LD50s in the references were expressed as µg/g, but were converted to µg/larva for comparison with LD50s from Estacion Cuauhtemoc and Weslaco. LD50s of fenvalerate, permethrin and methomyl were 0.013, 0.0071 and 0.78 µg/larva for the Orange County, CA, strain (Brewer and Trumble 1989**).** LD50 for fenvalerate for the same strain was 0.014 µg/larva (Brewer and Trumble 1989 and Brewer and Trumble 1991). LD50s for fenvalerate were equal in both references. LD50s for the same strain were 0.02 µg/larva for fenvalerate and 0.34 µg/larva for methomyl (Brewer and Trumble 1994). All LD50s for this strain were <1.0 µg/larva.

LD50s for chlorpyrifos, emamectin benzoate, and methomyl were determined by topical application to larvae of ICI-DOW strain at Weslaco, TX, USA, and Estacion Cuauhtemoc, TA, MX. Larvae of ICI-DOW, reared from individual egg masses, were treated with methomyl, esfenvalerate and fenvalerate by topical application to determine if there were differences in toxicity to larvae that eclosed. Also, LD50s of these three insecticides to a strain from the USDA-ARS laboratory at Phoenix, AZ, were determined in 1990 and again in 1996 to determine if strain would indicate resistance or if one would be susceptible.

Materials and Methods

Technical of chlorpyrifos was obtained from DOW Agrosciences, Inc., Indianapolis, IN, emamectin benzoate from Merck, Inc., Bridgeport, NJ and esfenvalerate, fenvalerate and methomyl from Dupont Crop Protection, Inc. Wilmington, DE.

Senior author obtained the sample of this strain from Al Rehmke of ICI, Richmond, CA in 1990, 1991 and 1992. The original strain of ICI-DOW was obtained by DOW from the USDA-ARS, Stoneville, MS (Torres, L). This strain was then lost. A replacement for the lost colony was also obtained from the USDA-ARS, Stoneville, MS prior to our evaluations here (Hendrix W.). The replacement was then combined with the remnants of the strain from ICI, Richmond, CA. The source of the remnant strain was unknown.

Doses of technical insecticides, diluted in acetone, were applied to larvae as described (Wolfenbarger and Brewer 1994). Doses of chlorpyrifos, emamectin benzoate and methomyl were 0.048 to 25.0, 0.006 to 1.0 and 0.39 to 50 µg/larva, respectively. Doses were diluted in 1 µl and placed on the dorsum of the thorax (Wolfenbarger and Brewer 1993). In Weslaco larvae were treated when they weighed 15 ± 6 mg. In Estacion Cuauhtemoc larvae were treated when they weighed 25 ± 3 mg (Teran-Vargas 1997). Larvae were sized daily and removed for treatment.

LD50s, their 95% Confidence Intervals (CI) and slope ± standard error (SE) were determined by probit analysis (SAS 1988). The total number of larvae treated with each insecticide was also determined. When CI values did not overlap they were significantly different from each other; overlapping CIs indicate equal response. Slopes <1.0 were considered to be flat while those >1.0 showed degrees of steepness.

Tests were also conducted to determine toxicity of different doses of methomyl, esfenvalerate and fenvalerate to larvae which eclosed from individual egg masses. Each egg mass of ICI-DOW strain was placed in a cup of diet after cutting them out from their oviposition site of plastic paper. Each emerging neonate larva was then placed individually in a 30 ml cup with 10 to 15 ml of diet and topically treated as described.

Results and Discussion

Of interest was the significant difference in LD50s of the laboratory strain from Phoenix to methomyl in 1990 and 1996 (Table 1). LD50s of methomyl were significantly greater for the same strain in 1990 compared to that determined in 1996. The LD50 of chlorpyrifos for larvae of the laboratory from Phoenix, AZ, was equal to the LD50 of chlorpyrifos of ICI-DOW. LD50s of emamectin benzoate and chlorpyrifos were equal. LD50s for emamectin benzoate were determined at Weslaco and Estacion Cuauhtemoc and the USDA-ARS laboratory in Phoenix and were equal. Slopes were variable; slopes of ICI-DOW and USDA-ARS Phoenix strains, treated with emamectin benzoate and methomyl were flat (<1.0) ranging from 0.46 to 0.75. For chlorpyrifos the slopes of the USDA-ARS Phoenix and ICI-DOW strains were 1.61 and 1.74, respectively, indicating steeper slopes. Homozygosity was not exhibited for emamectin benzoate and methomyl.

Table 1. Toxicity of contact insecticides of two susceptible strains of beet armyworm from two locations. 1996

Insecticide	Number treated	Slope ± SE	LD50 (µg/larva)	95% CI
		Weslaco ICI-DOW		
emamectin benzoate	191	0.46 ± 0.17	0.062	0.0054-5.6 X 10^{18}
chlorpyrifos	179	1.74 ± 0.66	0.12	1.92 X 10^{-9}-0.76
methomyl	122	0.68 ± 0.16	0.5	0.16-1.12
		Phoenix (USDA laboratory)		
emamectin benzoate	107	0.7 ± 0.29	0.017	∞-∞
chlorpyrifos	193	1.61 ± 0.53	0.25	0.068-5.7
methomyl	234	0.75 ± 0.12	0.15	0.062-0.28
		Estacion Cuauhtemoc ICI-DOW		
emamectin benzoate	320	1.55 ± 0.14	0.008	0.005-0.21

There was no difference in LD50s of emamectin benzoate to larvae of different weights (15 mg to larvae treated at Weslaco and 25 mg to larvae treated at Estacion Cuauhtemoc). CIs overlapped the strain from the USDA laboratory at Phoenix and the ICI-DOW strain treated at Estacion Cuauhtemoc and Weslaco.

A maximum LD50 <1.0 could be used as a threshold to separate resistance from susceptibility to chloropyrifos and emamectin benzoate. Methomyl and methyl parathion had LD50s >1.0; this value could be used to separate these insecticides from chlorpyrifos.

When larvae from individual egg masses of the ICI-DOW strain were treated with methomyl there was no relationship between percentage kill and number larvae/egg mass (Table 2). Trend shows greater mortality of <5 larvae/egg mass. Results indicate variation ranged from 30% to 60% mortality of larvae from the egg masses. Larval numbers/egg mass for this strain ranged from 1 to 15 larvae. Whether other strains show similar numbers of larvae remains to be determined.

Table 2. Toxicity of methomyl to larvae of ICI-DOW strain from individual egg masses. 1995

	Mortality (%) at µg/larva			
	0.39	0.78	1.56	3.125
1	0	0		
2	25	0	0	
3		0	0	
4	33			
5		30	10	60
6	25	50	25	83
7	15	26		
8	57	38	50	
9	0		22	56
10	20	60		
11	0	32		
14	43			86
15	40			86
mean mortality	24	26	20	74

Esfenvalerate was more toxic than fenvalerate to larvae of ICI-DOW among the individual egg masses (Table 3). When one to five larvae/egg mass were treated mortalities for fenvalerate were 33%, 0% and 23% greater at 0.024, 0.048 and 0.096 µg/larva than when six to 13 larvae/egg mass were treated.. Range of toxicity was 13% to 100% among the individual egg masses.. Number of larvae/egg mass ranged from 1 to 13.

Table 3. Toxicity of larvae of ICI-DOW with esfenvalerate and fenvalerate from individual egg masses. 1995.

	Mortality (%) by µg/esfenvalerate (E) or fenvalerate (F)					
	0.024		0.048		0.096	
Larvae/ Egg Mass	E	F	E	F	E	F
1	0	0	100	80	100	
2	100		100	25		
3		0	67	50	67	
4	50	0		25		50
5						100
6		0	83	83	83	67
7			29	57		
8		13		50		38
9	67	0		0	77	
10				70		
11		0		0		
12					42	
13		0		54		
mean mortality	31	2	28	44	74	64

Mortalities of larvae which eclosed from egg masses for methomyl with esfenvalerate and fenvalerate. Mortalities of larvae treated with methomyl were greater than when six to 15 larvae eclosed than when one to five eclosed. The reverse was true for esfenvalerate and fenvalerate; mortalities were greater when one to five larvae eclosed than when six to 13 eclosed.

In conclusion, larvae of ICI-DOW were consistently susceptible to all insecticides in 1993, 1995 and 1996 regardless of the location where they were treated. Toxicity of methomyl, esfenvalerate and fenvalerate to larvae from individual egg masses of this strain was variable, indicating heterozygosity. .

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