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Resistance to Azinphosmethyl and Reversion to Susceptibility by a Soil Weevil (Curculionidae: Coleoptera) Population

D. A. Wolfenbarger[1], H. W. Ray[2] and E. J. Villavaso[3]

[1]*Research Entomologist USDA, ARS, SPA, SARL, CIRU (Retired), 2413 E. Highway 83, Weslaco, TX 78596; now 55 Calle Cenizo, Brownsville, Texas 78520*
[2]*Novartis Corporation, 7145 58th Ave, Vero Beach, FL 32961-1090 (Retired)*
[3]*Research Entomologist, USDA, ARS Boll Weevil Research Laboratory, P.0. Box 5367, Mississippi State, MS 39762*

ABSTRACT

Following 36 applications to one cotton field at Vero Beach, FL in 1991 and three collection dates, LD_{50}s of azinphosmethyl for dead and dead + moribund boll weevils, *Anthonomus grandis* Boheman, were 3.33 and 1.24 µg/weevil, respectively, in generation one. These are the greatest values determined for this insecticide against this insect. LD_{50} for dead + moribund weevils was 0.29 µg azinphosmethyl/weevil when collected five times from three cotton fields after 14, 28 and 35 applications of azinphosmethyl, respectively. During this same growing season an LD_{50} of 0.044 µg azinphosmethyl/weevil was determined for a laboratory susceptible reference strain. It was significantly lower than shown for the mean value of all five collections. In generation one there was no resistance to methyl parathion by these populations. In generation two, LD_{50} of 0.11 and 0.059 µg azinphosmethyl/weevil were determined for dead and moribund + dead, respectively. Strain was susceptible since LD_{50}s of azinphosmethyl for dead + moribund of the field collected strain and the reference strain were equal. In this same generation none of the three pyrethroids tested were significantly more toxic than azinphosmethyl against weevils in dead and dead + moribund category. In generation three, LD_{50}s of azinphosmethyl and methyl parathion for selected and non-selected strains were equal. LD_{50} of azinphosmethyl by the reference strain was significantly greater than LD_{50} of the same insecticide to selected strain in this generation.

RESUMEN

Los valores DL_{50} del azinfós-metilo en la primera generación de picudos del algodón, *Anthonomus grandis* Boheman, al término de 36 aplicaciones a un campo de algodón en Vero Beach, FL en 1991 y tres fechas de colecta fueron 3.33 y 1.24 µg/picudo en el caso de picudos muertos y muertos + moribundos, respectivamente. Estos son los mayores valores determinados para este insecticida en contra de este insecto. La DL_{50} para picudos muertos + moribundos fue 0.29 µg de azinfós-metilo/picudo cuando se colectaron de tres campos de algodón después de 14, 28 y 35 aplicaciones de azinfós-metilo. Se determinó una DL_{50} de 0.044 µg de azinfós-metilo/picudo durante esta misma estación de crecimiento para una raza de laboratorio susceptible usada como referencia. Este valor fue significativamente menor que el valor promedio de todas las cinco colectas. En la primera generación, no ocurrió resistencia al paratión de metilo por parte de estas poblaciones. En la segunda generación, se determinaron valores DL_{50} de 0.11 y 0.059 µg de azinfós-metilo/picudo en las categorías de muertos y de muertos + moribundos respectivamente. La raza fue susceptible ya que las DL_{50} del azinfós-metilo para muertos + moribundos de la raza colectada en campo y de la raza de referencia fueron similares. En esta misma generación, ninguno de los tres piretroides probados fue significativamente más tóxico que el azinfós-metilo en contra de los picudos en las categorías de muertos y de muertos + moribundos. En la tercera generación, las DL_{50} del azinfós-metilo y del paratión de metilo para las razas seleccionadas y las no seleccionadas fue igual. La DL_{50} del azinfós-metilo para la raza de referencia fue significativamente mayor que la DL_{50} del mismo insecticida para la raza seleccionada en esta generación.

Azinphosmethyl and methyl parathion have been the standard insecticides for control of the boll weevil, *Anthonomus grandis* Boheman, in cotton in the United States since they were registered in the 1950's. All boll weevil populations in the United States are considered to be susceptible to both of these insecticides. However, producers in the Lower Rio Grande Valley of Texas have stated that azinphosmethyl is not always as effective today in the field as it was when first introduced for commercial use. Producers in the Lower Rio Grande Valley of Texas have not stated that methyl parathion has lost its effectiveness against this pest.

No LD_{50} values for azinphosmethyl and methyl parathion have been determined for boll weevils for cotton grown near Vero Beach, FL. Damage to squares by this pest in 1991 was heavy and continuous despite multiple applications of azinphosmethyl for its control. This scenario fits the description for resistance to azinphosmethyl by this insect. First we had to determine if this insect is resistant to these insecticides. Then we had to determine if the population would maintain its resistance or susceptibility. If a population was determined to be resistant we wanted to know if it would revert to susceptibility.

MATERIALS AND METHODS

Fields. Three cotton fields were planted in Vero Beach, FL, on 9 April, 7 May and 9 June, 1991, so fruiting would be continuous over several months. Fields were 0.32, 0.41 and 0.82 ha, respectively.

Squares (300 to 500) were collected at random on 1 August from field 1, 23 August from field 2 and 9, 15 and 31 October from field 3 and sent to Weslaco, TX. Each collection was considered to be a strain. Here, a strain is a portion of the population of boll weevil from Vero Beach and it is collected at one location at one time. This was done in generation one. The strains for generations two and three were then combined from the strains collected in generation one.

In field one 14 applications of azinphosmethyl were made weekly to biweekly from 20 May to 30 July; in field two 28 applications were made biweekly to four times/week from 19 July to 21 October; and in field three 36 applications were made biweekly to thrice-weekly from 3 June to 31 October. Applications in all fields were made with a 12-row high-boy sprayer at 151 liters/0.405 ha through 2 twin-jet number 6 (Spraying Systems Bellview, IL) nozzles/row at 70 g/cm^2 and 6.4 km/h. Azinphosmethyl was applied at 0.56 kg (AI)/ha on 4, 7, 9, 12, 16, 17, 18 and 21 October and 0.28kg (AI}/ha on all other dates. It was the only insecticide applied to the three fields.

Laboratory. Technical azinphosmethyl (94%), bifenthrin (90%) and cyfluthrin (89%), deltamethrin (100%), malathion (98%) and methyl parathion (98%) were obtained from Bayer, Inc., Kansas City, KS; FMC, Princeton, NJ; Bayer, Inc.; Rhone Poulenc, Research Triangle, NC; American Cyanamid, Princeton, NJ and Monsanto, St. Louis, MO, respectively. In Weslaco insecticides were diluted in acetone and each dose was topically applied in 1 µl to the dorsum of the thorax as described by Anonymous (1968), Walfenbarger et al. (1986) and Loera-G. et al. (1997).

Before treating, adults were allowed to feed on squares or diet and imbibe 5% sugar water for 48 to 120 h so the cuticle could harden. Adults were randomly selected and treated at 0.04 to 24, 0.0005 to 0.39, 0.0006 to 1.25, 0.000475 to 125, 0.002375 to 10 and 0.0155 to 24 µg/weevil with azinphosmethyl, methyl parathion, bifenthrin, cyfluthrin, deltamethrin and malathion, respectively. Number of doses tested ranged from 9 to 13. Untreated check adults were included with each replicate.

A total of ≥10 weevils of both sexes was treated per dose in all tests. Each weevil was placed in a 30-ml capped plastic cup after treatment and held at 25 ± 3°C. Weevils were classed as moribund (those that moved their legs, antenna or proboscis after gentle probing by a blunt instrument), dead or alive after 48 h. Not all doses were tested in all replicates because not enough weevils were available for testing. Moribund weevils were determined because some survived after generations one and two. Untreated moribund weevils were returned to non-selected populations. Dead weevils did not move their appendages following probing. Live weevils were upright and walked.

After treatment, boll weevils from each of the five strains were sent overnight to USDA-ARS Boll Weevil Research Laboratory, Mississippi State University. At the laboratory they were merged and fed on and oviposited in fresh green squares and standard diet in wax coated plugs (Lindig 1979) prepared at that laboratory. Larvae of generations two and three, in diet,

Table 1. Toxicity (after 48h) of azinphcsrnethyl and methyl parathion to boll weevil in first generation reared from cotton flower buds collected near Vera Beach, FL, 1991.

Dates Collected	Field	Number Tested	Condition	Slope ± Standard Error	LD_{50} (µg/weevil)	(95% Confidence Interval)
			Azinphos-methyl[a]			
All from 8-1 to 10~31[b]	1,2 and 3	1317	moribund + dead	0.39 ± 0.039	0.29[f]	(0.19-0.44)
		1317	dead	0.10 ± 0.037		
All from 8-1 and 8-23[c]	1 and 2	647	moribund + dead	0.16 ± 0.071	0.11	(0.0073-1.40)
		647	dead	0.099 ± 0.071		
All from 10-9 to 10-31[d]	3	670	moribund + dead	0.77 ± 0.068	1.24	(0.89-1~68)
		670	dead	0.85 ± 0.070	3.33	(2.5-4.57)
			Methyl Parathion			
All from 8-1 to 10-31[e]	1, 2 and 3	378	moribund + dead	1.35 ± 0.16	0.004	(0.0026-0.0056)
		378	dead	1.23 ± 0.13	0.0096	(0.0068-0.013)

[a]Aziflphos-methyl applied from 5-20 to 10-21 to cotton at 1 to 7 day intervals.
[b]Check mortality of 14.8% abd 5.0% for 121 dead + moribund and dead weevils, respectively.
[c]Check mortality 15.7% and 0 % for 70 dead + moribund and dead weevils, respectively.
[d]Check mortality of 13.7% and 11.8% for 51 dead + moribund and dead weevils, respectively.
[e]Check mortality of 8.6% and 0% for 23 dead + moribund and dead weevils, respectively.
[f]At 24 µg Azinphos-methyl 17.2% of weevils tested were killed.

Table 2. Toxicity (after 48 h) of insecticides to insecticide selected boll weevil strain of second generation.

Insecticide[a]	Condition	Number Tested	Slope ± SE	LD_{50} (µg/weevil)	(95% Confidence Interval)
Azinphos-methyl	moribund + dead	515	1.00 ± 0.28	0.059	(0.0094 - 0.19)
	dead	515	0.92 ± 0.24	0.11	(0.022 - 0.38)
Bifenthrin	moribund + dead	383	1.71 ± 1.49	[b]	
	dead	383	0.50 ± 0.16	0.11	(0.027 - 10.70)
Cyfluthrin	moribund + dead	370	1.08 ± 0.20	0.04	(0.018 - 0.076)
	dead	370	0.89 ± 0.22	0.50	(0.23 - 3.73)
Deltamethrin	moribund + dead	568	1.21 ± 0.28	0.018	(0.0058 - 0.04)
	dead	568	0.69 ± 0.15	0.32	(0.12 - 1.85)
Malathion	moribund + dead	374	2.09 ± 1.19	[b]	
	dead	374	1.98 ± 1.43	[b]	
Methyl parathion	moribund + dead	412	3.20 ± 0.52	0.070	(0.058 - 0.091)
	dead	412	324 ± 0.59	0.073	(0.059 - 0.098)

[a]Check weevil mortalities ranged form 8.4% fir 24 weevils to 13.9% for 72 weevils for insecticides tested.

[b]Unable to determine LC_{50} values because slope/SE ratio was not significant at t=≤1.96.

were returned to Weslaco overnight. Generation two and three were weevils of the combined strains. Adults of generation two and three were treated 48-120 h after they emerged from the diet. Emergence of adults from larval diet required 5 to 20 d.

Weevils treated with a dose of any insecticide in generation one and two were selected. In the first generation the untreated check weevils were non-selected. They were reared separately at the USDA laboratory in Mississippi at the same time as selected weevils. Generation one included all live and moribund weevils reared from squares. Larvae were reared on artificial diet developed by Lindig [1979]. Generation times of selected and non-selected weevils were the same. Weevil populations overlap in the laboratory because female weevils live 30 to 60 d and generation time is 20 to 30 d.

In addition to treating the field collected strains we also treated a laboratory reference strain from Gast Rearing Laboratory, Mississippi State, MS, Loera-Gallardo et al (1997). Weevils were treated at the same time with the same methods and LD_{50} was determined as described here.

Number of dead and moribund + dead boll weevils was totaled separately for each insecticide and subjected to probit analysis SAS [1988]. Untreated weevils were used to correct for mortalities in all three generations of both the dead and moribund + dead weevils separately with procedures of SAS (1988]. Fourteen and five replications were made for azinphosmethyl and methyl parathion in generation one, respectively. Three to five replicates of weevils from each collection were tested with the insecticides in generation two and three. A replicate was each day of treating. Differences between LD_{50} values were indicated when 95% confidence interval (C.I.) did not overlap. When ratio of slope per standard error (SE), was $t_{0.05} < 1.96$, P=∞ a non-significant regression was indicated.

RESULTS AND DISCUSSION

LD_{50} of azinphosmethyl was higher for boll weevils classed as dead when reared from squares collected 9-31 October in field 3 was higher than any previously reported (Table 1). LD_{50}s of azinphosmethyl for dead + moribund weevils from 9-31 October and 1-23 August in fields 1-2 were similar because they had overlapping confidence intervals. $L0_{50}$ of azinphosmethyl was 0.29 µg/weevil for dead + moribund boll weevils reared from squares of all five collection dates (fields 1, 2 and 3).

An LD_{50} of 0.044 µg/azinphosmethyl Loera-Gallardo et al. [1997] for a laboratory reference strain was determined during the 1991 growing season. It was significantly lower than any LD_{50} determined from the mean or any one of the five field collections. The greatest confidence interval of the reference strains was less (0.06 µg/azinphosmethyl/weevil) than the lowest confidence interval of the five field-collected strains. Slope values of weevils from the five collections treated with azinphosmethyl were extremely flat (<1). Results suggest that

Table 3. Toxicity (after 48 h) of insecticides to selected and nonselected boll weevil strains of third generation.

Strain	Condition	Number Tested	Slope ± SE	LD_{50} (µg/weevil)	(95% Confidence Interval)
		Azinphos-methyl[a]			
Selected	moribund + dead	535	0.51 ± 0.18	0.0022	($5.3X10^{-8}$ - 0.011)
	dead	535	0.44 ± 0.16	0.0038	($4.42X10^{-7}$ - 0.019)
Unselected	moribund + dead	531	0.65 ± 0.22	0.0070	(0.000067 - 0.033)
	dead	531	0.68 ± 0.23	0.014	(0.00060 - 0.088)
		Methyl Parathion[a]			
Selected	moribund + dead	96	4.81 ± 1.69	0.036	(0.016 - 0.046)
	dead	96	2.57 +± 0.52	0.031	(0.0212 - 0.042)

[a]Check weevil mortalities ranged from 7.4% for 31 weevils to 11.6% for 20 weevils.

more factors are involved in the response of these field-collected strains than are involved in response of the ebony strain whose slope value was 1.95.

LD_{50} of azinphosmethyl for moribund + dead weevils from first two collection dates from fields 1 and 2 were equal to mean of all five (fields 1, 2 and 3) and the last three collection dates [field 3). However, LD_{50}s of azinphosmethyl for moribund + dead of fields 1, 2 and 3 were significantly less than those of field 3. The reverse was true for the dead weevils. Mortalities of weevils treated after the first collection (field 1) were so variable that the results were combined with those treated in the second collection (field 2). Dose-mortality response of dead weevils from first two collection dates (fields 1 and 2) were not significant because ratio slope/SE was <1.96.

There was no cross resistance by this population of boll weevils to methyl parathion. Boll weevil was susceptible to methyl parathion regardless of whether the LD_{50} was determined from dead and moribund + dead weevils (Table 1). LD_{50}s ranging from 0.02 to 0.08 mg methyl parathion or azinphosmethyl/weevil are typical for this insect (Anonymous 1988). Slope values were greater for methyl parathion than for azinphosmethyl. Maximum time of mortality to the boll weevil by both these organophosphorus insecticides is about 48 h. In generation one 72 h mortality was determined, but the increase of dead and moribund weevils was <5% so the determination was discontinued.

These levels of resistance to azinphosmethyl were probably induced by extreme selection pressure following the spray applications to the weevil population present in the three fields. Despite the multiple applications, weevil damage was moderate to high in the fields at time of collection. If each weevil lived an average of 30 days during these 140 days then each weevil was exposed to about four or five applications during its life. Weevils could readily disperse from one field to another because the three fields were within 100 m of each other.

The second generation of selected weevils showed no resistance when treated with azinphosmethyl and methyl parathion (Table 2). LD_{50}s of the strain and the laboratory reference strain were not significantly different based on weevils in the dead + moribund category. Incorporation of moribund adults with live adults in the first generation did not cause an increase in levels of resistance to either insecticide in the second generation. LD_{50}s of azinphosmethyl and methyl parathion ranged from 0.05 to 0.11 µg/weevil for dead and dead + moribund weevils. $L0_{50}$s of the pyrethroids were lower than those of azinphosmethyl and methyl parathion. Of interest was the significant difference between LD_{50}s after 48 h and the steeper slopes of cyfluthrin and deltamethrin of the dead and moribund + dead. The slower action of pyrethroids compared to organophosphorus insecticides partially explains the difference observed Rathinam (1979). Leonard et al. (1989] reported that LD_{50}s for cyfluthrin from two field collections from Louisiana were statistically equal to the LD_{50} for cyfluthrin determined here for dead + moribund boll weevils.

In the second generation a non-significant regression was shown with malathion indicating no difference in mortality with doses which encompassed three log cycles. Toxicity by all the doses of malathion was low. Perhaps there is more resistance in this combined population to malathion than azinphosmethyl. A non-significant regression was determined when weevils were treated with bifenthrin and classed as moribund + dead. LD_{50}s of all three pyrethroids were statistically similar when only the dead weevil category was used.

In the third generation, LD_{50}s for azinphosmethyl were determined for selected and non-selected weevils and no significant difference in LD_{50}s was determined for weevils classed as dead and moribund + dead (Table 3). The 1991 lower confidence interval of 0.031 µg azinphosmethyl/weevil to the laboratory reference strain Loera-Gallardo et al. [1997] was greater than the greatest confidence interval for azinphosmethyl. Selected weevils, treated with methyl parathion, were as susceptible in this generation as they were in generations one and two.

Rearing both moribund and live weevils together was important; if some moribund weevils survive that mechanism should be incorporated into progeny of the treated adults. Non-moribund weevils that survive might have a different mechanism than moribund weevils.

It was obvious that first generation weevils were resistant to azinphosmethyl following selection pressure of field applications. Resistance was then lost in the second and third generation when the weevils were reared on artificial diet in the laboratory. This suggests that non-genetic factors are involved because the factors in generation one were not present in the next. Resistance levels in the second and third generation may have been equal to levels shown in generation one if larvae had been reared on squares.

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