Single Pairs of Tobacco Budworm: Toxicity of Cypermethrin to Larvae and Adults

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ABSTRACT

In 1989, immatures of tobacco budwarm, *Heliothis virescens* (F). were collected weekly from 4 cotton fields in late June to early August across the Lower Rio Grande Valley (LRGV) of Texas and reared to adults. Pest populations were low during the season, but 12 single pairs produced progeny. Single pairs, one male and one female moth, are the smallest phenotypic population of this insect. They are the smallest population which could exhibit variability of response to an insecticide. One-half of the larvae of these single pairs was topically treated with cypermethrin, (±)-alpha-cyano-3-phenoxybenzyl-(+)-cis, trans-3.{2,2-dichlorovinyl}.2,2-dimethylcyclopropanecarboxytate), and showed LD_{50} values ranging from 0.043 to 0.26 pg cypermethrin/larva. These two values differed about 7-fold and were significantly different. The single pair with the greatest LD_{50} value was from one male moth collected from a cotton field north of Edinburg and one female moth collected from a field south of Edinburg. Female and male moths, reared from the other half of the larvae of the 12 single pairs, were bioassayed in vials coated with cypermethrin. LC_{50} values of both sexes of moths ranged from 3.44 to 72.4 μg/vial, a 21-fold difference. The greatest LC_{50} value was determined for adults reared from progeny of one pair of moths collected from a field near Brownsville. Larvae, reared from this same single pair, were intermediate in response to cypermethrin. LC_{50} value for females was equal to LC_{50} value of males from the same single pair. The coefficient of determination (R^2) of LD_{50}s of the larvae show little relationship to LC_{50}s of adults. About 3% of the regression could be attributed to toxicity by cypermethrin to larvae. When the LD_{50} and LC_{50} of single pair seven was excluded from the analysis the coefficient of determination showed a positive significant relationship; 72% of the regression was attributed to toxicity by cypermethrin.

RESUMEN

Se colectaron semanalmente estadios inmaduros del gusano de las yemas del tabaco, *Heliothis virescens* (F). de 4 campos de algodón desde finales de Junio hasta los principios de agosto de 1989 a lo largo del Bajo Valle del Río Grande de Texas y se criaron hasta el estadio adulto. Las poblaciones fueron bajas durante la estación pero doce parejas individuales produjeron progenie. Las parejas independientes, un palomilla macho y una hembra, son la población fenotípica más pequeña de este insecto. Estas constituyen la población más pequeña que podría exhibir variabilidad de respuesta a un insecticida. La mitad de las larvas de estas parejas individuales fueron tratadas con cipermetrina, (±)-alpha-cyano-3-phenoxybenzyl-(±)-cis, trans-3.{2,2-dichlorovinyl}.2,2-dimethylcyclopropanecarboxytate, y mostraron valores de DL_{50} que fluctuaron entre 0.043 a 0.26 pg cipermetrina/larva. Estos dos valores difirieron cerca de 7 veces y fueron significativamente diferentes. La pareja individual con el mayor valor DL_{50} se obtuvo de una palomilla macho colectada de un campo de algodón al norte de Edinburg y una palomilla hembra colectada en un campo al sur de Edinburg. Las palomillas hembras y machos criados a partir de la otra mitad de las larvas de las doce parejas individuales fueron sujetas a bioensayos en viales cubiertos por cipermetrina. Los valores de CL_{50} de ambos sexos de palomillas variaron de 3.44 a 72.4 μg/vial, una diferencia de 21 veces. El mayor valor de CL_{50} fue determinado para los adultos criados a partir de la progenie de uno de los pares de palomillas colectadas de un campo cerca de Brownsville. Las larvas, criadas a partir de esta misma pareja tuvieron una respuesta intermedia a la cipermetrina. El valor CL_{50} para las hembras fue igual al valor CL_{50} de los machos de la misma pareja. El coeficiente de determinación (R^2) de los DL_{50}s de las larvas muestra poca relación con los CL_{50}s de los adultos. Cerca del 3% de la regresión puede ser atribuida a la toxicidad por cipermetrina a la larva. El coeficiente de determinación mostró una relación positiva significativa cuando el DL_{50} y el CL_{50} de la pareja individual se excluyo del análisis; el 72% de la regresión fue atribuida a la toxicidad por cipermetrina.

From 1979 to 1995 there was wide variation in LD_{50} values to cypermethrin by topical application to larvae obtained from multiple pairs of the tobacco budworm, *He//otlnis vitescens* (F), collected from cotton fields in the Lower Rio Grande Valley (LRGV) of Tamaulipas, Mexico and Texas, USA (Wolfenbarger & Vargas-Camplis 1997). The vial bioassay was used to treat males of tobacco budworm with doses of cypermethrin from 1986 to 1988 in the LRGV (Plapp et al. 1990). Plapp et al. (1990) did not determine LC_{50} values from his results as shown for our results here. Values best describe the variation in response of populations in an area.

Riley (1988) proposed topical applications to larvae as a precise definition of response. He suggested that the vial test for adults could be used as a regular monitoring test. Roush and Luttrell (1987) showed a significant correlation between LD_{50} values of either cypermethrin or permethrin to third-instar of tobacco budworm applied topically or by terminal bud bioassays. Then Micinski et al. (1991) showed that there was a significant correlation in seasonal tolerance between adults in a vial test bioassay and neonate larvae leaf dip bioassay. Comparisons were made with multiple pair matings of brother-sister of different laboratory reference and field-collected strains. Single pairs were not used by Roush and Luttrell (1987) and Micinski et al, (1991).

An experiment was conducted in 1989 to determine toxicity of cypermethrin to larvae and to adults of tobacco budworm when reared from the same single pairs collected from cotton in the LRGV. No comparison of toxicity by cypermethrin has been conducted with progeny from single pairs. Single pairs were used because they were the smallest phenotypic size of this species. Determination of toxicity by these two bioassay techniques should prove to be valuable in the interpretation of response by populations of larvae and adults of this pest. Authors were concerned that the kill of moths was not related to kill of larvae. If toxicity of adults cannot be related to kill of larvae and larvae cause all the damage to cotton then the results on toxicity to adults is moot. Collections of immatures from the fields of cotton were made at mid-fruiting across most of the cotton producing areas in the LRGV to determine this relationship.

MATERIALS AND METHODS

Technical of cypermethrin (>93%) was obtained from FMC Corp., Inc., Princeton, NJ. In 1989, eggs and larvae of tobacco budworm were collected weekly from 4 cotton fields in late June to early August near Brownsville, Weslaco, one field north and one field south of Edinburg in the LRGV, Texas. Edinburg is 120 km northwest of Brownsville and Weslaco is between these cities. Ten (83%) of the single pairs were collected from a field near Brownsville.

Larvae were reared to pupation on artificial diet (Shaver and Raulston 1974). Pupae were sexed and males and females were paired singly for their lifetime. Our priority was to pair adults from the same field. If a male or female moth from the same field was unavailable, a moth of the opposite sex from any of the fields which emerged within 24-48 h was used for pairing. Moths may only live three to 15 days. We considered it important to pair moths within 48 h of emergence because females emerge 24-48 h prior to males. Females are listed first in crosses. Fifteen pairs were established, but moths of three pairs died prematurely, failed to mate or the eggs were not fertile. Twelve single pairs produced progeny.

All neonate larvae were placed singly in 30-ml plastic cups containing 10 ml of diet. Half of the larvae were treated when they weighed 22 ± 7 mg or 4 to 7 days. All available larvae were treated. Doses of cypermethrin were 0.003875, 0.007 75, 0.0155, 0.031, 0.0625, 0,125, 0.25, 0.5, 1.0 and 1.25 g/larva in 1 l acetone. A micro-applicator was used to apply cypermethrin to the dorsum of the thorax of each larva by methods of Anonymous (1970). For each single pair two to 58 larvae/dose/replicate/day were treated. Each replicate was conducted on a different day.

Mortalities were determined after 72 h. LD_{50}, 95% confidence interval (CI), as µg/larva, slope ± standard error (SE) were determined by probit analysis {SAS Technical Report 1988). Log 10 was used to transform doses. A significant difference in LD_{50} values was determined by non-overlapping of the 95% CI. Equal LD_{50} values have overlapping CI values. LD_{50} values were ranked from lowest to greatest.

The other half of the larvae were reared to pupation, sexed and allowed to emerge for vial bioassays. One male or female

Table 1. Toxicity of cypermethrin to larvae from single pairs by topical appliction (72 h). Lower Rio Grande Valley, Texas. 1989.

Cross(♀x ♂)	Larvae Tested	Slope ± SE	LD_{50} (µg/larva)	95% Confidence Interval
Weslaco x Brownsville	49	2.16 ± 0.5	0.043	0.025-0.071
Brownsville x Brownsville (1)	364	1.98 ± 0.2	0.044	0.036-0.054
Brownsville x Brownsville (2)	249	1.81 ± 0.2	0.069	0.053-0.092
Brownsville x Brownsville (3)	123	1.04 ± 0.4	0.078	∞-∞
Brownsville x Brownsville (4)	47	1.39 ± 0.4	0.081	0.041-0.25
Brownsville x Brownsville (5)	149	1.61 ± -.4	0.086	0.043-0.3
Brownsville x Brownsville (6)	137	1.17 ± 0.5	0.086	∞-∞
Brownsville x Brownsville (7)	107	1.92 ± 0.5	0.091	0.057-1.31
Brownsville x Brownsville (8)	198	1.2 1 ± 0.2	0.1	0.072-0.16
Brownsville x Brownsville (9)	130	1.09 ± 0.2	0.19	0.12-0.47
Brownsville x Brownsville (10)	226	1.09 ± 0.2	0.23	0.15-0.42
Edinburg (south) x Edinburg (north)	119	1.35 ± 0.3	0.28	0.16-0.81

moth, reared from half of the larvae of 12 single pairs, was placed in a 20 ml glass vial coated with 2.5, 5, 10, 30, 50 or 100 µg cypermethrin/vial with methods described by Norman et al. (1990) and Sparks et al. (1990). They were also bioassayed in 1990 and 1992 with these methods. All moths were treated. Moths were 24 to 48 h old when placed in the vial. Depending on availability of moths five to 10 untreated vials, each containing a moth of either sex of each single pair, were used as the check to correct for natural mortality.

Mortalities were determined after 24 h (Norman et al. 1990 and Sparks et al. 1990). LC_{50}, slope ± SE and 95% CI, as µg/adult, were determined by the same probit program. Log 10 was used to transform doses. LC_{50}s were ranked from lowest to greatest. A significant difference in LC_{50} values was determined by non-overlapping of the 95% CI. A nonsignificant regression was indicated when ratio of slope/SE was t<1 .96 for infinity at P>.05. This meant the regression did not differ from zero.

A coefficient of determination (R^2) was determined by linear regression (Y=a+bX) analysis by LINREG (SAS Institute 1988) for 11 LD_{50} values of larvae and the corresponding LC_{50} values of both males and females. LD_{50}s for males and females were also determined separately. Then, coefficients were determined when (1) one LD_{50} and LC_{50} of single pair seven of Brownsville x Brownsville were excluded, (2) low CI was substituted for LC_{50} of single pair seven or (3) LD_{50} values and their corresponding LC_{50} values which had infinity for CI. Another coefficient was determined for 7 LD_{50}s of larvae and LC_{50}s of adults which excluded CIs of infinity and LD_{50} and LC_{50} of single pair seven. Coefficients of determination for the same linear regression were also determined for slopes of larvae and moths with 12 single pairs with the same data used for the LD_{50} values and LC_{50} values. The slope was tested because it might be flatter or steeper as the LD_{50} or LC_{50} became greater or lower.

RESULTS AND DISCUSSION

Larvae. LD_{50}s of progeny from 12 single pairs from four fields ranged from 0.043 to 0.28 g/larva (Table 1). These values were 7-fold different and significantly different from each other. The greatest value was obtained from the single pair collected as larvae or eggs from two fields near Edinburg. Field populations of tobacco budworm eggs or larvae were considered to be at low density across the field near Brownsville.

Ten of the 12 swigle pairs collected near Brownsville had LD_{50} values which ranged from 0.044 to 0.23 µg/larva, a 5-fold difference. The 10 single pairs from Brownsville were numbered;

Table 2. Toxicity of cypermethrin to adults from surviving larvae by vial bioassay (24 h). Lower Rio Grande Valley, Texas. 1989.

Cross(♀x♂)	Adults Tested	Slope ± SE	LD_{50} (µg/larva)	95% Confidence Interval
		Both Female and Male		
Brownsville x Brownsville (3)	24	0.97 ± 0.5	3.44	∞-∞
Brownsville x Brownsville (5)	71	0.79 ± 0.3	4.45	0.77-12.93
Weslaco x Brownsville	54	1.10 ± 04	5.84	∞-∞
Brownsville x Brownsville (1)	228	0.97 ± 0.2	7.1	4.71-11.24
Brownsville x Brownsville (4)	30	1.32 ± 0.5	7.4	2.48-42.39
Brownsville x Brownsville (6)	62	2.09 ± 0.5	8.57	5.41-17.42
Brownsville x Brownsville (2)	147	1.37 ± 0.3	8.89	6.11-13.87
Brownsville x Brownsville (9)	86	1.23 ± 0.3	9.21	5.78-16.69
Brownsville x Brownsville (8)	74	1.88 ± 0.5	10.84	7.15-19.5
Edinburg (south) x Edinburg (north)	116	1.44 ± 0.5	22.8	∞-∞
Brownsville x Brownsville (7)	120	0.84 ± 0.3	72.4	25.38-74.67
		Female		
Brownsville x Brownsville (5)	39	0.82 ± 0.4	2.38	zero-8.12
Brownsville x Brownsville (6)	27	4.84 ± 1.9	8.71	5.46-16.54
Brownsville x Brownsville (7)	30	1.87 ± 0.7	9.4	4.19-27.67
Brownsville x Brownsville (2)	75	1.41 ± 0.6	10.14	?-?
Brownsville x Brownsville (1)	120	1.04 ± 0.3	10.75	6.36-24.16
Edinburg (south) x Edinburg (north)	53	1.77 ± 0.5	13.93	8.2-36.65
		Male		
Brownsville x Brownsville (1)	108	0.96 ± 0.2	4.31	∞-∞
Weslaco x Brownsville	28	1.47 ± 0.6	6.15	1.72-19.5
Brownsville x Brownsville (9)	63	1.22 ± 0.4	6.77	3.27-15.1
Brownsville x Brownsville (2)	72	1.33 ± 0.4	7.72	4.25-15.13
Brownsville x Brownsville (6)	35	1.88 ± 0.6	8.06	4.07-18.27
Brownsville x Brownsville (8)	32	0.82 ± 0.5	9.46	∞-∞
Brownsville x Brownsville (5)	44	2.12 ± 0.8	13.28	6.31-74.06
Edinburg (south) x Edinburg (north)	63	1.2 ± 0.5	41.0	17.2-3466.2
Brownsville x Brownsville (7)	90	1.09 ± 0.5	53.0	19-2437.06

the numbering followed the adults. Most (58%) of the LD50 values were <0.099 µg/larva. Five of the single pairs from Brownsville and the Edinburg had single pairs with LD50 values which ranged from 0.091 to 0.28 µg/larva. Their values were equal. The lowest two LD50 values from Brownsville x Brownsville and Weslaco x Brownsville were equal.

Slope values ranged from 1.09 to 2.16 for the 12 pairs; 92% ranged from 1-2, and 8% were >2. No trend was shown for location, slope or SE values.

There was no relationship between number of larvae treated for each single pair and the LD_{50}. These ranged from 47 to 364 for each single pair. This range is considered 'average' for field-collected single pairs. We treated 49 larvae of the single pair with the lowest LD_{50} and then treated 364 larvae at the next LD_{50} (Table 1).

The 95% CI value for each single pair overlapped the 95% CI of the next greatest value. This is a continuum of response for single pairs which is a typical pattern for response to cypermethrin when single pairs are compared. This result may not be a typical pattern when multiple pairs from any source(s) are collated for oviposition and their progeny are treated (Wolfenbarger and Vargas-Camplis 1997). When using multiple pairs, second and third matings of each female may occur with different males. These results can change LD_{50}s of cypermethrin to larvae and adults because proportion of genotypes are different.

Adults. The greatest LC_{50} values from 1986 to 1992 show an increasing trend. In 1986, 1987 and 1988 LC_{50} values of 0.056, 3.4 and 4.15 for males of tobacco budworm in the ERGV were shown by Plapp et al. (1990). In 1989, LC_{50} values of cypermethrin for both sexes reared from 11 single pairs ranged from 3.44 to 72.4 µg/vial, a 21-fold difference (Table 2). Results show ever increasing LC_{50} values from 1986 to 1989. Single pair 10 of Brownsville x Brownsville showed a nonsignificant regression; for 51 moths a slope ± SE of 0.39 ± 0.4 was determined. In 1990, moths treated and their LC_{50} (CIs) and slope ± SE values were 400,83.8 (∞-∞) and 1.19 ± 0.4, respectively. In 1992, three separate collections were made in June and July. One had a significant regression; number of moths treated, LC_{50} (CIs) and slope ± SE were 232, 91.25 (60.7-175.46) and 1.23 ± 0.4, respectively. The two collections showed a non-significant regression. For number of moths treated and its slope ± SE, collections one and two were 400 and 1.19 ± 0,4 and 116 and 1.61 ± 1.39 respectively. The CI values show no significant difference in the LC_{50}s of the male moths in 1989 (greatest value), 1990 and 1992.

Slopes for combined mortalities of females and males, females alone and males alone ranged from 0.79 to 2.08; 25% were <1, 68% ranged from 1-2 and 8%were>2 (Table 2). This was the same trend shown for larvae (Table 1). SE values were consistent and showed no trend. Number of adults treated was less than number of larvae treated; which was expected because adults do not develop from all progeny.

All LC_{50} values for females were equal because CI values overlapped; they ranged from 2.38 to 13.93 µg/vial, a 6-fold difference (Table 2). Six regressions were not significantly different from zero. For single pairs 3, 4, 8, 9 and 10 and Weslaco x Brownsville, slopes ± SE were 1 .05 ± 0.7, 0.74 ± 04, 1.1 ± 0.7, 1.31 ± 07, -0.045 ± 0.4, and 0.78 ± 0.5, respectively. These values are shown because females are never captured by pheromone traps. LC_{50} values for males ranged from 4.31 to 53.0 g/vial, a 12-fold difference. All LC_{50} values of males were equal; their CI values overlapped. Three regressions were not significantly different from zero. For single pairs 3, 4 and 10 slope ± SE were 0.84 ± 09, 1.7 ± 0.9 and 1.41 ± 08 respectively. Of interest were the slopes for the male, both sexes, and the female, respectively, for non-significant regressions of single pair 1 0; slope of the male was the steepest (>1) while slope of the female was the flattest (<0.05). Slope of both sexes was intermediate.

In 1988 there were no significant differences in toxicity of field collected males and females of this insect (Sparks et al. 1990). This same relationship was shown for male and female moths in Louisiana (Micinski et al. 1991). LC_{50} values of males and females of all crosses were equal (Table 2). Our results confirm previous information. LD_{50} values and LC_{50} values of Edinburg x Edinburg single pair were greatest or second greatest for all the bioassays.

Slope values of significant or non-significant regressions of females for 12 pairs were 33% <1, 58% range of 1-2 and 8% for >2. Slope values of significant or non-significant regressions of males were 22% <1, 67% range of 1-2 and 11% for >2. Slope values of the females and males were about equal. SE values for each of the sexes were about equal.

Comnarative toxicity of larvae and adults. Linear regression analysis of 11 LD_{50} values for larvae as the dependent variable and LC_{50} values for combined sexes of adults showed R^2=0.025, df 9, P>.65. Results show little relationship between LD_{50} values and LC_{50} values. Only 3% of the regression could be attributed to toxicity by cypermethrin. Regression of doses tested was not significantly different from zero in 8% (both sexes) to 50% (females alone) for vial bioassay and 0% for topical application.

We examined the LD_{50} values and LC_{50} values for causes of the nonsignificant regression. Moths of both sexes from single pair seven of the Brownsville crosses had the greatest LC_{50} while the LD_{50} for larvae was intermediate. When linear regression analysis of LD_{50} values of larvae and LC_{50} values of adults were determined without single pair seven the coefficient was 0.72, df 8, P>.002. This was a significant correlation coefficient; 72% of the regression could be attributed to toxicity by cypermethrin. Other coefficients were determined, but none were significant. When the low CI was used in place of LC_{50} of single pair seven a coefficient of 0.32, df 9, P>.073 was determined. When the three LC_{50} values which showed infinity for their CI values were deleted a coefficient of 0.6 df, P>99 was determined. When both the LD_{50} of single pair seven and the LD_{50} values which showed infinity were deleted a coefficient of 0.12, df 5, P>.5 was determined.

Then we compared the slope values of 12 LD_{50} values and slope values of their corresponding LC_{50} values for the same coefficient of determination. Coefficient of slopes of all pairs was 0.083,10 df, P>.36. Coefficient of slopes without slope of single pair seven was 0.059, 9 df, P>.47. Neither showed a significant coefficient.

CONCLUSION

Results of these two bioassays show variation in response to cypermethrin by larvae and adults of the tobacco budworm from the same single pair. We showed both resistance and susceptibility among larvae from the single pairs collected across the LRGV. There was little relationship between toxicity to larvae and toxicity to adults when a coefficient of determination was made for all single pairs. This means that toxicity to adults does not always mean that there is toxicity to larvae. When a single pair was deleted there was a positive significant coefficient between toxicity to larvae and toxicity to adults. Larvae, reared from single pairs, provide the most consistent response to cypermethrin.

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