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Cypermethrin and the Tobacco Budworm: Two Approaches to Determine Sample Sizes Required to Estimate Mortalities of Moths Bioassayed in Vials

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ABSTRACT

Vial and petri dish bioassays with cypermethrin were conducted using tobacco budworm, *Heliothis virescens* (F.), males (n=10790) collected at three locations in Texas during the 1988 cotton season. Sample sizes were based on the binomial distribution, i.e. dead or alive, for mean mortality of moths·dose^{-1}. Sample size, as moths·dose^{-1}, is an important factor when estimating LC_{50} values by probit analysis. In the first approach, a Monte Carlo study using sample sizes of 10, 20, 30, 40 and 50 moths·dose^{-1} indicated that the greatest variation was found when 10 moths·dose^{-1}·day^{-1} of bioassay were tested and the least variation was obtained when 50 moths·dose^{-1} were tested. Based on this study, a sample size of 40 to 50 moths·dose^{-1} for each of 6 or 7 doses provided an adequate estimate of the LC_{50} value. In the second approach, sample size necessary to reduce the Coefficient of Variation (C.V.) and the 95% 1/2 Confidence Interval (C.I.) by 10% and 30% was calculated. The C.V. or 95% 1/2 C.I. was determined from mortalities of an untreated check and 6 and 10 doses of microgram cypermethrin·vial^{-1} and kg cypermethrin (A.I.)·0.4 kg^{-1} in petri dishes, respectively. Based on this approach the estimated sample size to decrease the C.V. and C.I. by 30% required 387, 2327 and 510 moths compared to 190, 1140 and 250 moths actually tested during the three month season in the untreated check, determined in the Lower Rio Grande Valley, the Brazos Valley, and Uvalde-LaPryor, respectively.

RESUMEN

Se realizaron bioensayos en viales y en cajas de petri con cipermetrina utilizando machos (n=10790) del gusano de la yema del tabaco, *Heliothis virescens* (F.), colectados en tres localidades en Texas durante la estación algodonera de 1988. Los tamaños de la muestra se basaron en una distribución binomial, esto es muertos o vivos, para determinar la mortalidad promedio de palomillas·dosis^{-1}. El tamaño de la muestra, dada en palomillas·dosis^{-1}, es un factor importante cuando se estiman los valores de CL_{50} para el análisis probit. En el primer intento un estudio Monte Carlo usando tamaños de muestra de 10, 20, 30, 40 y 50 palomillas·dosis^{-1}·día^{-1} indicó que la variación más grande se encontró cuando se muestrearon los bioensayos de 10 palomillas·dosis^{-1}·día^{-1} y la menor variación se obtuvo cuando se muestrearon 50 palomillas·dosis^{-1}. En base a este estudio, un tamaño de muestra de 40 a 50 palomillas·dosis^{-1} por cada una de las 6 o 7 dosis dio una estimación adecuada del valor CL_{50}. En el segundo intento, se calculó el tamaño de muestra necesario para reducir el coeficiente de variación (C.V.) y el 95% 1/2 intervalo de confianza (I.C.) por un 10% y un 30%. El C.V. o 95% 1/2 I.C. se determinó a partir de las mortalidades de un testigo no tratado y de 6 y 10 dosis de microgramos de cipermetrina·vial^{-1} y de kilogramos de cipermetrina (I.A.)·0.4 ha^{-1} en cajas de petri, respectivamente. En base a este método, el tamaño de muestra estimado para disminuir el C.V. y el I.C. en un 30% requirió de 387, 2327, y 510 palomillas en comparación con 190, 1140 y 250 palomillas muestreadas realmente en el control no tratado durante los tres meses que duró la estación de muestreo, durante la determinación realizada en la porción baja del Valle del Rio Grande, en el Valle del Brazos y en Uvalde-LaPryor, respectivamente.

Plapp et al. (1987) first published the results of bioassays where male moths of the tobacco budworm, *Heliothis virescens* (F.), were exposed to various dosages of cypermethrin coated on glass vials. This bioassay technique was widely accepted in Texas, Louisiana and Arkansas to determine response of moths because it is comparatively easy to conduct. But no information exists on the sample size of moths to be determined with this bioassay. Roush and Miller (1986) stated that hundreds of individuals per location are needed to reliably detect response of a sample of a population with a discriminating dose.

In 1988, male moth mortalities, which conformed to the

binomial distribution, i.e. dead or alive, were determined from untreated and cypermethrin treated vials or petri dishes from the Lower Rio Grande Valley (LRGV), Brazos River (BV), and Uvalde-LaPryor (U-LP), Texas. From these bioassays two approaches to estimate sample sizes were made. In the first approach we evaluated the variation of the LC_{50} when calculated using 10, 20, 30, 40 and 50 moths·dose^{-1} at nine different doses. In the second approach number of moths required when the coefficient of variation (C.V.) and calcu-lated 95% confidential interval (C.I.) were decreased by 10 and 30% for various vial (microgram·vial^{-1}) and field use rates (kg (A.I.)·0.4 ha^{-1}). The study was conducted to determine optimal of number male tobacco budworms for response to cypermethrin.

MATERIALS AND METHODS

Doses, calculated as micrograms per vial or as kg A.I. per 0.4 ha of technical cypermethrin (FMC Corporation, Princeton, NJ) in acetone, were applied with methods described by Gage and Hatfield (1989). Vials were coated with acetone alone or acetone with appropriate dosage of cypermethrin and placed on a vial roller for one hour. After the acetone evaporated, vials were capped and held at room temperature until used 72 to 96 h later. Then we evaluated mortalities following application of 10 field use rates. Sprays were applied by airplane at 7. 6 gallons·A^{-1} to 9 cm diameter glass petri dish surfaces placed at various locations in the field.

Male moths were collected from 26 virelure-baited pheromone traps with removable tops from June through August for bioassay in all tests at LRGV, BV and U-LP. Traps were placed 2-5 m from the field edge inside 8 to 15 fields of cotton at each location. Moths were collected daily at sunrise, brought to the laboratory and placed in the refrigerator. After 15-30 min moths were placed singly in treated or untreated vials or glass petri dishes and capped with caps or dish tops, respectively. Mortalities were determined 24 h later and summarized as mean number dead, moribund or live moths. Bioassays were conducted 3 to 25 times in June and July for each dose.

At BV, 6 vial doses (1, 5, 10, 30, 100 and 300 µg·vial^{-1}) and 10 field use rates (0.000625, 0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 kg (A.I.)·0.4 ha^{-1}) were tested. At U-LP, the same vial doses and field use rates of 0.000625, 0.00125, 0.0025, 0.005, 0.01, 0.02 and 0.04 lb (a.i.)·A^{-1} were tested. At LRGV, vial dosages of 1, 5, 10 and 30 µg·vial^{-1} and 0.01, 0.02 and 0.04 lb (a.i.)·A^{-1} rates were tested. Untreated check vials or petri dishes were used at all 3 locations. These doses, or rates, were used because mortalities were known to range from 98% for the greatest to 8% for the lowest dose tested.

Sample Size Estimate

For our first approach, a mortality response test was conducted on August 22 in BV using 5 doses of cypermethrin and 100 moths·dose^{-1}. A Monte Carlo study was conducted to evaluate the variation of the LD_{50}'s when the number of moths per vial was randomly chosen to be 10, 20, 30, 40 or 50 moths per vial. In each case the probit analysis (SAS 1988) was corrected for a control. The maximum, minimum and median LD_{50} value was recorded for each moth/vial set. We also compared the width of the LD_{50} fiducial limits from the probit analysis as moths·dose^{-1} changed.

For our second approach, the number of moths to sample was determined by 10 and 30% less than the Karandinos (1976) coefficient of variation (C.V.) (Karandinos used standard error (SE) instead of standard deviation (SD) in C.V. calculations) and 2) the 95% confidence interval (C.I.). The 10 and 30% reductions were selected because they represented a range of reasonable reduction. If we selected 50% reduction, sample sizes would have been exceedingly large. If we selected 5% reduction, sample sizes would have not been much greater than the number sampled.

First, the SE of the mean for the normal approximation of the binomial distribution was calculated (Steel and Torrie 1960). Where p or x = the percentage dead, q = the percentage living (q = 100 - percentage dead), t = students t, and n = the total number of insects tested.

$$SE=\sqrt{\frac{(p)\ (q)}{n}} \qquad \text{Eq. 1}$$

Then, coupled with the t at P=0.05 for df 29, 40, 60, 120 and infinity, depending upon the actual sample size (Karandinos 1976, Steel and Torrie 1960 and Cochran 1961), the SE of the mean was used to calculate both the Karandinos C.V. and the one-half 95% confidence interval.

An estimate of the sample size when decreasing the C.V. by a given percentage, where the fraction or percent of decrease,, using the "Karandinos" C.V. is:

$$C.V.\ (100-Z) = \frac{SD\ /\ \sqrt{n}}{x}\ (100) \qquad \text{Eq. 2}$$

$$n = \left[\ \frac{STANDARD\ DEVIATION\ (100)}{(C.V.)\ (1.0)\ X}\ \right]^2 \qquad \text{Eq. 3}$$

The calculation of n (number of moths to test) is the number of moths needed when decreasing the C.I. by a proportion:

$$C.I.\ (1-Z) = \frac{(100)}{\sqrt{n}} \qquad \text{Eq. 4}$$

$$n = \left[\ \frac{(t)}{(C.I.)\ (1.0\ -\ Z)}\ \right]^2 \qquad \text{Eq. 5}$$

where percent decrease of 0.1 (10%) or 0.3 (30%) from the C.V. or 95% ½ C.I. The sample size for both the C.V. or 95% ½ C. I. is the same because the SE of the mean was used in both equations.

Test for Normality

Before any of the same size estimates were made the arcsine

Table 1. Toxicity of cypermethrin based on different number of moths/dose of 4 dosages and the untreated check. BV, Texas. 1988.

Range of LD_{50}	Number of Moths·Dose^{-1} Tested with 9 Tests				
	10	20	30	40	50
			LC_{50} (µg/vial)		
Lowest	16.2	16.3	18.5	19.2	19.2
Median	24.7	22.7	24.0	22.1	20.9
Greatest	35.9	29.8	28.3	26.8	26.4
Range	19.7	13.5	9.8	7.6	7.2
			Average width of the 95% Fiducial Limits		
	39.96	23.8	17.8	15.0	13.2

*individual readings in the table are averages of five disks read

Table 2. Example calculations estimating sample size (n) when decreasing the coefficient of variation (C.V.) and the 95% ½ confidence interval (C.I.) by 30%.

$$CV = \left[\frac{P=Mortality\ (8=1-P)}{n\ =\ 190}\right]^{½} \qquad \text{Eq. 1}$$

$$CV = \frac{7218}{1}\ x\ 100\ =\ 72.18$$

n = the number of moths to be sampled in order to decrease the C.V. by 30%:

$$n = \left[\frac{(99)^a\ (100)}{72.18^b\ (1)\ 0.70}\right]^2 \qquad \text{Eq. 2}$$

$$n\ =\ 387$$

n = the number of moths to be sampled in order to decrease the 95% C.I. by 30% or proportion of 0.3:

$$95\%\ ½\ C.I.\ =\ (t)\ (SE)\ =\ (1.96)\ (0.7218)\ =\ 1.414806 \qquad \text{Eq. 3}$$

$$n = \left[\frac{(9.949874)\ (1.96)^c}{(1.414806)^d\ (1-0.30)}\right]^2$$

$$n\ =\ 387$$

[a]SD determined by (p) (q) for binomial distribution

[b]Determined by $\dfrac{0.7218\ x\ 100\%}{p\ =\ 1\%\ or\ number\ respond}$

[c]Infinity of t.

[d]95% Confidence Interval of check for LRGV in Table 2.

transformation was applied to the mortalities from the BV observed in the check and the 10 and 100 µg/vial. These data were selected because they represented a low (3%), mid (44%) and high (87%) mortality of 1140, 800 and 820 moths, respectively. We also considered this to be a large sample size and be representative of all other data. We wanted to determine if it should be applied to normalize the percentage mortality. This transformation is recommended for binomial distribution (Steel and Torrie 1960).

RESULTS AND DISCUSSION

Test for Normality

The arcsine transformation was applied to mortalities from BV for the check, 10 and 100 µg cypermethrin·vial^{-1} treatments. The transformed mean ± standard error of the mean was 5 ± 0.42, 45 ± 1.7 and 87 ± 1.1, respectively for the low, mid and high mortality and was either the same or no more than 2, 1 or 6% less than that determined with the untransformed mortalities of the untreated check (data not shown). This difference was not considered to be of enough importance to apply the transformation to the remaining data. Thus the remaining data were analyzed without transformation.

Sample Size Estimates

With the first approach, the low, median and high LC_{50} values per sample date were statistically similar because their 95% confidence interval overlapped (Table 1) . Also the LC_{50} value for all moths tested (100·dose^{-1}) on the date was 22.5 µg/vial which was ± 3% of any median value. Range of LC_{50} values (difference between greatest and lowest value) was similar for 30, 40 and 50 moths·dose^{-1} per sample date based on percentage SD of mean. Ten moths·dose^{-1}, as expected, were the most variable while 20 was intermediate.

For our second approach an example (Table 2) shows calculations to determine number of moths to be sampled when using a level of 30% less than the C.V. or the 95% ½ C.I. for both bioassay methods, i.e. vial and petri dish. In the LRGV, 387 moths were needed to sample the untreated check. With the vial bioassay, 7480 moths were tested. As expected, sample size required to decrease the C.V. or the ½ 95% C.I. by 10% required fewer samples than a decrease of 30%. Sample sizes can be calculated for any proportion change in the C.V. and ½ 95% C.I. using the formula in Table 2 and substituting the proportion of change desired.

The number of moths to sample following calculations with C.V. or ½ 95% C.I. for 10% and 30% for check and mortalities of doses was equal [variable] (Table 3), and no relationship was evident. Check values were shown because we wanted to compare sample sizes where few insects are killed versus the doses where many insects are killed. At 30% of C.V. the number of moths to sample ranged from 81 to 1877. At 30% of C.V. 5, 30 and 10 µg·vial^{-1} and the check 1000 or greater moths needed to be sampled and all occurred in the BV. Nine concentrations ranged from 100 to 796 moths to sample. Mean number moths to sample at each concentration from 1 to 300 µg·vial^{-1} were 494 at 10% and 816 at 30% C.V. or ½ 95% C.I. Our results agree with Roush and Miller (1986) as hundreds of moths need to be treated per dose for a three month season even though the objectives for the determinations were not similar.

Field rates of cypermethrin, as kg (A.I.)·0.4 ha^{-1}, are shown with mean moth mortality, actual C.V. and 95% C.I. and number of moths tested at the same 3 locations (Table 4). No bioassay information has been found showing mortalities of moths with

Table 3. Number moths to test to determine mortalities of tobacco budworms tested in untreated vials or vials treated with various concentrations as µg cypermethrin·vial^{-1} at two levels of precision, Texas, June-August, 1988.

Dose (µg·Vial^{-1})	Location[a]	Number (n) Moths Tested	Mortality + SE[b]	Actual		No Moths to test at	
				C.V.	95% ½ C.I.	10% C.V.[c] or C.I.[d]	30% C.V.[c] or C.I.[d]
0	LRGV	190	1 ± 0.72	72	1.42	235	387
0	BV	1140	3 ± 0.51	17	0.97	1407	2327
0	U-LP	250	6 ± 1.50	25	2.99	308	510
1	BV	660	8 ± 1.06	13	2.07	815	1347
1	U-LP	40	10 ± 4.74	47	9.6	50	81
1	LRGV	100	13 ± 3.36	26	6.67	123	204
5	BV	870	37 ± 1.64	4.2	3.21	1074	1776
5	LRGV	180	49 ± 3.73	7.6	7.34	222	367
10	U-LP	390	41 ± 2.49	6.1	4.9	481	796
10	BV	800	44 ± 1.75	4.0	3.44	988	1633
10	LRGV	200	56 ± 3.51	6.3	9.9	247	408
30	U-LP	240	65 ± 3.08	4.7	7.97	296	490
30	BV	920	70 ± 1.51	2.2	3.0	1135	1877
30	LRGV	180	75 ± 3.23	4.3	6.37	222	368
100	U-LP	130	85 ± 3.13	3.68	6.19	160	265
100	BV	820	87 ± 1.17	1.35	2.3	1012	1673
300	LRGV	370	89 ± 1.63	1.83	3.20	456	755
300	U-LP	100	98 ± 1.40	1.43	2.77	123	204

[a] LRGV = Lower Rio Grande Valley
BV = Brazos Valley
U-LP = Uvalde-LaPryor

[b] Calculated using the standard error of the mean (SE)

[c] Coefficient of Variation

[d] ½ length of 95% Confidence Interval

Table 4. Number moths to test to determine mortalities of tobacco budworms in vials treated with cypermethrin as kg (A.I.)·0.4 ha^{-1} concentrations at two levels of precision and methods of calculation, June-August, Texas, 1988.

Rate (lb as·$Acre^{-1}$)	Location[a]	Number (n) Moths Tested	Mortality + SE[b]	Actual		No Moths to test at	
				C.V	95% 1/2 C.I.	10% C.V.[c] or C.I.[d]	30% C.V.[c] or C.I.[d]
.000625	BV	100	17 ± 3.76	22	7.52	123	204
.000625	U-LP	80	33 ± 5.26	16.0	10.46	98	163
.00125	BV	180	25 ± 3.23	12.9	6.36	222	367
.00125	U-LP	100	58 ± 4.94	8.5	9.7	123	204
.0025	BV	270	42 ± 3.0	7.15	5.91	333	551
.0025	U-LP	110	63 ± 4.6	7.3	9.11	136	225
.005	U-LP	60	47 ± 6.44	14	12.88	75	124
.005	BV	240	48 ± 3.22	6.7	6.35	297	490
.01	LRGV	30	56 ± 9.06	16	18.5	37	60
.01	BV	340	66 ± 2.57	4	5.06	420	694
.01	U-LP	110	84 ± 3.50	4	6.93	133	221
.02	U-LP	280	75 ± 2.59	3	5.09	345	571
.02	BV	380	76 ± 2.19	3	4.31	467	775
.04	U-LP	380	76 ± 2.19	3	4.31	467	775
.04	LRGV	120	91 ± 2.61	3	5.15	149	244
.04	BV	250	94 ± 1.5	2	2.96	307	509
.06	BV	170	95 ± 1.67	2	3.33	209	346
.08	BV	100	97 ± 1.71	2	3.38	124	204
.1	BV	110	97 ± 1.63	2	3.22	135	224

[a]LRGV = Lower Rio Grande Valley
BV = Brazos Valley
U-LP = Uvalde-LaPryor
[b]Calculated using the standard error of the mean (SE)
[c]Coefficient of Variation
[d]1/2 length of 95% Confidence Interval

cypermethrin following applications by airplane applied at field rates for this insect. The total number of moths tested was 3310. The number of moths to bioassay was variable for the 10 concentrations and 3 locations. At 30% of C.V. number of moths to be bioassayed ranged from 60 to 775 by C.V. and 41 to 526 by 30% of 95% 1/2 C.I. Fewer insects are required to be sampled when using the petri dishes treated with field use rates than the vial doses. Mean number of moths to bioassay at 0.000625 to 0.1 kg (A.I.)·0.04 ha^{-1} were 221 at 10% and 366 at 30% C.V. or 95% C.I.

In conclusion, we suggest that 30 to 50 moths·$dose^{-1}$ should be bioassayed on a given day to adequately estimate an LC_{50} for male tobacco budworm. The vial or petri dish technique indicates that the number of moths to bioassay be 200 to 300 moths·$dose^{-1}$ for the 3 month season. Additionally, because variables such as trapping method, trap location, population levels of tobacco budworm and cultural practices at trap sites may influence collection efficiency, it is suggested that a sufficient number of traps be used in a given area to obtain a suitable number of moths to provide a reasonable level of precision in the results. This may also require that all available moths on consecutive days be bioassayed and results combined until the appropriate sample size is obtained. We suggest that the level of precision used here is an appropriate level to aid in drawing specific conclusions from monitoring program data and is thus a suitable sample size.

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