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Estimating resistance to methomyl in the tomato pinworm (Lepidoptera: Gelechiidae) using a pheromone trap bioassay

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The tomato pinworm, *Keiferia lycopersicella* (Walsingham), is an important pest of tomatoes in the southern and southwestern United States and Mexico. A field-based method for assessing resistance of adults to the carbamate insecticide, methomyl, was developed by incorporating varying doses of technical insecticide into the adhesive of pheromone traps. The mortalities of male and female adults were similar whether exposed to methomyl, either by topical or pheromone trap bioassays. Larvae were less susceptible than adults to methomyl by topical application. Therefore, adult male susceptibility provides a good estimate of adult female susceptibility but may overestimate larval susceptibility. Nevertheless, using the pheromone trap bioassay and making comparisons of field populations with a laboratory strain suggested that all field populations evaluated were less susceptible to methomyl than the laboratory strain. Populations in Mexico were more susceptible than populations in California, where methomyl is used more often. Populations in Florida were either intermediate in susceptibility or were similar to those in California. Copyright © 1996 Elsevier Science Ltd.

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The tomato pinworm, *Keiferia lycopersicella* (Walsingham), is a serious pest of tomatoes in the southern and southwestern United States (Elmore, 1937) and Mexico (Alvarado-Rodriguez and Rivera-Rubio, 1990). Newly hatched larvae bore into leaves forming serpentine mines (Elmore, 1937) and, although larvae can complete their development on foliage, inflicting substantial defoliation, the greatest damage occurs when they bore into fruit. Feeding is usually restricted to the fruit core and can be difficult to detect since entry holes are covered with silk. If damaged fruit are undetected in the packing process, they can be infected with secondary rot organisms and can deteriorate during shipment or storage, thereby affecting uninfested fruit. In order to avoid fruit loss, growers routinely spray insecticides. Growers may rotate different classes of insecticides with applications based upon larval thresholds (Peña *et al.*, 1986; Wiesenborn, Trumble and Oatman, 1990) or on adult action threshold using pheromone traps (Van Steenwyk, Oatman and Wyman, 1983). Nevertheless, reduced susceptibility to the pyrethroid, fenvalerate, has been documented in Mexico and California (Brewer *et al.*, 1993) and suspected in Florida (Schuster, personal observation). The carbamate, methomyl, is also used to manage the tomato pinworm, particularly in California and Florida, and some growers in Florida report increased difficulty in managing the tomato pinworm with this insecticide (Schuster, personal observation).

Brewer *et al.* (1993) adapted the pheromone trap bioassay of Brewer and Trumble (1989) for estimating field resistance of tomato pinworm adults to fenvalerate. In this self-dosing bioassay, adult males are captured in pheromone traps with insecticide-laced adhesive and their mortality subsequently determined.

The purpose of the present investigation was to develop baseline toxicity levels of methomyl to a laboratory strain of the tomato pinworm using topical and pheromone trap bioassays and to use the pheromone trap bioassay to document levels of adult susceptibility in the field in Mexico, California and Florida.

Materials and methods

Baseline toxicity bioassays

A laboratory colony of *K. lycopersicella* that had been maintained continuously on tomato seedlings at the Gulf Coast Research & Education Center, Bradenton,

Florida since 1978 was used for baseline toxicity studies. Since both larvae and adults may be targets of insecticides in the field, the relationship between larval and adult susceptibility was studied using topical application. Because pheromone traps capture almost exclusively males, the susceptibilities of male and female adults were also compared with both topical and pheromone trap bioassays. In both bioassays, technical methomyl (98% w/w; E. I. du Pont de Nemours & Company, Wilmington, DE) was diluted in acetone.

Excluding the solvent only checks, five concentrations ranging from 100 to 1600 μg/ml were applied to ≤2 day old male and female adults and 10 concentrations ranging from 100 to 8000 μg/ml were applied to third or fourth instar larvae. Prior to dosing, adults and larvae were anesthetized with CO_2 and weighed in groups of 10 to the nearest 0.01 mg. Weights were averaged to convert doses to μg methomyl per g body weight. After chilling the insects at 2–3°C in Petri dishes, 0.2 μl were applied to the dorsum of each insect with an Arnold microapplicator (Burkard, Rickmansworth, Herts, UK). Adults were transferred to plastic cups with a cotton ball moistened with a 5% honey solution. Larvae were placed on excised tomato leaflets (10/leaflet) in plastic containers. Mortality was recorded 24 h later. Each bioassay was replicated at least four times with 10–25 insects per concentration and included solvent only checks.

For the pheromone trap bioassay, 1 ml of appropriate serial dilutions of methomyl in acetone were incorporated into 100 g of Tangle-Trap™ insect trap adhesive (Tanglefoot Co., Grand Rapids, MI, USA) to yield concentrations of 1.25, 2.5, 5, 10, 20 and 40, μg (AI)/g adhesive. Other trap adhesives, such as Stikem Special™ (Seabright Laboratories, Emeryville, CA, USA), should not be used since they may be too liquid and result in excessive mortality of trapped adults. Adhesive/methomyl mixtures and an adhesive/acetone control mixture were applied evenly onto pressed wax-coated cardboard inserts (325 cm^2) cut to fit the bottoms of Pherocon 1C wing traps (Trece, Salinas, CA; Concep, Bend, OR, USA).

Between 10 and 20 ≤ 1-day-old males and females were confined *c.* 5 h in plastic containers with a cotton ball saturated with a 5% honey solution. The moths were chilled for 5–10 min at 5°C and dropped onto the coated inserts. Inserts were suspended vertically in a plastic camp cooler with *c.* 1.3 cm of water in the bottom to provide *c.* 95% RH. The cooler was held 72 h in a room at *c.* 21°C. Mortality was observed after 12, 24, 36, 48, 60 and 72 h of incubation. Only moths attached to the adhesive by the ventrum were considered in mortality observations since this is the position in which most moths are captured in the field. A moth was considered dead if no movement of wings, legs, head, or antennae were observed after probing with a dissecting needle. Bioassays were replicated three times.

To determine the effect of moth age on estimates of susceptibility, newly emerged males were held in plastic containers with cotton balls saturated with a 5% honey solution for 1–2, 3–4 and 5–6 days. Moths were chilled as before and dropped onto inserts coated with either mixtures of 0 or 5 μg (AI)/g adhesive. Inserts were incubated under the above conditions for 36 h after which mortality was observed. The experiment was replicated three times.

Field toxicity bioassays

For field evaluations, at least six concentrations ranging from 2 to 2000 μg (AI)/g adhesive were applied to inserts that were placed in pheromone traps baited with tomato pinworm sex attractant pheromone lures (Scentry, Goodyear, AZ; Concep, Bend, OR, USA). A trap with an insert coated with only solvent/adhesive was included in each replicate as a control. Traps were placed ≥20 m apart, usually after 1500 h, in randomized complete blocks designs in commercial tomato fields in Mexico, California and Florida. To minimize variation among localities caused by different field conditions, traps were left in the field no more than 18 h and usually were retrieved before 0800 h. After retrieval, the traps were returned to the laboratory and the inserts were removed and incubated as in the baseline bioassays. Mortality was observed *c.* 24 h after trap retrieval which corresponded to an incubation time of 36–40 h, assuming most moths were captured in the traps within 4 h of sunset (McLaughlin *et al.*, 1979). Only moths without noticeable loss of scales were included in mortality observations.

Six sites were sampled in Mexico: one in 1988 and five in 1989. One site was sampled twice in 1989. In California, one site was sampled three times in the fall of 1986 and once in the spring of 1988. Seven sites were sampled late in the spring crop of 1994 in Florida. Populations at all sites were compared to the laboratory colony by calculating resistance ratios (RR) at the lethal concentration (LC_{50} and LC_{90} levels (LC_n) of the field population divided by the LC_n of the laboratory colony).

Data analyses

The data were pooled for each concentration over all replications prior to probit analysis using POLO-PC (LeOra Software, Berkeley, CA, USA). This program uses a correction for control mortality based upon the formula of Abbott (1925). For some sampling dates, data were analyzed without pooling replications and tested for batch (replication) heterogeneity. The Pearson χ^2 was used to determine goodness-of-fit to the Probit model. For the effect of male age on susceptibility, the proportion of dead moths was calculated and the data were transformed (arcsine square root) prior to performing ANOVA (SAS Institute Inc., 1988).

Results and discussion

Baseline toxicity bioassays

Mortality estimates and slopes of probit lines were similar for males and females using the topical bioassay (*Table 1*); however, slopes and lethality estimates differed between adults and larvae. The LC_{50} value for larvae was about 1.5 times that of adults but, the LC_{90} value was about between five and six times that of adults. Therefore, adult male susceptibility is a good indicator of adult female susceptibility but may overestimate larval susceptibility, especially at higher doses.

With respect to insecticide resistance, adult susceptibility still may be a conservative estimator of resistance or change in susceptibility of larvae because of the difference in adult and larval susceptibility at the higher doses (*Table 1*).

A standard incubation period of 36 h was selected for pheromone trap bioassays since LC_{50} values were stable at 36 and 48 h incubation and since mortality in the control at 36 h was less than 10% but exceeded 10% at 48 h (*Figure 1*). Using 36 h incubation, mortality estimates and probit slopes for males and females in the laboratory were similar (*Table 1*). Therefore, estimates of adult male susceptibility using the pheromone trap bioassay are good relative indicators of female susceptibility.

The age of adult males affected mortality in the pheromone trap bioassay (*Table 2*). Moths 3–4 and 5–6 days old had greater mortality than moths 1–2 days old even with only the adhesive/acetone mixture. McLaughlin *et al.* (1979) observed that males would respond to the pheromone on the second night following eclosion but that the response was greatest on the third night. Therefore, traps placed in the field are likely to capture populations of mixed age groups. To avoid this source of variation, moths with noticeable loss of scales such that wing patterns were less distinct were not included in mortality observations in field bioassays. While the rate of loss of scales is variable, it is the only way of detecting aged moths in the field.

Estimation of larval susceptibility to methomyl using the topical bioassay was laborious and time consuming. Larvae had either to be dissected from foliage or collected as they exited excised foliage as it desiccated, a process that can stress the larvae and affect their responses to insecticides. The topical bioassay, therefore, is not well-suited for field monitoring for resistance in this species. Conversely, the pheromone trap bioassay is ideally suited for use in the field since adults are self-dosed upon capture and a reproducible estimate of susceptibility can be obtained in less than 48 h. Since both topical and pheromone bioassays provided consistent estimates of adult susceptibility and since estimates of susceptibility at the lethal dose LD_{50} level provided similar estimates of relative susceptibility of both adults and larvae, the pheromone trap bioassay was used for all field estimations of susceptibility.

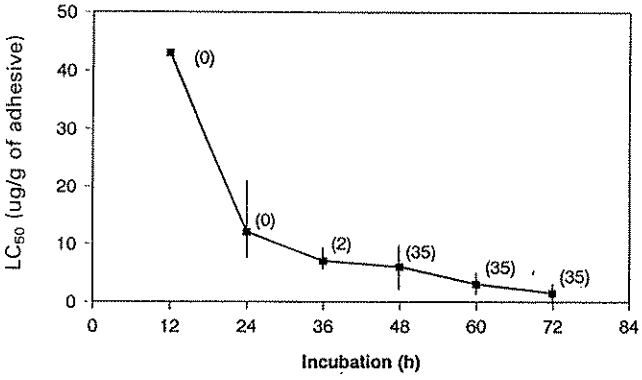

Figure 1. Influence of incubation time on LC_{50} values of tomato pinworm males exposed to methomyl in the adhesive of pheromone traps. Vertical lines are 95% fiducial limits and values in parentheses are the percent mortality of controls

Field toxicity bioassays

Populations at all sites sampled were less susceptible to methomyl than the laboratory population (*Table 3*). RR_{50} values ranged from as low as 2.1 for the May 1989 sample at the Ruiz Cortinez site in Mexico to as high as 62.7 for a late spring site in Florida. Slopes and RR_{50} values generally were lower in Mexico than in California and Florida. Pyrethroid insecticides, including fenvalerate and permethrin, are used more than methomyl in Mexico. At the Ruiz Cortinez site, RR_{50} values increased from May to June perhaps owing to methomyl use over this time. In California, methomyl is used more than pyrethroids and this is reflected in the high, late season RR_{50} values. However, unlike the Ruiz Cortinez site in Mexico, RR_{50} values decreased at the California site as the season progressed, although there was some overlap of fiducial limits of LC_{50} values. The RR_{50} value at the California site for spring 1988 was *c.* 6–10% of the values for the fall of 1986.

Variability among populations was greater in Florida than in Mexico. With the exception of the Culiacan site in 1988, probit slopes in Mexico were very similar but low, suggesting uniform but heterogeneous populations. LC_{50} values were similar in 1989 with the exception of the May sample at the Ruiz Cortinez site. In Florida, slopes varied by a factor of almost 6 and RR_{50} values by a factor of 5.5.

Table 1. Comparisons of susceptibility of adults and larvae of a laboratory strain of *K. lycopersicella* to methomyl using topical and pheromone trap bioassays

	Topical			Pheromone trap	
Parameter	Male	Female	Larvae	Male	Female
n	299	362	418	320	307
Mortality	1.5	1.2	1.2	1.8	5.7
Weight	1.30	1.60	1.91	1.40	1.68
Slope ± SE	3.74 ± 0.41	4.20 ± 0.41	1.48 ± 0.15	3.16 ± 0.43	3.46 ± 0.77
LD or LC_{50}	42.1	38.2	61.4	6.62	8.52
95% FL	32.6–52.3	34.2–42.4	48.2–76.1	5.38–7.81	4.74–10.2
LD or LC_{90}	92.8	77.1	452	16.8	20.0
95% FL	71.6–146	67.0–93.0	323–721	13.7–22.8	16.0–48.0
P	0.38	0.95	0.55	0.60	0.13

n, total number of insects tested excluding the control; Mortality, percentage of the control mortality; Weight, average mg pretest body weight; Slope (SE), slope of the probit line and the standard error of the estimate; LD or LC_x, mortality estimates based on µg/g body weight for topical bioassays (LD) and µg/g of adhesive for surface treated bioassays (LC); 95% FL, fiducial limits for the preceding mortality estimate; *P*, heterogeneity χ^2

Table 2. Effect of age on percent mortality of *K. lycopersicella* adult males exposed to two doses of methomyl in a pheromone trap bioassay

Dose (μg/g)	Age (days)* 1–2 *n*	1–2 % (SE)	3–4 *n*	3–4 % (SE)	5–6 *n*	5–6 % (SE)
0	58	8.7 (1.8)a	57	20.3 (3.5)a	32	45.1 (7.3)b
5	58	43.0 (11.2)a	49	85.1 (3.4)b	35	100.0 (0.0)c

*Means within rows followed by the same letter are not significantly different ($P < 0.05$; Duncan's (1955), multiple range test after the arcsine square root of the proportion transformation)

All populations in Florida indicated less susceptibility than those in Mexico, but some were more susceptible than the one population sampled in California in 1986. The low levels of susceptibility to methomyl indicated in Florida in this study are not surprising since many growers have reported difficulty in managing the tomato pinworm with this insecticide. While methomyl was used at all sites sampled in Florida, the number of applications varied among growers and did not relate consistently to the levels of susceptibility observed. For instance, methomyl was applied eight times at the Ft. Hammer site where the LC_{50} value was 203, twice at the Waterbury-B site where the LC_{50} value was 223, and three times at the Waterbury-A site where the LC_{50} value was 94. The Waterbury sites were located on adjacent farms and were separated by less than a half a kilometer.

Analyses of selected unpooled data from Florida suggested heterogeneity between and within replications on those sampling dates with the large χ^2 values. While the heterogeneity could have more than one possible explanation, heterogeneity of the tomato pinworm population is perhaps the most likely. All sampling in Florida was done late in the fresh market tomato season. All fields were at or nearing the end of commercial harvest of mature green fruit and many were open to consumer harvesting of pink or red fruit

Table 3. Methomyl pheromone trap bioassay of *K. lycopersicella* collected from Mexico and the USA

Site	Date (mo/yr)	*n*	% control mortality	Slope ± SE	LC_{50} (95% FL)*	RR_{50}†	LC_{90} (95% FL)*	RR_{90}†	*P*
				Mexico					
Culiacan, Ciapan	May/88	824	10.8	0.79 ± 0.08	20.8‡	3.1	857‡	51.0	<0.01
Guasave, Batamote	May/89	353	9.5	1.52 ± 0.20	46.1 (19.7–78.9)	7.0	320 (181–865)	19.0	0.13
Ruiz Cortinez	May/89	323	5.6	1.37 ± 0.23	13.6 (5.76–22.2)	2.1	116 (79.7–200)	6.9	0.82
Ruiz Cortinez	June/89	286	0.0	1.58 ± 0.18	42.6 (30.8–56.3)	6.4	275 (192–453)	16.4	0.70
Los Mochis	May/89	110	13.3	1.59 ± 0.37	46.4 (3.89–110)	7.0	297 (126–3400)	17.7	0.21
Corretera	May/89	404	3.2	1.44 ± 0.17	42.4 (16.6–75.6)	6.4	330 (164–1688)	19.6	0.01
Del Fuerte	May/89	110	9.1	1.53 ± 0.34	41.7 (5.56–92.3)	6.3	286 (128–2893)	17.0	0.21
				USA – California					
Orange Co.	Aug/86	969	7.6	5.28 ± 0.61	305 (208–384)	46.1	534 (421–923)	31.8	<0.01
Orange Co.	Sept/86	588	10.3	6.31 ± 0.86	200 (176–224)	30.2	320 (282–383)	19.0	0.95
Orange Co.	Oct/86	462	2.5	7.72 ± 2.26	174 (106–254)	26.3	575 (378–1245)	34.2	0.78
Orange Co.	Apr/88	204	0.0	1.29 ± 2.26	17.8‡	2.8	176‡	10.3	0.57
				USA – Florida					
Manatee Co. Ft. Hammer	11 May/94	259	9.7	3.96 ± 1.13	203 (46–256)§	32.7	428 (330–773)§	25.5	0.48
Manatee Co. Ft. Hammer	17 May/94	552	5.5	3.45 ± 0.33	87.1 (67.2–106)	14.0	205 (164–282)	12.2	0.31
Manatee Co. Waterbury–A	12 May/94	1060	7.9	2.27 ± 0.12	94.0 (59.6–131)	15.2	343 (238–614)	20.4	<0.01
Manatee Co. Waterbury–B	16 May/94	1606	5.5	4.45 ± 0.30	223 (198–248)	36.0	433 (380–519)	25.8	0.26
Hills. Co. Ruskin	18 May/94	896	0.7	2.96 ± 0.15	87.6 (70.8–107)	14.1	237 (184–343)	14.1	0.02
Manatee Co. Parrish	23 May/94	179	5.0	1.82 ± 0.29	87.2 (40.6–156)	14.1	439 (229–1900)	26.1	0.32
Manatee Co. Lorraine	24 May/94	6359	4.2	3.95 ± 0.15	199 (137–248)	32.1	420 (329–720)	25.0	<0.01
Hills. Co. Willow	25 May/94	4527	3.7	10.7 ± 0.52	389 (330–495)§	62.7	513 (428–887)§	30.5	<0.01

*95% Fiducial limits for the mortality estimate
†Resistance ratios based upon LC_{50} and LC_{90} values of 6.62 and 16.8, respectively, for calculating the RR_{50} and RR_{90} values, respectively
‡Fiducial limits were not computed because $g \geq 0.50$ (criterion based on Finney, 1971)
§90% Fiducial limits were computed because $g \geq 0.50$ at the 95% (criterion based on Finney, 1971)

only. Thus, the frequency of insecticide applications was reduced and tomato pinworm populations increased. Furthermore, some fields had already been abandoned and others had been sprayed with a desiccating herbicide prior to field destruction. Thus, the propensity for interfield movement of tomato pinworm adults was high. Pheromone traps without methomyl-laced adhesive were placed in a field with a very low infestation of tomato pinworm larvae and captured *c.* 20–100 moths per trap per night. This apparent movement of tomato pinworm moths within the tomato-growing area would most likely decrease the uniformity of tomato pinworm populations being sampled and could explain why the mortality data failed to fit the probit model in 50% of the samples.

Despite the heterogeneity of responses indicated on some sampling dates, the pheromone trap bioassay was an effective, low cost method for detecting differences in susceptibility of the tomato pinworm to methomyl in California, Florida, and Mexico. The bioassay provides a rapid assessment of susceptibility relative to a susceptible laboratory strain. The technique could be useful in managing insecticide resistance by detecting initial decreases in susceptibility and allowing growers to alter their choice of insecticide before control difficulty or failure occurred. The technique could also be useful in rapidly determining the role of reduced susceptibility as a cause for control difficulty or failure using either methomyl or fenvalerate.

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References

Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**, 265–267

Alvarado-Rodriguez, B. and Rivera-Rubio, E. (1990) Tomato pinworm (Lepidoptera: Gelechiidae): an increasing pest on tomatoes in Sinaloa, Mexico. *Fla. Entomol.* **73**, 677–680

Brewer, M.J. and Trumble, J.T. (1989) Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **82**, 1520–1526

Brewer, M.J., Schuster, D.J., Trumble, J.T. and Alvarado-Rodriguez, B. (1993) Tomato pinworm (Lepidoptera: Gelechiidae) resistance to fenvalerate from localities in Sinaloa, Mexico and California, USA. *Trop. Agric. (Trinidad)* **70**, 179–184

Elmore, J.C. (1937) The tomato pinworm. *U.S. Dept. Agric. Cir.* No. 440, 8 pp

Finney, D.J. (1971) *Probit Analysis*, 3rd edition. Cambridge University Press, London and New York

McLaughlin, J.R., Antonio, A.Q., Poe, S.L. and Minnick, D.R. (1979) Sex pheromone biology of the adult tomato pinworm *Keiferia lycopersicella* (Walsingham). *Fla. Entomol.* **62**, 35–41

Peña, J.E., Pohronezny, K., Waddill, V.H. and Stimac, J. (1986) Tomato pinworm (Lepidoptera: Gelechiidae) artificial infestation: effect on foliar and fruit injury of ground tomatoes. *J. Econ. Entomol.* **79**, 957–960

SAS Institute Inc. (1988) *SAS/STAT™ User's Guide*, release 6.03 edition. SAS Institute Inc., Cary, NC

Van Steenwyk, R.A., Oatman, E.R. and Wyman, J.A. (1983) Density treatment level for tomato pinworm (Lepidoptera: Gelechiidae) based on pheromone trap catches. *J. Econ. Entomol.* **76**, 440–445

Wiesenborn, W.D., Trumble, J.T. and Oatman, E.R. (1990) Economic comparison of insecticide treatment programs for managing tomato pinworm (Lepidoptera: Gelechiidae) on fall tomatoes. *J. Econ. Entomol.* **83**, 212–216