# Tomato pinworm (Lepidoptera: Gelechiidae) resistance to fenvalerate from localities in Sinaloa, Mexico and California, USA

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**The cultivated tomato *Lycopersicon esculentum Mill.* is an important host of the tomato pinworm *Keiferia lycopersicella* (Walsingham) (Lepidoptera: Gelechiidae) in Mexico and the southern United States of America. Because of reports of high fruit infestation in the Guasave and Del Fuerte valleys in Sinaloa, Mexico, despite frequent use of pyrethroid insecticides, resistance was suspected. A portable bioassay that assayed adult male susceptibility was used to determine the existence of resistance in this region. These data were compared with data collected in southern California where control was adequate. Comparison of adult male susceptibility with adult female and larval susceptibility indicated that male susceptibility was a good indicator of female and larval susceptibility. Using likelihood ratio tests to compare each field strain with a laboratory susceptible strain, all field strains were less susceptible to fenvalerate than the laboratory strain. Resistance ratios at the $LC_{50}$ were greater than 3 in all cases and were as high as 2,141 at the $LC_{90}$. Tomato pinworm collected in Sinaloa consistently showed lower susceptibility to fenvalerate than those collected in California which corresponded to the use of higher annual rates of pyrethroids in Sinaloa than in California. Tomato pinworm resistance to fenvalerate in Sinaloa localities appeared to be extreme and should be a serious concern to management of this insect.**

Keywords: *Keiferia lycopersicella*; Insecticide resistance; Fenvalerate; Tomato

The tomato pinworm *Keiferia lycopersicella* (Walsingham) (Lepidoptera: Gelechiidae) is oligophagous, with host preference for the Solanaceae. The cultivated tomato *Lycopersicon esculentum* Mill. is an important host in Mexico (Anon., 1983) and the southern United States of America (USA) (Oatman, 1970; Batiste *et al.*, 1970). Larvae mine and fold foliage and bore into fruit (Elmore and Howland, 1973; Lin and Trumble, 1986). Infested fruit are difficult to market, particularly in the USA where there is low tolerance to insect presence and damage on fresh market produce. As a result, insecticides have been used in an attempt to prevent infestation of the fruit in major tomato-producing regions in the southern USA and Sinaloa, Mexico. Sinaloa producers need to meet the expectations of the USA tomato market because they export to this market.

The pyrethroids fenvalerate and permethrin have

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been used to control this and other tomato-feeding Lepidoptera in these regions. Because of reports of high fruit infestation in the Guasave and Del Fuerte valleys in Sinaloa despite frequent use of these insecticides (Alvarado-Rodriguez and Rivera-Rubio, 1990), resistance to one or both pyrethroids was suspected. In contrast, the authors were not aware of control failure reports in the southern USA. These two regions differed in amounts of insecticide used to control tomato pinworm; but were similar in that they contained major tomato-producing regions and used pyrethroids as one method of tomato pinworm control. The objectives of this study were to document tomato pinworm susceptibility to fenvalerate in Sinaloa and compare these data with those from tomato pinworm collected in southern California, USA.

## Materials and methods

The bioassay used to document resistance to fenvalerate in both laboratory and field strains was adapted from the self-dosing adult susceptibility test reported by Brewer and Trumble (1989). Methods and modifications to the methodologies are briefly stated here. Moths were caught in adhesive–fenvalerate mixtures applied to insects placed in pheromone wing traps (Pherocon 1C, Trece, Salinas, CA). A series of concentrations of fenvalerate mixed in adhesive (Tanglefoot Company, Grand Rapids, MI) were prepared by first serially diluting fenvalerate (94%; E.I. du Pont de Nemours & Company, Wilmington, DE) in 9:1 hexane–ethanol. Addition of 1 ml of the appropriate dilutions $100 g^{-1}$ of adhesive yielded concentrations of 2, 10, 20, 40, 80, 160, 320, 500, 640, 1,000, 2,000, and 4,000 µg (a.i.) $g^{-1}$ of adhesive. A control consisted of adhesive containing 1 ml of the solvent alone 100 $g^{-1}$ of adhesive. The treated adhesive (5.5 g) was spread evenly onto pressed wax-coated cardboard inserts (325 $cm^2$) cut to fit into the base of the pheromone trap. Inserts of at least seven concentrations of each insecticide, including the solvent only control, were used in all pheromone trap tests. Each concentration was replicated using at least three inserts.

### Baseline tomato pinworm susceptibility

Mortality data from a laboratory strain were used as a reference to compare with data from field strains. The strain had been maintained continuously since 1978 on tomato seedlings (Schuster and Burton, 1982). Bioassays consisted of adhesive –fenvalerate mixtures (20, 40, 80, 160, 320, and 640 µg $g^{-1}$ of adhesive) and solvent only controls applied to trap inserts as previously described. Five to 20 ≤ 1-day-old males and females $concentration^{-1}$ were tested. Moths of each sex were confined for approximately 5 h with a cotton ball saturated with 5% honey solutions before testing. The moths were dropped onto the coated inserts after being chilled at approximately 5°C for 5–10 min. Only moths attached to adhesive by the ventrum were used as this positioning was most common when insects were caught in pheromone traps in the field. Inserts were kept at 21 ± 2°C and ≥95% relative humidity (RH). Each bioassay was replicated at least three times. Mortality was observed after incubation times of 12, 24, 36, 48, 60, and 72 h. A moth was considered dead if no movement of wings, legs, or head occurred after a wing was lifted from the adhesive. The same criteria were used for field studies. Data from each concentration were pooled before probit analysis of data collected for each incubation time (POLO-PC; LeOra Software, 1987). The data were tested for fit to the probit model using the $\chi^2$ goodness-of-fit test in this and subsequent tests. The incubation time chosen for all other laboratory and field tests was the longest incubation time in which control mortality was <10% and in which the data set was adequately described ($P \geq 0.10$) by the probit model.

### Comparison of larval and adult susceptibility

Larval testing is a laborious process that requires microapplication equipment that is not readily portable as well as removal of larvae from leaf tissue. In this study, a portable bioassay was necessary because resources were not available to transport multiple insect collections to a central rearing facility, maintain these collections until testing, and test insects from each collection using microapplicator equipment. Alternatively, the pheromone trap test required preparation of adhesive–fenvalerate mixtures in a laboratory. The bioassay was done by self-dosing moths as they were caught in a pheromone trap, and incubation was done in a building with temperature control. Necessary humidity could be maintained by placing the inserts into a closed container with adequate moisture (Brewer and Trumble, *ibid.*).

Because both larvae and adults of tomato pinworm are targets of insecticide control in the field, the relationship of larval and adult susceptibility was investigated to determine if adult susceptibility was an adequate measure of larval susceptibility. Because the pheromone traps only attract males, sex differences in susceptibility were also tested. For these comparisons, fenvalerate was applied topically to larvae and adults of the laboratory strain. Six concentrations were applied to larvae (third or fourth instars) and seven concentrations to male and female (≤2-day-old) adults. Fenvalerate was diluted in acetone. The test concentrations were 12.5–200 µg $ml^{-1}$ of acetone for larval tests and 5–160 µg $ml^{-1}$ of acetone for adult tests. An Arnold microapplicator (Burkard, Rickmansworth, Herts, England) was used to deliver 0.2 µl to the dorsum of each insect. Groups of 10–20 insects of each replication were weighed. Weights were averaged to report toxicity on a µg $g^{-1}$ body weight basis. Ten to 30 $insects\ concentration^{-1}$ were tested; each bioassay was replicated at least four times. Mortality was recorded 24 h after application. Data of each concentration were pooled before probit analysis (POLO-PC; LeOra Software, *ibid.*). Likelihood ratio (*LR*) tests (Savin *et al.*, 1977) which generated a $\chi^2$ statistic were used to compare probit regression lines and slopes calculated from each

data set (POLO-PC; LeOra Software, *ibid.*). *LR* was used to avoid confusion with the $\chi^2$ goodness-of-fit test that was also used.

## Susceptibility of field populations

For field studies, the treated inserts were placed in pheromone traps baited with tomato pinworm pheromone lure (Scentry, Phoenix, AZ). To sample tomato pinworm at a particular location and date, pheromone traps representing a full bioassay (at least seven adhesive–fenvalerate mixtures ranging from 10 to 4,000 μg $g^{-1}$ of adhesive) were placed ≥20 m apart in a randomised complete block design. The concentrations were represented within each block and the blocks were replicated four times. To reduce variation in mortality caused by different field conditions among localities, inserts were left in the field for <18 h. About 75% of males are captured in pheromone traps during the twilight period (McLaughlin *et al.*, 1979); therefore traps were placed in the field after 1500 h to minimize exposure to sunlight. Traps were retrieved the next morning before 0700 h. After retrieval, inserts were removed from the traps and held at 21 ± 2°C and ≥95% RH. Moths were inspected between 0800 and 1100 h the day after traps were removed from the field. This inspection time corresponded to an incubation period of 36–40 h assuming moths were caught in the traps within 4 h of sun set. A similar approach was used by Haynes *et al.* (1987) for detecting pyrethroid resistance in another gelechiid, the pink bollworm, *Pectinophora gossypiella* (Saunders).

Insects were sampled at three locations in Sinaloa during May and June 1989 and one location in California. Locations in Sinaloa were separated by at least 20 km. The California location was sampled for three consecutive months in 1986 and once in 1988. Two other locations in California were sampled but tomato pinworms were not caught in sufficient numbers for analysis (see criteria below).

For each test, mortality data from all inserts of a concentration were pooled. The data were analyzed using probit analysis (POLO-PC; LeOra Software, *ibid.*). A field bioassay was omitted from analysis if control mortality was >15%, the number of insects caught was <90, or the pooled number of moths from traps of a concentration was <20 for three or more concentrations. All data sets which met these criteria were adequately described by the probit model ($P \geq 0.10$; Table 1); therefore susceptibility of different field strains were compared using this model. Likelihood ratio tests were used to compare probit estimates among Sinaloa and California locations (POLO-PC; LeOra Software, *ibid.*). Laboratory results at 36 h incubation were compared with field results using the likelihood ratio test. Resistance ratios (RR) were calculated as $LC_x$ field strain/$LC_x$ of the laboratory strain, *x* representing 50 or 90% lethality.

**Table 1** Susceptibility to fevalerate in tomato pinworm populations sampled in Sinaloa, Mexico and California, USA

| Site | Date | *n* | % Control mortality | Slope (SE) | $LC_{50}$ (95% FL) | $RR_{50}$[a] | $LC_{90}$ (95% FL) | $RR_{90}$[a] | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Guasave Valley A | May '89 | 233 | 0.0 | 0.62 (0.15) | 1,718 (747–15,697) | 17.3 | 202,440 (19,503–4.6 × $10^9$) | 378 | 0.40 |
| Los Mochis | May '89 | 406 | 0.0 | 0.71 (0.13) | 3,522 (1,550–39,301) | 35.4 | 223,290 (25,440–6.4 × $10^8$) | 417 | 0.17 |
| Guasave Valley B | June '89 | 153 | 0.0 | 0.49 (0.17) | 2,853 (1,035–79,873) | 28.7 | 1,145,365 (53,002–5.7 × $10^{10}$) | 2,141 | 0.59 |
| Guasave Valley B | May '89 | 348 | 6.0 | 0.63 (0.12) | 1,726 (663–6,808) | 17.3 | 191,280 (26,657–8.7 × $10^7$) | 358 | 0.34 |
| Guasave Valley B | May '89 | 233 | 0.0 | 0.61 (0.15) | 1,820 (754–25,336) | 18.3 | 228,131 (19,381–6.1 × $10^{10}$) | 426 | 0.36 |
| Orange Co. | Aug. '86 | 722 | 14.9 | 10.6 (3.2) | 465 (407–500) | 4.7 | 615 (556–854) | 1.1 | 0.51 |
| Orange Co. | Sept. '86 | 878 | 8.1 | 2.26 (0.21) | 474 (354–598) | 4.8 | 1,752 (1,266–3,035) | 3.3 | 0.10 |
| Orange Co. | Oct. '86 | 410 | 7.4 | 2.19 (0.34) | 1,124 (901–1,443) | 11.3 | 4,331 (2,913–8,671) | 8.1 | 0.75 |
| Orange Co. | April '88 | 312 | 2.0 | 1.80 (0.24) | 347 (222–496) | 3.5 | 1,784 (1,105–4,396) | 3.3 | 0.42 |

*n*, total number of insects tested excluding control; Slope (SE), slope of probit line and the standard error of the estimate; $LC_x$ lethal concentration needed to kill *x*% of the test population; 95% FL, 95% fiducial limits of the $LC_x$; P, probability of the $\chi^2$ associated with testing the null hypothesis that the data were adequately described by the probit model

[a]Laboratory strain data used as baseline susceptibility data: 99.5 and 535 μg $g^{-1}$ adhesive for $LC_{50}$ and $LC_{90}$, respectively, resistance ratios ($RR_x$) = $LC_x$ of field strain/$LC_x$ of laboratory strain

## Results and discussion

### Baseline tomato pinworm susceptibility

A standard incubation period of 36 h was used in the pheromone trap test. $LC_{50}$ of males were stable at 24, 36, and 48 h incubation times (Figure 1). At 48 h incubation, mortality in the control increased beyond 10%. Using a 36-h incubation, mortality did not exceed 10% in the laboratory test (Figure 1; Table 2) and in eight of nine tests subsequently done in the field (Table 1). Also, the laboratory strain data were adequately described by the probit model at the 36-h incubation time ($P > 0.10$, Table 2). Thus, the probit estimates calculated from the male susceptibility data of the laboratory strain at a 36-h incubation were compared with probit estimates of field strain susceptibility data.

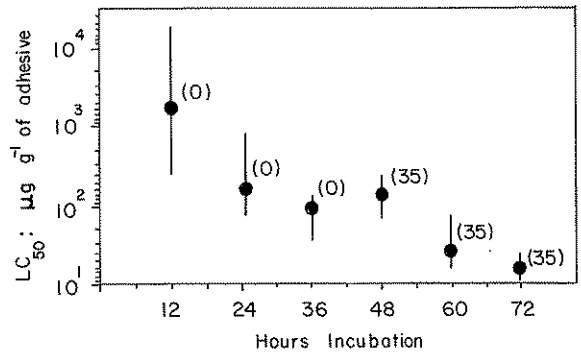


**Figure 1** Effect of incubation time on male susceptibility to fenvalerate using the pheromone trap bioassay. Vertical lines are 95% fiducial limits; values in parentheses are percentage of control mortality

### Comparison of larval and adult susceptibility

Susceptibility to fenvalerate did not differ between males and females using the topical application test ($LR = 0.20$; df = 2; $P = 0.90$) or the pheromone trap test ($LR = 0.58$; df = 2; $P = 0.75$). These data suggested that male susceptibility was a good indicator of female susceptibility. Comparison of male and larval susceptibility using the topical application test also suggested that male susceptibility was a good indicator of larval susceptibility. Probit slopes calculated from the two data sets were similar ($LR = 2.21$; df = 1; $P = 0.14$) and the $LD_{50}$ and $LD_{90}$ values differed by less than two-fold. Using methomyl (a carbamate) as the toxicant, large differences in male, female, and larval susceptibility were detected (unpublished data). Therefore, this process of comparing growth stages and sexes is suggested if susceptibility of one stage is to be used as a predictor of susceptibility of another stage or sex.

### Comparison of field and laboratory strains

Using *LR* tests to compare each field strain with the laboratory strain, all field strains were less susceptible to fenvalerate than the laboratory strain (in nine comparisons $LR > 51$; df = 2; $P < 0.001$. Resistance ratios at the $LC_{50}$ were greater than 3 in all cases and were calculated as high as 2,141 at the $LC_{90}$ (Table 1). All data from Sinaloa strains yielded lower slope estimates than the laboratory strain (in five com-

**Table 2** Comparisons of adult and larval susceptibility to fenvalerate of a laboratory strain using topical and pheromone trap bioassays

| | Topical | | | Pheromone trap | |
|---|---|---|---|---|---|
| | Male | Female | Larvae | Male | Female |
| *n* | 304 | 390 | 389 | 302 | 394 |
| Mortality | 2.8 | 1.3 | 2.6 | 0.0 | 2.0 |
| Weight (mg) | 1.33 | 1.73 | 1.74 | 1.16 | 1.79 |
| Slope (SE) | 3.03 (0.42) | 3.23 (0.49) | 2.41 (0.24) | 1.75 (0.18) | 1.68 (0.18) |
| LD or $LC_{50}$ | 6.71 | 6.90 | 4.98 | 99.5 | 110 |
| 95% FL | 5.55–7.93 | 5.12–8.80 | 4.11–5.90 | 69.8–142 | 87.6–137 |
| LD or $LC_{90}$ | 17.7 | 17.2 | 16.9 | 535 | 636 |
| 95% FL | 14.0–25.6 | 12.4–38.7 | 13.6–22.6 | 322–1,322 | 455–1,024 |
| *P* | 0.90 | 0.29 | 0.98 | 0.40 | 0.77 |

*n*, total number of insects tested excluding control; Mortality, percentage of 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, lethality estimates based on µg $g^{-1}$ body weight for topical bioassays (LD) and mg $g^{-1}$ of adhesive for surface treated bioassays (LC); 95% FL, 95% fiducial limits for the preceding lethality estimate; *P*, probability of the $\chi^2$ associated with testing the null hypothesis that the data were adequately described by the probit model

parisons $LR > 22$; df = 1; $P < 0.001$, estimates given in Tables 1 and 2); whereas data from California strains yielded slope estimates as high or higher than the laboratory strain (in two comparisons $LR < 1.5$; df = 1; $P > 0.22$ and in two comparisons $LR > 3.8$; df = 1; $P < 0.05$, estimates given in Tables 1 and 2).

Susceptibility to fenvalerate in field strains varied considerably (Table 1). Comparing each Sinaloa strain with each California strain, Sinaloa strains were less susceptible to fenvalerate than the California strains (in 15 comparisons $LR > 24$; df = 2; $P < 0.001$). These differences coincided with annual region-wide pyrethroid use in Sinaloa that was higher than use in California (Table 3). Before 1987, fenvalerate was used in Sinaloa about as frequently as current permethrin use.

Comparing sampling locations and dates within Sinaloa and California, less variation in resistance was detected among locations in Sinaloa than among sampling dates in California. Among Sinaloa locations, no differences in susceptibility were detected ($LR = 10.7$; df = 8; $P = 0.22$); whereas tomato pinworm sampled on four dates at the California location differed in susceptibility ($LR = 180$; df = 6; $P < 0.001$). The lack of detectable variation among Sinaloa locations did not appear to be due to lack of differences in insecticide use among locations (Table 3). Among location, differences may be masked by the large variation within locations as indicated by low slopes and resulting wide fiducial limits (Table1). The increasing resistance with time detected at the California location in 1986 may be a consequence of selection of resistant insects as selection pressure increases during the growing season. When the same field was sampled early in the growing season (April 1988), tomato pinworm susceptibility was high relative to the late season 1986 data. A similar temporal increase in resistance in Sinaloa was not detected in the study because all data were taken within a short time period (Table 1). Tomato pinworm resistance to fenvalerate in Sinaloa was high in all cases.

Although resistance ratios are measures of physiological levels of resistance and there are no threshold values indicative of field failure known for this insect, the authors suspect that resistance ratios of >300 at the $LC_{90}$ will result in field failure. This suspicion is based on the observation that fenvalerate application levels used in 1989 did not provide adequate control as judged by fruit inspection (Trumble and Alvarado-Rodriguez, personal observation). Although the data in Table 1 do not provide information to determine a threshold <300 related to field failure, ratios >300 at the $LC_{90}$ appear to be related to field failure of fenvalerate. Thus reliance on fenvalerate should be minimized because of the likelihood of field failure. The timing of the reincorporation of fenvalerate into a sustained management programme for tomato pinworm control in Sinaloa still remains to be answered.

The pheromone trap test detected a suspected resistant population and provided supporting data that the difficulty in control of tomato pinworm in Sinaloa was due in part to resistance to fenvalerate. Because the test is portable, susceptibility can be monitored over time and in different locations. Such data would aid in determining resistance stability with time under different management strategies. Laboratory studies aimed at measuring heritability of resistance would compliment these field studies. Use of the pheromone trap bioassay for resistance management would be simplified by the development of a diagnostic dose (Brewer and Trumble, 1991). The current system does allow for rapid assessment of resistance ratios and verification of reports of reduced tomato pinworm control using fenvalerate.

**Table 3** Average frequency of fenvalerate and permethrin use at field sites during survey

| Region | Site | Plantings | Insecticide applications[a] | |
|---|---|---|---|---|
| | | | Fenvalerate | Permethrin |
| Sinaloa[b] | Guasave Valley A | 3 | 3 | 7 |
| | Los Mochis | 3 | 3 | 13 |
| | Guasave Valley B | 3 | 3.5 | 19.5 |
| California | Orange County | 1 | 7 | 0 |

[a]All applications were within label specifications. Applications, average number of applications of insecticide crop$^{-1}$

[b]Mixture of two or three insecticides (pyrethroid, carbamates, and organophosphates) were used approximately 65% of the time but were recorded as separate applications for pyrethroid use. Amount of insecticide application$^{-1}$ was the same if used alone or in mixture

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