Monitoring Insecticide Resistance in *Liriomyza trifolii* (Diptera: Agromyzidae) with Yellow Sticky Cards

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J. Econ. Entomol. 82(4): 1011–1018 (1989)

ABSTRACT The use of commercially available yellow sticky cards coated with thin layers of insecticide-sticker mixtures as a bioassay was explored to determine insecticide resistance in adult *Liriomyza trifolii* (Burgess). Young flies emerging from celery or chrysanthemum foliage collected in the field were captured on sticky cards treated with various amounts of permethrin or chlorpyrifos. The control included no insecticide. Mortality of the flies was used to estimate the degree of resistance of a given population to these insecticides. Duration of exposure to insecticide before mortality evaluation was standardized at 24 h. Control mortality increased with increasing amounts of sticker on the cards and with increasing adult age. Males were slightly more susceptible to both insecticides than females, and small flies were slightly more susceptible to permethrin than large flies. Dose–mortality estimates produced by the sticky card procedure are comparable with those produced by a topical application bioassay. The sticky card bioassay is a simple procedure that is accurate, repeatable, and usable for field or greenhouse populations.

KEY WORDS Insecta, resistance management, bioassay, serpentine leafminers

A SERPENTINE LEAFMINER, *Liriomyza trifolii* (Burgess) is a world-wide pest of numerous vegetable and ornamental crops (Lindquist 1983). This pest is notoriously difficult to control because of resistance to insecticides. Resistance in this species was first detected in Florida in the late 1940s with a resistance to toxaphene (Wolfenbarger 1957). Since then, numerous insecticides have failed to provide control of *L. trifolii* after only 2–3 yr of use in the field. The celery (*Apium graveolens* L.) industry was on the verge of collapse in 1981 because of the failure of insecticides to control this pest (Talbot 1981).

In California, economic losses associated with chemical control and celery damage were estimated at $19–20 million during 1984 (Trumble 1985a, Trumble et al. 1985). Chrysanthemums (*Chrysanthemum morifolium* Ramat) also have sustained considerable damage. A survey of chrysanthemum growers in California (Newman & Parrella 1986) revealed that, between 1982 and 1985, growers spent an average of nearly $14,800 per hectare per year on insecticides for leafminer control and still lost 23% of the crop; this was equivalent to a $93 million loss over this 4-yr period.

Obviously, the capability of *L. trifolii* to develop resistance to insecticides must be taken into consideration when integrated pest management programs are developed for crops where this leafminer is a problem (Trumble 1985b, Parrella & Jones 1987). A vital part of any such program is development of a monitoring technique that allows the rapid and accurate detection of resistance levels in this pest (Brent 1986). Keil et al. (1985) developed a technique using standard topical application procedures, but their method is not suited to the rapid processing of large numbers of field samples. Based on a method in which insecticide resistance was assessed in adult lepidoptera caught in pheromone traps (Haynes et al. 1987), a bioassay method was tested where resistance in adult *L. trifolii* was determined with insecticide-laced sticker spread on yellow cards (Haynes et al. 1986). This technique appeared to be an easy and accurate method for processing numerous field samples. However, questions concerning the validity of this technique remained.

In the studies described here, we examined this technique in more detail. Specifically, effects of the duration of exposure to insecticide, the amount of sticker used, and adult age, sex, and size on bioassay results were investigated. In addition, we compared results obtained using the sticky card bioassay with results from standard topical application procedures.

Materials and Methods

Standard Procedure. The procedure of Haynes et al. (1986) was used with some modifications. Technical insecticide (permethrin, FMC Corporation, Philadelphia) and chlorpyrifos, Dow Chemical Company, Midland, Mich.) was dissolved in a 90:10 hexane/ethanol solution and diluted serially to desired concentrations. The dilutions plus a control solution without insecticide were added to 5 to 10 g of insect adhesive (Tangle-Trap Sticker,

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0022-0493/89/1011–1018$02.00/0

Tanglefoot Company, Grand Rapids, Mich.) in small flint glass jars (0.2 ml/g sticker). The resulting mixtures were each stirred continually for 5 min with a glass rod for thorough mixing and stored in a refrigerator at approximately 3°C.

In preparation for a bioassay, the desired insecticide–sticker concentrations were first stirred again for about 1 min; a small amount was then applied with a stirring rod to a yellow vinyl card (8.5 by 15 cm, 10 gauge) and spread evenly (2 to 4 mg of sticker per square centimeter) with a glass microscope slide over a 7- by 7-cm area. Two separate concentrations were applied to each card. Four to seven concentrations plus a control were used for each test; five concentrations were most commonly used. After the cards were prepared in this manner, they were left on a dust-free shelf overnight to permit solvent evaporation.

Tests were done with four strains of *L. trifolii*. Two of these were originally collected from southern California commercial chrysanthemum (Ch-S strain) and celery (Ce-S strain) fields, but they had been kept separately in laboratory culture without insecticide exposure for several years. Two field strains that had been sprayed moderately (Ch-M strain) and heavily (Ch-H strain) with various insecticides (including permethrin and chlorpyrifos) were collected from chrysanthemum greenhouses in southern California.

Flies were obtained by collecting infested plant material from the field or from laboratory colonies and suspending the leaves on wire screening over trays filled with sand (Keil et al. 1985); this provided a pupation site for emerging last instars. After several days, the pupae were sifted from the sand into small vials and held in a sleeve cage provisioned with honey and several potted chrysanthemum plants. Three days after initial adult emergence, the plant material was removed and each yellow sticky card was held in the sleeve cage until 60 to 160 flies had been captured. Approximately equal numbers of flies were caught on each card. After each card was removed from the cage, flies on the card were inspected and any that were upright or not firmly entangled were gently rolled on their sides with a fine brush to prevent escape and to assure fairly uniform contact for each fly.

All of the cards for a given bioassay were then placed on shelves inside a clear plastic box (30 by 16 by 9 cm) that was provisioned with cotton saturated with water to maintain high relative humidity, sealed with a lid, and held at 21°C and continuous light for 24 h. Mortality was then evaluated under a stereomicroscope by gently prodding each fly and looking for any motion, including movement of legs, wings, mouthparts, or genitalia. Flies showing no movement were considered dead. Mortality data were corrected for control mortality (Abbott 1925) and analyzed by the probit analysis computer program of Raymond (1985). This standard procedure was used in all tests with exceptions noted below.

Duration of Exposure. An estimate of how long flies should be exposed to the insecticide on cards before evaluation of mortality was needed. Haynes et al. (1986) arbitrarily evaluated mortality after 24 h. However, we wanted to determine the longest convenient time period after which control mortality would be consistently <10%. An established time period would minimize the amount of insecticide required to produce reliable mortality data.

Periodic observations of mortality were made between 7 and 48 h after capture of young flies from the two field populations (Ch-H and Ch-M), and from the laboratory colony (Ch-S). The field populations were each tested with permethrin (10, 20, 30, 50, and 100 mg [AI]/g sticker), and the laboratory population was tested with permethrin (1, 2, 5, and 10 mg [AI]/g sticker) and chlorpyrifos (0.3, 1, 3, 5, and 10 mg [AI]/g sticker). The optimal duration of exposure was evaluated by plotting the LC_{50} (with associated 95% fiducial limits [FL]), plus the corresponding control mortality, at each interval in each test, against time.

Amount of Sticker. To determine whether the amount of the insecticide–sticker mixture influenced mortality, flies from the Ch-S strain were caught on cards coated with insecticide–sticker mixtures of permethrin (10 mg [AI]/g sticker), chlorpyrifos (5 mg [AI]/g sticker), and an insecticide-free control, each spread evenly over cards at 2.1, 6.3, and 11.5 mg mixture per square centimeter. Two cards were prepared for each insecticide and thickness combination, and mortality was evaluated after 24 h. Within each insecticide treatment, differences in the proportion of flies killed among the three amounts of sticker were evaluated with a multiple comparison test for proportions (Ryan 1960).

Adult Age. Preliminary observations indicate that older flies may be more susceptible to insecticide and likely to die more quickly on the sticky cards with and without a toxicant than younger flies of the same population. If so, bioassays of field populations with varying age distributions could yield inconsistent results with unacceptable control mortalities.

To evaluate *L. trifolii* of various ages for this bioassay technique, adults were allowed to age and then tested. Vials containing Ch-S strain pupae were placed into sleeve cages provisioned with potted chrysanthemums and honey; adults were allowed to emerge for a specified time after which the vials were removed. In this way, adults were produced in age classes of 0–3, 6–7, and 8–11 d. The populations were each tested with sticky cards with permethrin (0.3, 1, 2, 3, 5, and 10 mg [AI]/g sticker) and chlorpyrifos (0.156, 0.312, 0.625, 1.25, 2.5, and 5.0 mg [AI]/g sticker), plus controls.

Sex and Size. In a topical application bioassay, Keil et al. (1985) found that male *L. trifolii* were slightly more susceptible to permethrin than females, and suggested that this disparity may be caused by differences in weight between the sexes

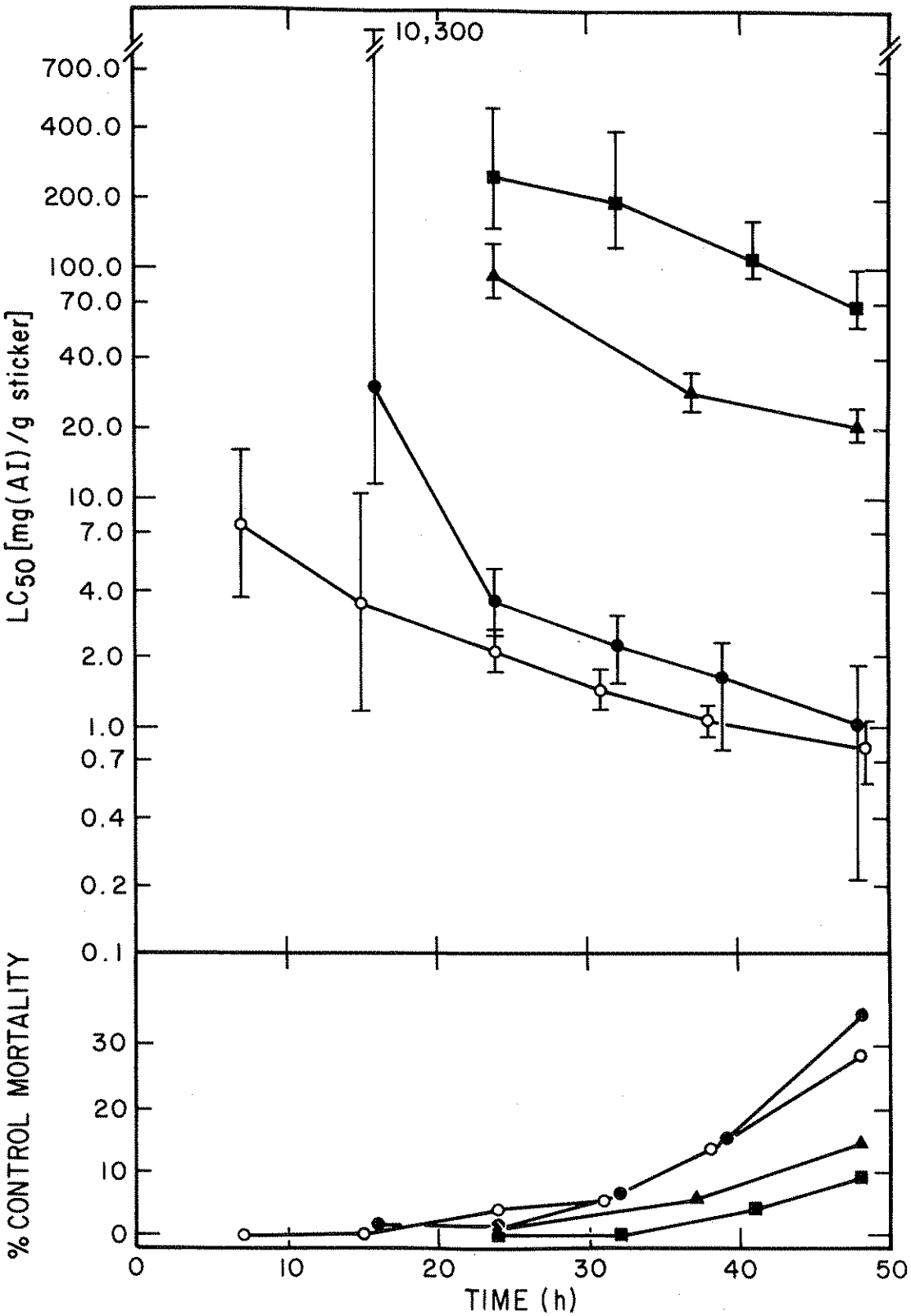

Fig. 1. LC_{50} estimates and corresponding control mortalities through time of three populations of *L. trifolii* adults. Ch-H strain (permethrin) (■); Ch-M strain (permethrin) (▲); Ch-S strain (permethrin) (●), and (chlorpyrifos) (○).

Table 1. Mortality of laboratory (Ch-S, Ce-S) and field (Ch-M, Ch-H) strains of *Liriomyza trifolii* adults in the sticky card bioassay

Chemical	Strain	*n*	Slope ± SE	LC_{50}[a]	95% FL	Resistance ratio[b]
Chlorpyrifos	Ch-S	277	3.49 ± 0.41	2.10	1.75–2.45	1.0
	Ch-H	209	2.47 ± 0.45	24.4	19.6–34.0	11.6
Permethrin	Ch-S	238	1.30 ± 0.28	3.49	2.40–5.20	1.0
	Ce-S	243	1.30 ± 0.28	5.59	3.79–12.0	1.6
	Ch-M	448	1.99 ± 0.25	90.66	73.6–122.4	26.0
	Ch-H	187	1.67 ± 0.35	248.3	187–403	71.1

[a] mg (AI)/g sticker.
[b] LC_{50} of test strain/LC_{50} of Ch-S strain (the most susceptible strain).

(males are usually smaller than females). Therefore, we compared the responses of the sexes as well as that of flies of different sizes. The length of more than 300 Ch-H and Ch-M strain puparia were individually measured with a dissecting microscope fitted with a calibrated ocular micrometer. A frequency distribution of pupal lengths was then calculated for each strain. Based on inspection of these frequency distributions, arbitrary boundaries were established to classify pupae into small (<1.8 mm), intermediate (1.8–1.95 mm), and large (>1.95 mm) length classes. For each strain, lengths of all pupae to be tested were then measured and pupae were sorted into one of these three size classes. A separate bioassay with permethrin was done on adults emerging from the large and small size classes of each strain. The intermediate size class was excluded to ensure no overlap between lengths of individuals of the large and small size classes. Furthermore, immediately after capture on the sticky cards, the length of each fly was measured, and any "small" flies that were ≥1.85 mm or "large" flies that were ≤1.85 mm were discarded. Five concentrations (10, 20, 30, 50, and 100 mg [AI]/g sticker) plus a control were used in each test, and mortality was evaluated after 48 h. The sex of each fly was recorded, and data from each strain were analyzed separately.

Responses of flies of each sex and size were first analyzed to test for independence of size and sex. The total number of living and dead flies was summed (over all dosages tested) for each combination of size and sex. These data were then analyzed in a 2 × 2 × 2-way test of independence (sex × size × mortality response) with a *G* statistic (Sokal & Rohlf 1969). Mortality of each size class (without regard to sex) was then analyzed by probit analysis. Mortality of each sex (without regard to size) was examined in four separate tests: males and females of the Ch-S strain were tested with permethrin (0.3, 1, 2, 3, 5, and 10 mg [AI]/g sticker) and chlorpyrifos (0.312, 0.625, 1.25, 2.5, and 5.0 mg [AI]/g sticker), and the males and females of the Ch-M strain were tested with permethrin (10, 20, 30, 50, and 100 mg [AI]/g sticker), whereas those of the Ch-H strain were tested with chlorpyrifos (5, 10, 20, and 40 mg [AI]/g sticker).

Comparison with Topical Application Bioassay. Data from sticky card and topical application bioassays (Keil et al. 1985) from the same strains were compared. The topical application procedure of Keil et al. (1985) was used with several modifications. Four groups of 15 flies each were tested at concentration (30 males and 30 females). After treatment, the flies were placed into polycarbonate vials (90 ml) fitted with screened lids and provisioned with dental wicks saturated with a 20% honey and water solution. A preliminary study indicated no difference in control mortality when these vials were used rather than the more complicated vial system used by Keil et al. (1985) that contained a chrysanthemum cutting. Flies were held at 21.1°C and continuous light for 24 h, after which mortality was assessed.

For comparison of the two techniques, the Ch-S strain flies were tested with permethrin and chlorpyrifos by topical application and sticky card bioassays. Chlorpyrifos was used on the sticky cards at concentrations of 0.312, 0.625, 1.25, 2.5, and 5.0 mg (AI)/g sticker, and at topical application concentrations of 0.03125, 0.0625, 0.10, 0.125, 0.25, and 0.50 mg (AI)/ml acetone. Permethrin was used on the sticky cards at concentrations of 0.3, 1, 2, 3, 5, 10, and 20 mg (AI)/g sticker, and at topical application concentrations of 0.0313, 0.0625, 0.125, 0.25, 0.50, and 1.00 mg (AI)/ml acetone. Ch-M and Ch-H strain flies were tested by both procedures with either permethrin (sticky card concentrations: 10, 20, 30, 50, and 100 mg [AI]/g sticker; topical application concentrations: 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 mg [AI]/ml acetone) or chlorpyri-

Table 2. Mortality of *L. trifolii* adults (Ch-S strain) captured on sticky cards coated with increasing amounts of insecticide–sticker mixtures

mg sticker/cm²	Treatment					
	Control		Permethrin[a]		Chlorpyrifos[b]	
	n	% Mortality	*n*	% Mortality	*n*	% Mortality
2.1	138	0a	149	71.1a	131	80.1a
6.3	145	2.8a	133	70.7a	140	83.6a
11.5	129	15.5b	137	79.6a	124	83.9a

Percentages in the same column followed by the same letter are not significantly different ($P > 0.05$; Ryan's [1960] multiple comparison test for proportions).
[a] 10 mg (AI)/g sticker.
[b] 5 mg (AI)/g sticker.

Table 3. Control mortalities and mortality responses of *L. trifolii* adults (Ch-S strain) of three age classes

Chemical	Adult age (d)	*n*	% Control mortality	Slope ± SE	LC_{50}[a]	95% FL[a]
Permethrin	8–11	183	44.2	NE[b]	NE	NE
	6–7	195	13.5	2.43 ± 0.43	1.44	0.98–1.83
	0–3	228	6.8	2.76 ± 0.44	5.06	4.21–6.25
Chlorpyrifos	8–11	226	13.0	2.25 ± 0.34	0.52	0.23–1.08
	6–7	146	3.4	3.29 ± 0.50	0.88	0.70–1.08
	0–3	245	2.0	2.93 ± 0.32	0.96	0.80–1.13

[a] mg (AI)/g sticker.
[b] NE, not estimated because of excessive control mortality.

fos (sticky card concentrations: 1.25, 2.5, 5, 10, 20, and 40 mg [AI]/g sticker; topical application doses: 0.5, 1, 2, 3, 4, and 6 mg [AI]/ml acetone), respectively. Results were compared by contrasting the LC_{50} and LC_{90} resistance ratios [e.g., LC_{50} (suspected resistant strains Ch-M, Ch-H)/LC_{50} (suspected susceptible strain Ch-S)], the estimates of the slope and associated standard error of the concentration/response lines, and the 95% FL of each chemical with each procedure.

Results

Standard Procedure. As demonstrated by Haynes et al. (1986), the sticky card bioassay can be used to detect resistance in *L. trifolii* (Table 1). The ratio of the LC_{50} of the Ch-H strain to that of the Ch-S strain with permethrin and chlorpyrifos (71.1 and 11.6, respectively) demonstrates the degree of resistance that this species can attain. Resistance ratios of the strains are consistent with their expected levels of resistance, given histories of insecticide use, and incidences of control failures in their respective environments. This relatively simple bioassay appears to give acceptable results over a range of resistance levels.

Duration of Exposure. Control mortalities increased and LC_{50}'s decreased with longer exposure times (Fig. 1). The rate of increase in control mortality through time was variable and, in two of the four tests, became unacceptable (>10%) as early as the interval between 31 and 38 h after capture. In all but one test, the control mortality was unacceptable by 48 h. We evaluated mortality after 24 h because control mortalities were consistently low and the 95% FL for the LC_{50} estimate was narrow in most cases. In addition, this was a convenient time to record mortality. Although control mortalities also were consistently low 31 h after capture, the additional exposure time did not result in substantially more reliable LC_{50} values (based on width of 95% FL) or significantly lower LC_{50}'s. The additional exposure time also would not appreciably reduce the amount of insecticide needed for reliable mortality data.

Amount of Sticker. Mortality increased significantly from 0% at 2.1 mg sticker per cm^2 to 15.5% at 11.5 mg sticker per cm^2 in the insecticide-free control, whereas no significant differences in mortality occurred among the various amounts of sticker with either insecticide (Table 2). Riedl et al. (1985) found that a thick adhesive layer (approximately 0.83 mm) shortened the lifespan of male codling moths, *Cydia pomonella* (L.), captured on pheromone traps compared with those captured on a thin layer (approximately 0.19 mm). Haynes et al. (1986) noted minimal control mortality in *L. trifolii* with a sticker thickness of 4 mg sticker per cm^2. Although no control mortality occurred at 2.1 mg sticker per cm^2 in our test, capture of larger

Table 4. Mortality of adult *L. trifolii* of each sex separately and combined, from three strains

Chemical	Strain	Sex	*n*	Slope ± SE	LC_{50}[a]	95% FL[a]	Resistance ratio[b]
Permethrin	Ch-S	♂	163	1.71 ± 0.28	2.20	1.50–3.05	
		♀	220	1.29 ± 0.32	4.47	2.24–8.90	2.03
		Combined	383	1.42 ± 0.28	3.26	1.87–5.61	
	Ch-M	♂	228	2.68 ± 0.44	66.0	54.4–87.2	
		♀	218	1.47 ± 0.31	139.9	90.8–349.3	2.12
		Combined	446	1.99 ± 0.25	90.7	73.6–122.4	
Chlorpyrifos	Ch-S	♂	199	2.22 ± 0.31	1.73	1.37–2.17	
		♀	196	2.35 ± 0.33	2.58	2.08–3.38	1.45
		Combined	395	2.27 ± 0.22	2.10	1.81–2.48	
	Ch-H	♂	131	4.72 ± 0.76	8.56	7.30–10.03	
		♀	172	4.17 ± 0.53	18.69	16.20–21.5	2.18
		Combined	303	3.34 ± 0.31	13.35	11.79–15.08	

[a] mg (AI)/g sticker.
[b] LC_{50} (females)/LC_{50} (males).

Table 5. Mortality of *L. trifolii* adults of two size classes (lengths), within each of two strains, to permethrin

Strain	Length (mm)	*n*	Slope ± SE	LC_{50}[a]	95% FL[a]	Resistance ratio[b]
Ch-H	<1.85	312	2.28 ± 0.37	54.63	44.61–69.95	2.33
	>1.85	179	1.09 ± 0.34	127.06	70.30–1,024.4	
Ch-M	<1.85	186	2.86 ± 0.51	19.39	14.30–24.06	1.56
	>1.85	177	3.25 ± 0.59	30.29	23.02–37.12	

[a] mg (AI)/g sticker.
[b] (LC_{50} of flies >1.85 mm)/(LC_{50} of flies <1.85 mm).

flies was often not adequate on the cards. Because the amount of sticker can influence mortality in some cases (Table 2), the sticker–toxicant mixture should be applied to the cards in a uniformly thin layer at amounts between 4 and 6 mg/cm². This will prevent most captured flies from escaping, yet cause minimal mortality.

Adult Age. Control mortalities of 8- to 11-d-old flies in tests with chlorpyrifos and permethrin were unacceptably high, as was that of 6- to 7-d-old flies in the permethrin test (Table 3). Only 0- to 3-d-old flies had control mortalities consistently <10%. Therefore, fly age should be standardized at 0 to 3 d for use with this technique. Keil et al. (1985) also mentioned that standardizing *L. trifolii* adult age at 0 to 3 d significantly reduced variability and control mortality in their topical application procedure.

Probit analyses of the data indicated that susceptibility to permethrin and chlorpyrifos increased with adult age (Table 3). These data show that flies cannot be collected directly in the field for use in this test because their age would not be standardized.

Sex and Size. Statistical analysis revealed that the mortality responses of flies were a joint function of both size and sex ($G = 122.1$; df = 2; $P < 0.01$). The proportion of large males (length > 1.85 mm) that died was similar to that of small males (length < 1.85 mm) (65.3 and 64.5%, respectively), but fewer large females (length > 1.85 mm) died than did small females (length < 1.85 mm) (51.8 and 60.7%, respectively). Although this interaction of sex and size is biologically interesting, the magnitude of the interaction is probably not sufficient to affect the repeatability of this bioassay when it is used in the field. Furthermore, the sex ratio of flies emerging from field-collected foliage is nearly always about 1:1 (Zehnder & Trumble 1984, Parrella 1987), and fly size is fairly consistent from a given host plant species provided that intraspecific larval competition is minimal (Parrella 1983). Therefore, the repeatability of bioassays with wild flies should not be greatly affected by the interaction of sex and size from trial to trial.

Given that the sex-size interaction was not biologically strong, mortality for the two sizes and for the two sexes were analyzed separately by probit analysis. The LC_{50}'s of males were consistently less than those of females in all tests (Table 4) with resistance ratios [LC_{50} (female)/LC_{50} (male)] ranging from 1.45 to 2.18. The 95% FL of the LC_{50} estimates of each sex did not overlap in the chlorpyrifos bioassay of the Ch-H strain and the permethrin bioassay of the Ch-M strain, suggesting that males are more susceptible than females.

Keil et al. (1985) concluded that the response of males was more variable than that of females based on the standard error of the slope of the dose–mortality regression for each sex. In our studies, however, the standard error of the slope for males tended to be less than that for females in half the tests (Table 4). Moreover, combined male and female mortality data produced the lowest standard error of the slope in each test, probably as the result of increased sample size. Because of these results, the relatively minor difference in susceptibility between males and females, and the inconvenience of determining the sex of each fly, data should be taken without regard to sex for use in this bioassay.

The ratio of the LC_{50} of large flies (length > 1.85 mm) to that of small flies (length < 1.85 mm)

Table 6. Comparison of mortality to permethrin of two strains of *L. trifolii* adults tested with sticky cards or topical application

Assay method	Strain	*n*	Slope ± SE	LC_{50}[a]	95% FL[a]	LC_{90}[a]	95% FL[a]	Resistance ratios	
								LC_{50}[b]	LC_{90}[c]
Sticky cards	Ch-S	741	2.28 ± 0.15	4.10	3.65–4.61	14.96	12.35–18.99	22.1	26.7
	Ch-M	446	1.99 ± 0.25	90.66	73.6–122.4	400	252–848		
Topical application	Ch-S	474	1.98 ± 0.16	0.10	0.08–0.12	0.45	0.35–0.61	36.6	34.5
	Ch-M	344	2.07 ± 0.24	3.70	3.04–4.66	15.44	10.68–27.12		

[a] mg (AI)/g sticker.
[b] LC_{50} (Ch-M strain)/LC_{50} (Ch-S strain).
[c] LC_{90} (Ch-M strain)/LC_{90} (Ch-S strain).

Table 7. Comparison of mortality to chlorpyrifos of two strains of *L. trifolii* adults tested with sticky cards or topical application

Assay method	Strain	n	Slope ± SE	LC_{50}[a]	95% FL[a]	LC_{90}[a]	95% FL[a]	Resistance ratios	
								LC_{50}[b]	LC_{90}[c]
Sticky cards	Ch-S	395	2.27 ± 0.22	2.10	1.81–2.48	7.72	5.87–11.35	6.4	4.2
	Ch-H	303	3.34 ± 0.31	13.35	11.79–15.08	32.27	27.19–40.54		
Topical application	Ch-S	364	2.58 ± 0.27	0.29	0.24–0.35	0.90	0.67–1.40	6.4	3.9
	Ch-H	301	4.58 ± 0.46	1.86	1.65–2.05	3.34	3.14–4.13		

[a] mg (AI)/g sticker.
[b] LC_{50} (Ch-H strain)/LC_{50} (Ch-S strain).
[c] LC_{90} (Ch-H strain)/LC_{90} (Ch-S strain).

indicated that large flies were 1.56 and 2.33 times more resistant to permethrin than small flies for the Ch-M and Ch-H strain, respectively (Table 5). This differential response may in part be due to differences in body weight or the smaller surface to volume ratio of the larger flies, as well as the interaction between size and sex. To minimize the effect of size, very small pupae could be sifted from a sample of pupae before adult emergence and thus eliminated from the bioassay.

Comparison with Topical Application Bioassay. Both procedures produced estimates of the standard error of the slope of the concentration–response regressions that were reasonably small, and 95% FL that were reasonably narrow (Tables 6 and 7). With permethrin, the slopes were similar within and between strains (Table 6). Resistance ratios at LC_{50} and LC_{90} were fairly consistent within each procedure, reflecting the parallel concentration–response regressions. However, the topical application procedure produced somewhat greater resistance ratios than did the sticky card procedure (Table 6).

With chlorpyrifos, resistance ratios at LC_{50} and LC_{90} differed within each procedure but were similar between procedures (Table 7), indicating consistent results. Both procedures produced similar slope estimates of the concentration–response regression of the Ch-S strain. Slope estimates for the Ch-H strain differed, although both procedures produced higher slope estimates for the Ch-H strain than for the Ch-S strain (Table 7).

In general, the two procedures produced similar slope estimates and associated standard errors, fiducial limit widths, and resistance ratios. The two procedures therefore produce fairly comparable results, although the sticky card procedure requires less sophisticated equipment and is easier to use.

Discussion

The sticky card bioassay is a quick, reliable, and accurate method for assessing insecticide resistance in *L. trifolii*. This technique can be used at the individual farm level (with the assistance of competent pest control applicators, farm advisors, or county agents) to determine insecticide resistance levels. All that is required is the collection of infested leaf material, a simple glass-topped sleeve cage to allow emergence of larvae and subsequent adults, and a fairly air-tight box (e.g., a plastic shoe box) provisioned with water-saturated cotton (to maintain high relative humidity) to hold the sticky cards after the flies have been captured. Obtaining sufficient numbers of flies per card per concentration is relatively easy in such a confined area, and the assessment of mortality after 24 h can be done with an ordinary stereomicroscope. Accurate mortality determination is critical and the person who records mortality data must be trained to take sufficient time to look carefully for any movement of each fly. Sticky cards treated with insecticide must be prepared at a research laboratory and then shipped to the test site. Preferably, leaf material might be shipped to the research laboratory where the bioassay can be done.

Estimation of concentration–mortality lines for *L. trifolii* populations requires that a minimum of four concentrations (including a control) be used (Robertson et al. 1984) and this may not be possible, based on the time of the operator and the number of flies in the sample (at least 240 adult flies are needed for a reliable bioassay [Robertson et al. 1984], excluding flies needed for control mortality estimation). In this situation, perhaps only one concentration (a diagnostic concentration) could be used (e.g., the LC_{90} or LC_{98} of the susceptible colony; Ch-S), which would provide some indication of the resistance level of the test population (Roush & Miller 1986).

The usefulness of the sticky card bioassay technique has not been tested in the field. Of critical importance is the relationship between LC_{50} and LC_{90} values obtained from the cards and mortality rates using field recommended rates of the pesticide for adults and larvae of *L. trifolii*. Preliminary results suggest that applications of field rates of permethrin or chlorpyrifos to adults of the Ch-S colony provides approximately 100% control, whereas little control (approximately 30%) is obtained when the Ch-H colony is tested with the same rates of these materials (J.P.S., unpublished data). In addition to evaluation of the relationship between adult and larval resistance levels, more research is needed in this area.

Not all insecticides can be used with this technique; insect growth regulators, microbial materials, and other compounds that must be ingested may not be suitable. Furthermore, the insecticide must be miscible in the sticker and not react with the insect adhesive. Those materials that rapidly decompose (e.g., by photooxidation) cannot be used with this technique. Nonetheless, the sticky card bioassay has the potential to assess resistance levels to many insecticides of numerous insects commonly caught on these traps. These include aphids, fungus gnats, whiteflies, and thrips. Given the glasshouse environment and the tremendous propensity for the development of resistance in these insects (Parrella & Jones 1987), sticky cards may be valuable for monitoring changes in population density as well as insecticide susceptibility.

Acknowledgment

We thank J. Bethke and W. Carson for technical assistance, and R. T. Roush for reviewing a previous draft of the manuscript. The research was supported in part by the California Department of Food and Agriculture, the Western Regional Pesticide Impact Assessment Program, and the American Floral Endowment.

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Received for publication 24 July 1984; accepted 12 September 1988.