Decline of Resistance in *Liriomyza trifolii* (Diptera: Agromyzidae) in the Absence of Insecticide Selection Pressure

MICHAEL P. PARRELLA AND JOHN T. TRUMBLE

Department of Entomology, University of California, Riverside, California 92521

J. Econ. Entomol. 82(2): 365–368 (1989)

ABSTRACT The decline of resistance to permethrin and chlorpyrifos in a population of *Liriomyza trifolii* (Burgess) in the absence of selection from these materials was determined. When the test began, LC_{50}'s (mg [AI]/g sticker) for permethrin and chlorpyrifos were 248.3 and 24.4, respectively. A susceptible population established at the same time had LC_{50}'s of 3.5 and 2.1 to permethrin and chlorpyrifos, respectively. After 15 generations (10 mo), LC_{50}'s for the decline population were 51.0 (permethrin) and 7.0 (chlorpyrifos). At rates recommended for field use, applications of permethrin to the decline and susceptible populations demonstrated that adults of the decline strain were still significantly more tolerant than adults in the susceptible population. No differences were found between populations with chlorpyrifos; however, this was not due to a decline in resistance, because mortality was very low for both populations when the test began. These results suggest that where *L. trifolii* has developed resistance to permethrin or chlorpyrifos, removal of one or both insecticides from a spray program for a period of 10 mo is insufficient to cause resistance reversion in this species.

KEY WORDS Insecta, leafminer, chrysanthemum, celery

DEVELOPMENT OF INSECTICIDE RESISTANCE by the leafminer, *Liriomyza trifolii* (Burgess), has been well documented (Keil et al. 1985, Broadbent et al. 1986, Smith 1986). This resistance problem is so serious that integrated pest management programs developed for control of *L. trifolii* have incorporated tactics to compensate for this resistance phenomenon (Trumble 1985, Parrella & Jones 1987).

Many operational (Georghiou & Taylor 1977, Georghiou 1983) and agronomic–control (Leeper et al. 1986) factors have been identified that, when manipulated at the grower level, act to reduce the development of insecticide resistance. Most of these are very general and theoretically apply to any arthropod under selection pressure from pesticides. In reality, each arthropod pest has its own set of factors that, when manipulated, could reduce the development of resistance. Few examples of successful manipulation of operational factors to manage insecticide resistance are available from agriculture; two such programs include management of the cattle tick in Australia (change in application methods) and planthoppers attacking rice in Japan (change tank mixes) (Nolan & Roulstan 1979, Saito & Miyata 1982).

One of the easiest operational factors for growers to put into practice is to use insecticides with different modes of action in rotation. Although this appears simple, real challenges exist in determining the optimal sequence of use and the point at which a change should be made. Of course, materials must be reciprocally unaffected by cross resistance, and no resistance to either material must be present at the start of the rotation. The concept of rotation of chemicals as an antiresistance measure assumes that individuals resistant to one chemical have substantially lower biotic fitness than susceptible individuals, so that the frequency of these individuals declines in the population during the interval between sprays of the same insecticide (Georghiou 1983). During 1986–1987, we examined the decline of resistance to permethrin and chlorpyrifos in resistant populations of *L. trifolii* in the absence of selection pressure.

Materials and Methods

Leafminer Populations. Two populations of *L. trifolii* were maintained in separate glasshouses (2.7 by 3.7 m); these were labeled as decline and susceptible populations. The decline population was started from larvae collected from a chrysanthemum grower in Orange County, Calif. These flies were under intensive insecticide pressure with one or two pesticide applications made every week throughout the year. Many pesticides were used at this location; permethrin and chlorpyrifos were commonly applied.

The susceptible population was started with larvae from a laboratory colony that has been maintained on celery at University of California at Riverside for the last five years. During this time, the colony has not been exposed to pesticides. Although

the colony was originally collected from untreated celery in Orange County, Calif., the leafminer population in the region had been treated with a variety of pesticides. Therefore, these flies were not truly susceptible, but they served as the susceptible population for this study. Probably, no truly susceptible population of *L. trifolii* exists (Keil et al. 1985).

Both populations of *L. trifolii* were started with at least 2,000 individuals. Although some celery (*Apium graveolens* L. cv. Tall Utah 52-70R) was used as host plants (one plant per week), chrysanthemums (*Dendranthema grandiflora* Tzvelev cv. Florida Marble) were used as the primary host plant. Ten chrysanthemum plants (each 30 d old) reared in a separate glasshouse were exposed to free-flying adults in the decline and susceptible glasshouses for 24 h. Plants were then covered with cheesecloth to prevent excessive oviposition and subsequent larval intraspecific competition (Parrella 1983). After 5 to 7 d, plants were placed horizontally on specially designed tip racks so that emergent larvae would fall into sand-filled trays at the base of the rack. The tip rack was located in the same glasshouse and also was covered with cheesecloth.

After larvae emerged and pupated, the sand was sifted and the pupae collected. In this way, daily production of *L. trifolii* could be monitored; a strong relationship between pupal weight and number of pupae greatly facilitated the estimation of pupal production (Parrella et al. 1989). Commonly, approximately 100 pupae per plant were produced per day. With 10 plants per day per glasshouse, about 1,000 *L. trifolii* were produced each day. Environmental conditions in the glasshouses fluctuated, with temperatures ranging between 21 and 28°C and relative humidity between 40 and 70%. Populations in all glasshouses were started in July 1986, and the study was done through March 1987.

Resistance Bioassay. The sticky card bioassay technique described by Haynes et al. (1986) and Sanderson et al. (in press) was used to assess the response of the decline and susceptible populations of *L. trifolii* to permethrin and chlorpyrifos. Initially, a range of doses was tested to determine those which provided between 20 and 80% mortality for both populations. Pupae collected from the susceptible and decline populations were isolated in small vials and allowed to emerge within small glass-topped sleeve cages. Each population was kept separate; honey and a chrysanthemum plant were supplied to the flies in each cage. After 3 d of emergence, cards containing the different doses of insecticides were exposed in these cages until approximately 50 adults were caught at each dose. All of the flies used in the bioassay were 3 d old or younger. Sex ratio was not determined; however, this rearing method produces a sex ratio of about 50:50 (Parrella 1987). Cards were held in plastic boxes specially designed to maintain high relative humidity at a constant 21°C, and mortality was checked after 24 h. After the colonies were started in July 1986, both populations were subjected to this bioassay procedure on a monthly basis. Data were analyzed by probit analysis (Raymond 1985). The criterion for significant differences at the LD_{50} was failure of 95% fiducial limits to overlap.

Efficacy Trials. After 15 generations (10 mo), field-recommended rates of permethrin (0.24 g [AI]/liter) and chlorpyrifos (0.60 g [AI]/liter) were applied to adults and larvae of the two populations. For adult trials, 100 adults <3 d old (five replicates of 20 per replicate) were lightly anesthetized with CO_2 and exposed to a spray of each material at field-recommended rates in a Kearns–March knockdown chamber (Kearns & March 1943) for 5 s. Controls were treated only with water. After exposure, flies were placed in small vials supplied with a chrysanthemum cutting and honey. Mortality was recorded after 24 h. Analysis of variance (ANOVA) or Duncan's multiple range test (SAS Institute 1982) was used to compare survivorship in each treatment to the control ($P = 0.05$). Percentage data were transformed to arcsine $\sqrt{x}$ before analysis.

Evaluations of permethrin and chlorpyrifos against later stage larvae were conducted as described by Parrella et al. (1982). Eighty standardized chrysanthemum plants were exposed to free-flying adults in both of the glasshouses for 2 h. Plants were then removed and placed on benches in an adjacent glasshouse. After 3 d, newly eclosed larvae on each plant were counted, and 60 plants were chosen with approximately equal numbers of larvae. When larvae were in the late second and early third stage, they were sprayed to runoff with the field-recommended rates of chlorpyrifos or permethrin (10 plants per material per population). Each plant was then tipped over a sand-filled tray to collect pupae that were held for adult emergence. Survivorship to the pupal and adult stages for both treatments was compared with a *t* test ($P = 0.05$) (SAS Institute 1982).

Results

The reduction in resistance to permethrin appeared to occur in two phases (Table 1). After 2 mo (three generations), resistance at LD_{50} had declined by approximately 50% (248.3 to 109.9). During the next 2 mo, LD_{50}'s increased, then resistance declined by another 50% (109.9 to 79.8). Thereafter, resistance was stable with no further decline in LD_{50} over the next 4 mo (7 to 8 generations). The slight increase observed during months 4 and 5 remains unexplained, but it may reflect natural variation.

The decline in resistance to chlorpyrifos at LD_{50} followed a similar pattern, with approximately 50% decline in 2 mo (24.4 to 12.9). Thereafter, resistance levels followed no specific trend, but a reduction of the LD_{50} to 7.0 was noted at the end of

Table 1. Response of the decline population of *L. trifolii* over a 10-mo period

Month/year	Permethrin				Chlorpyrifos			
	n	Slope ± SE	LC_{50}[a]	95% FL	*n*	Slope ± SE	LC_{50}[a]	95% FL
7/86	187	1.67 ± 0.35	248.3	187.0–403.0	209	2.47 ± 0.45	24.4	19.6–34.0
8/86	240	1.79 ± 0.34	288.4	223.0–451.0	237	3.16 ± 0.34	17.8	15.4–20.6
9/86	317	1.91 ± 0.26	109.9	89.6–131.0	308	2.80 ± 0.28	12.9	11.3–14.8
10/86	365	2.04 ± 0.37	164.5	103.6–168.8	364	3.39 ± 0.31	9.7	8.6–11.0
11/86	277	2.48 ± 0.33	269.6	132.2–207.7	279	3.60 ± 0.35	13.9	12.3–25.7
12/86	184	1.91 ± 0.14	79.8	79.4–80.1	195	2.93 ± 0.29	12.1	12.1–12.2
1/87	256	1.00 ± 0.44	68.0	67.5–68.5	243	2.18 ± 0.36	19.2	19.0–19.3
2/87	305	1.73 ± 0.60	36.0	35.1–36.9	277	3.26 ± 0.52	7.6	7.5–7.6
3/87	288	2.11 ± 0.51	48.1	47.4–48.8	204	3.40 ± 0.47	10.2	10.2–10.3
4/87	246	1.41 ± 0.28	51.0	50.4–51.7	219	1.98 ± 0.08	6.0	6.9–7.0

[a] mg [AI]/g sticker; 30–50 flies per dose, six doses/insecticide.

the trial (after an additional 7 mo). During month 7 of this study, a distinct peak occurred (LD_{50} = 19.2 with 95% fiducial limits not overlapping those for any other LD_{50}); this remains unexplained.

When applied to adults from the susceptible population at rates recommended in the field, permethrin and chlorpyrifos were not very effective, with only 56 and 17% mortality, respectively (Table 2). However, the decline strain was still significantly ($P = 0.05$) more difficult to kill with permethrin than the susceptible strain, even after 10 mo with a lack of selection pressure from this material. No significant difference was noted when mortality from the chlorpyrifos treatments was compared with the susceptible and decline populations. This result was expected because chlorpyrifos is recommended to control *L. trifolii* larvae, not adults. However, this material also was ineffective against larvae from the susceptible and decline populations, providing 17 and 5% mortality, respectively. No significant difference was observed between the chlorpyrifos and the control treatment.

Applications of permethrin to larvae of *L. trifolii* were ineffective in the susceptible and decline strains (Table 3). Resistance to permethrin by *L. trifolii* larvae was documented by Parrella (1983) and was thought to be widespread in California at that time. Applications of chlorpyrifos were more effective than permethrin, but no significant difference was detected when susceptible and decline strains were compared.

Table 2. Efficacy of field-recommended rates of permethrin[a] and chlorpyrifos[b] applied to adult *L. trifolii*

Population	$\bar{x}$ Percentage mortality		
	Permethrin	Chlorpyrifos	Control
Susceptible	56.0Aa	17.0Ab	0.0Ac
Decline	20.0Ba	5.0Ab	1.0Ab

Means within columns followed by the same letter (uppercase) are not significantly different ($P = 0.05$; ANOVA). Means within rows followed by the same letter (lowercase) are not significantly different ($P = 0.05$; Duncan's multiple range test [SAS Institute 1982]). Data transformed to arcsine$\sqrt{\bar{x}}$ before analysis.

[a] 0.24 g [AI]/liter. $n = 5$ replicates with 20 flies per replicate.

[b] 0.60 g [AI]/liter. $n = 5$ replicates with 20 flies per replicate.

Discussion

As mentioned earlier, the concept of using insecticide rotation to prevent the development of insecticide resistance is applicable only with an insect that has not developed resistance to either material. With *L. trifolii*, which has a long history of exposure to pesticides (Parrella & Keil 1985), this strategy may not be successful. An improvement of reduced fitness through coadaptation in resistant individuals has been observed when an insect is placed under continual pesticide selection (see Georghiou [1983] and references therein). In some cases, studies with other insects have shown that decline of resistance in the absence of selection pressure occurs too slowly to be of much practical value (see Leeper et al. [1985] and references therein). This is clearly the situation for *L. trifolii* resistance to permethrin and chlorpyrifos.

Many chrysanthemum growers have abandoned the use of permethrin and chlorpyrifos, because of their ineffectiveness. Avermectin b_1 (Abamectin)

Table 3. Efficacy of the field-recommended rate of permethrin[a] and chlorpyrifos[b] applied to larvae of *L. trifolii* in chrysanthemum

Population[c]	$\bar{x}$ Percentage survivorship to pupation[d]		$\bar{x}$ Percentage survivorship to adults[d]	
	Permethrin	Chlorpyrifos	Permethrin	Chlorpyrifos
Susceptible	0.94a	0.57a	0.46a	0.12a
Decline	0.89a	0.61a	0.65a	0.30a

[a] 0.24 g [AI]/liter.

[b] 0.60 g [AI]/liter.

[c] LC_{50} (mg [AI]/g sticker) estimated with yellow card bioassay in April 1988 for susceptible, 4.37 (permethrin), and 1.58 (chlorpyrifos). Decline data presented in Table 1.

[d] Ten plants per population with a $\bar{x}$ ± SEM of 20.8 ± 1.2 and 19.2 ± 0.4 (permethrin) and 19.2 ± 0.7 and 19.8 ± 1.2 (chlorpyrifos) larvae per plant in the susceptible and decline populations, respectively. Mean survivorship calculated per plant. Means in the same columns followed by the same letter are not significantly different ($P = 0.05$; t test [SAS Institute 1982]).

has a unique mode of action and is the primary material now used. Conceivably, growers could wait for long periods before using permethrin or chlorpyrifos again; our study suggests that they should wait longer than 15 generations. Many growers now rely exclusively on Abamectin for their leafminer control without rotation of insecticides. In addition, most use the highest recommended label rate. This kind of resistance management, the high dose strategy, renders resistant individuals functionally recessive by applying dosages sufficiently high to be lethal to susceptible and heterozygote individuals. When the heterozygotes are killed, the R genes are eliminated and resistance does not evolve provided that homozygote-resistant genotypes are not present in the population (Georghiou 1983). Such an effect is usual with untreated populations, but may not be the situation with *L. trifolii*, which has been under intensive selection pressure from many different compounds. Thus, this grower-adopted strategy may be doomed to eventual failure.

We examined the decline in resistance in the absence of potential migration of susceptible individuals; such migration would enhance the rate of decline. However, truly susceptible *L. trifolii* may be rare (Keil et al. 1985). Because field populations do not often reach the extreme levels of resistance found in the glasshouse environment (Haynes et al. 1986), decline in resistance required for field rates of pesticides to regain effectiveness would be proportionately less than for the glasshouse. However, this variable factor, as well as potential effects of the introduction of *L. trifolii* resistant celery varieties (Trumble & Quiros 1988) that suppress leafminer density, will require further research before conclusions on resistance trends in field populations can be drawn.

Acknowledgment

We thank John Sanderson, Ezatulah Yosafi, and James Bethke for technical assistance. This research was supported, in part, by the California Department of Food and Agriculture, the Western Regional Pesticide Impact Assessment Program, the Academic Senate at University of California–Riverside, the American Floral Endowment, and the California Celery Research Advisory Board. Karen Robb critically reviewed an earlier draft of this manuscript.

References Cited

Broadbent, A. B., M. Chita & B. D. McGarvey. 1986. Aldicarb on greenhouse chrysanthemums in Ontario: efficacy for control of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and residues in foliage. Can. Entomol. 118: 789–795.

Georghiou, G. P. 1983. Management of resistance in arthropods, pp. 769–792. *In* G P. Georghiou & T. Saito [eds.], Pest resistance to pesticides. Plenum, New York.

Georghiou, G. P. & E. P. Taylor. 1977. Operational influences in the evolution of insecticide resistance. J. Econ. Entomol. 70: 653–658.

Haynes, K. F., M. P. Parrella, J. T. Trumble & T. A. Miller. 1986. Monitoring insecticide resistance with yellow sticky cards. Calif. Agric. 40(11–12): 11–12.

Kearns, C. W. & R. B. March. 1943. Small chamber method for testing effectiveness of insecticides against houseflies. Soap Sanit. Chem. Feb.: 101–104.

Keil, C. B., M. P. Parrella & J. G. Morse. 1985. Method for monitoring and establishing baseline data for resistance to permethrin by *Liriomyza trifolii* (Burgess). J. Econ. Entomol. 78: 419–422.

Leeper, J. R., R. T. Roush & H. T. Reynolds. 1986. Preventing or managing resistance in arthropods, pp. 335–346. *In* E. H. Glass [chrmn.], Pesticide resistance: strategies and tactics for management. National Academy Sciences, Washington, D.C.

Nolan, J. & J. Roulstan. 1979. Acaricide resistance as a factor in the management of Acari of medical and veterinary importance, pp. 3–13. *In* D. Rodriguez [ed.], Recent advances in acarology. Academic, New York.

Parrella, M. P. 1983. Intraspecific competition among larvae of *Liriomyza trifolii* (Diptera: Agromyzidae): effects on colony production. Environ. Entomol. 12: 1412–1414.

Parrella, M. P. & V. P. Jones. 1987. Development of pest management strategies in floriculture. Bull. Entomol. Soc. Am. 33: 28–34.

Parrella, M. P. & C. B. Keil. 1985. Insect pest management: the lesson of *Liriomyza trifolii*. Bull. Entomol. Soc. Am. 30: 22–25.

Parrella, M. P., K. L. Robb & P. Morishita. 1982. Response of *Liriomyza trifolii* larvae to various insecticides with notes about efficacy testing. J. Econ. Entomol. 75: 1104–1108.

Parrella, M. P., J. T. Yost, K. M. Heinz & G. W. Ferrentino. 1989. Mass rearing of *Diglyphus begini* (Hymenoptera: Eulophidae) for biological control of *Liriomyza trifolii* (Diptera: Agromyzidae). J. Econ. Entomol. 82(2): 420–425.

Raymond, M. 1985. Presentation d'un programme Basic d'analyse log-probit pour micro-ordinateur. Cah. ORSTOM, Ser. Entomol. Med. Parasitol. 23: 117–121.

Saito, T. & T. Miyata. 1982. Studies on insecticide resistance in *Nephotettix cincticeps*, pp. 377–382. *In* K. L. Heong et al. [eds.], Proceedings, international conference plant protection in the tropics. Malayasian Plant Protection Society, Kuala Lumpur.

Sanderson, J. P., M. P. Parrella & J. T. Trumble. In press. Monitoring insecticide resistance in *Liriomyza trifolii* with yellow sticky cards. J. Econ. Entomol.

SAS Institute. 1982. SAS user's guide: statistics. SAS Institute, Cary, N.C.

Smith, R. F. 1986. Efficacy of selected insecticides against *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and residues in foliage. Can. Entomol. 118: 761–766.

Trumble, J. T. 1985. Integrated pest management of *Liriomyza trifolii* influence of avermectin, cyromazine, and methomyl on leafminer ecology in celery. Agric. Ecosys. Environ. 12: 181–188.

Trumble, J. T. & C. F. Quiros. 1988. Antixenotic and antibiotic resistance in *Apium* species to *Liriomyza trifolii* (Diptera: Agromyzidae). J. Econ. Entomol. 81(2): 602–607.

Received 23 December 1987; accepted 20 October 1988.