

# Intraspecific Variation in Levels of Pesticide Resistance in Field Populations of a Parasitoid, *Aphytis melinus* (Hymenoptera: Aphelinidae): The Role of Past Selection Pressures

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**ABSTRACT** Thirteen populations of *Aphytis melinus* DeBach, a key biological control agent of California red scale, *Aonidiella aurantii* (Maskell), were collected from the citrus-growing regions of California. Each population's history of exposure to insecticides was estimated by determining history of insecticide use at both local (in-grove) and regional (county-wide) geographical scales. Concentration/mortality regressions for five insecticides widely used in citrus were estimated for the populations. For each chemical, substantial variability existed in the responses of different populations. LC<sub>50</sub>'s were correlated with both in-grove and county-wide pesticide use histories; patterns of variability were best explained by results of a multiple regression analysis that combined the influences of these two histories. Roles of food limitation, migration, and host distribution in determining patterns and rates of evolution of pesticide resistance in arthropod biological control agents are discussed.

**KEY WORDS** *Aonidiella aurantii*, *Aphytis melinus*, biological control, citrus, integrated pest management, intraspecific variation, pesticide resistance

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PRACTITIONERS OF integrated pest management (IPM) in agricultural cropping systems may employ a diverse array of tactics to control noxious organisms. Many of these tactics may be grouped under the categories of biological control, chemical control, cultural control, and plant resistance. Most of these approaches are compatible; thus, biological control, cultural control, and the use of plant resistance may, with certain noteworthy exceptions (e.g., Campbell & Duffey 1979, Herzog & Funderburk 1985), be combined to good effect, as may chemical control, cultural control, and plant resistance. This mutual compatibility does not, however, often extend to biological and chemical control.

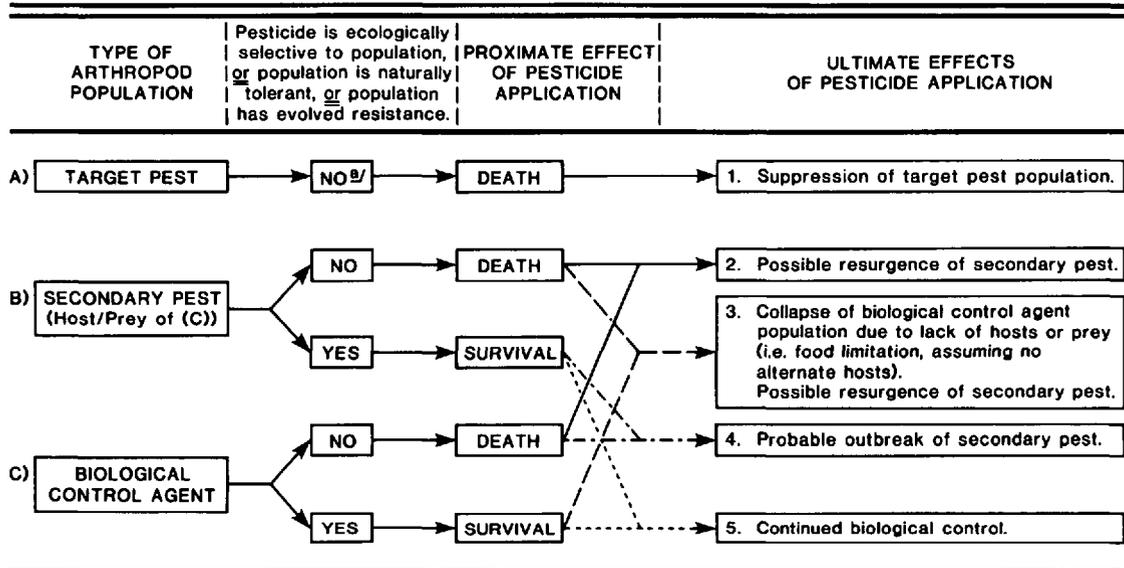
The application of broad-spectrum insecticides frequently disrupts the action of arthropod biological control agents, with resulting pest resurgences and secondary pest outbreaks (Bartlett 1964, DeBach 1974) (Fig. 1). This disruption may be due either to a direct toxic effect on predators and parasitoids, whose susceptibility to insecticides is often greater than that of the associated pest species (Croft & Brown 1975), or to an indirect effect via starvation, emigration, or lack of hosts after the reduction of the pest population by the pesticide (Huffaker 1971, Newsom 1974, Powell et al. 1985) (Fig. 1). This incompatibility represents a serious handicap in the development of sound IPM programs.

Attempts to overcome this handicap have taken several forms. One is the use of chemicals in an

ecologically selective manner (Hull & Beers 1985). Another is the use of materials with a physiological selectivity based upon either natural tolerance or evolved resistance of the biological control agent (Mullin & Croft 1985). The evolution of resistance in beneficial arthropods through natural selection in the field and artificial selection in the laboratory has recently been documented, and such resistant natural enemies have been successfully incorporated into IPM programs (Croft & Strickler 1983, Hoy 1985a).

*Aphytis melinus* DeBach is the major biological control agent of the California red scale, *Aonidiella aurantii* (Maskell), in California and in many other citrus-growing regions of the world (Rosen & DeBach 1979). Although the degree of control exerted by *A. melinus* ranges from partial to complete in the different citrus-growing regions of California, its effectiveness in all these regions is severely impeded by the use of insecticides (DeBach et al. 1971, Bellows et al. 1985, Griffiths et al. 1985). Chemicals commonly applied for control of *A. aurantii* and other key pests of citrus not under biological control, including the citrus thrips, *Scirtothrips citri* (Moulton), and several lepidopteran species, are toxic to *A. melinus* (University of California, Statewide IPM Project 1984; Morse & Bellows 1986), resulting in the destruction of resident populations and hindering programs of augmentative releases of insectary-reared parasitoids.

Our study was done to investigate whether *A.*



<sup>a/</sup> Only pesticides that are effective in killing the target pest are employed, hence there is no "yes" option. (Some IPM programs do, however, use effective chemicals at rates intended to kill only a proportion of the target pest population.)

Fig. 1. Some proximate and ultimate effects of pesticide applications on the ecology of arthropod parasitoid/host and predator/prey relationships: secondary pest resurgences and outbreaks due to the direct effects of pesticide toxicity and the indirect effects of food limitation on biological control agents. (For purposes of explanation, we present scenarios where populations show either 100% mortality or 100% survival; these outcomes represent the two ends of a continuum of possible responses. Outcomes that are intermediate to both the proximate and ultimate effects listed may occur.)

*melinus* has evolved increased levels of resistance in response to the selective pressures exerted by the use of insecticides. We determined if variability existed in the resistance levels of different field populations of *A. melinus*, and ascertained if a relationship existed between these resistance levels and the previous insecticide use in the localities where the colonies were collected. Finally, we considered the roles of food limitation, migration, and host distribution in determining patterns and rates of the evolution of pesticide resistance in *A. melinus*.

### Materials and Methods

**Colony Collection and Maintenance.** In October 1984, 11 populations of *A. melinus* were collected from the major citrus-growing regions of California (Fig. 2) by two techniques: 1) citrus fruits bearing parasitized *A. aurantii* were collected, and 2) trap fruits (lemons infested with *A. aurantii* in the laboratory) were placed in citrus trees to elicit oviposition by *A. melinus*. Both techniques were used at each collection site. In sites with heavy infestations of *A. aurantii*, the collection of scale-bearing citrus fruits generally yielded the greater number of parasitoids, whereas in sites with light infestations use of the trap fruits was the more effective approach. The number of field-collected parasitoids used to initiate each colony varied from 42 to 1,560 (average = 415). The

number of generations (ca. 0–4) required for populations to adapt to laboratory conditions and attain a normal rate of increase varied. During this period of adaptation the effective population size may have been substantially less than the total adult population. In addition to the 11 colonies collected as described above, 2 colonies, 1 collected in Ventura County in February 1983 (population 10), and the other a long-term laboratory colony (population 13), were provided by T. S. Bellows and R. F. Luck (Division of Biological Control, University of California, Riverside), respectively, for testing. Voucher specimens from each colony were confirmed as *A. melinus* by D. Rosen, Hebrew University, Rehovot, Israel.

Colonies were maintained in the laboratory at  $26 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  RH, and a photoperiod of 16:8 (L:D) on a uniparental strain of oleander scale, *Aspidiotus nerii* Bouché, that was grown at  $24 \pm 1^\circ\text{C}$  on pink banana squash, *Cucurbita maxima* Duchesne, under constant darkness. This rearing technique was adapted from that described by DeBach & White (1960). Undiluted honey was provided in cages as a carbohydrate source for *A. melinus*.

**Bioassays.** Adult male and female *A. melinus* (0–48 h old) were collected for testing with pesticides by placing squash bearing parasitized scale in an emergence cage similar to that described by Abdelrahman (1973). The cage consisted of a sealed plastic garbage can with 12 holes drilled in the

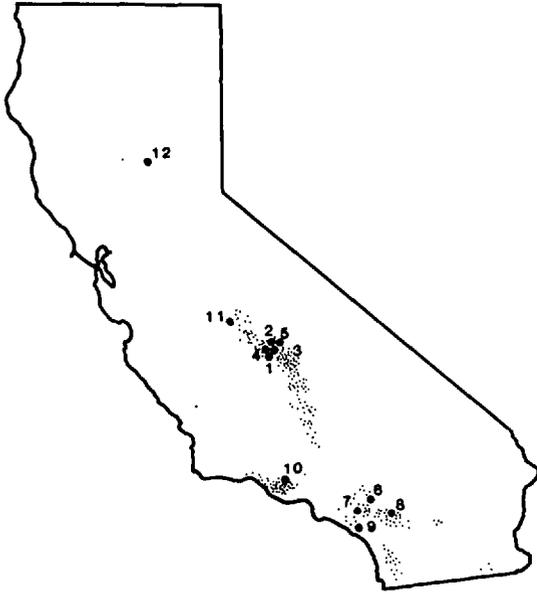


Fig. 2. Distribution of commercial citrus in California. Each dot represents 405 ha. Numbered circles are collection sites for *A. melinus* colonies.

cover. Each of these holes was filled with a glass test-tube (12 by 75 mm) containing streaks of honey. By illuminating from above, we then drew the parasitoids, which are positively phototropic and negatively geotropic, into the tubes, where they were harvested. Cages were emptied and cleaned every 48 h to provide parasitoids that were 0–48 h old.

Concentration/mortality data were generated by confining between 10 and 20 adult parasitoids in a treated disposable plastic cup (30 ml) capped with untreated polyester gauze upon which undiluted honey was provided. The cups were treated by dipping them for 5 s into insecticide solutions formulated in distilled water with a spreader (0.1% Triton AG-98). Cups were drained onto paper toweling and air dried. Three materials widely used for California red scale control (carbaryl [Sevin 80 sprayable], malathion [Malathion 25 sprayable], and methidathion [Supracide 2 emulsifiable concentrate]), one material used for thrips control (dimethoate [Cygon 400]), and one material used for control of both California red scale and a complex of lepidopterous pests (chlorpyrifos [Lorsban 4 emulsifiable concentrate]) were tested.

At least five concentrations and a water/spreader control were tested for each chemical. Vials were held at  $26 \pm 1^\circ\text{C}$ , 87% RH, and a 16:8 photoperiod for 24 h before the tests were scored. Individuals were considered dead if they were unable to maintain a normal posture or walk normally, covering at least 1 mm/s. With a given chemical, all populations were tested simultaneously. Each test was

repeated on at least three different days for a total of 4–18 replicates per concentration.

Data were analyzed by probit analysis with the POLO computer program (Russell et al. 1977). Hypotheses of parallelism (equal slopes) and equality (equal slopes and intercepts) were tested with likelihood-ratio tests (Savin et al. 1977). Populations were considered to have different tolerances if the hypothesis of equality was rejected ( $\alpha = 0.05$ ).

**Pesticide Use Histories.** To investigate the relationship of past exposure to insecticides to observed population resistance levels, we investigated each population's history of insecticide exposure. (Population 13, a laboratory colony, was not included in this analysis.) This was done indirectly by assessing 1) the past use of insecticides in the grove from which the colony was collected (the local or in-grove pesticide use) during the 5-year period (1980–84) and 2) the past use of insecticides in citrus groves in the surrounding areas (the regional or county-wide pesticide use) for the same period.

In-grove and county-wide pesticide use histories were obtained from individual growers and the California Department of Food and Agriculture, Division of Pest Management (unpublished records, Sacramento, Calif.), respectively. We assumed that results of these 5-year surveys would approximate the relative overall levels of historical pesticide use not only for 1980–84, but for an earlier period of time as well, during which *A. melinus* was present in California; the groves sampled were mature and had been under single ownership for more than the 5-year period surveyed. Also, patterns of pesticide use were consistent over time in those groves for which more extensive histories were available.

The influences of in-grove and county-wide pesticide use were evaluated both independently and in combination using bivariate (one independent variable) and multiple (two independent variables) regression analysis from the SPSS' computer statistical package (SPSS 1986, 662–686). For regressions involving tolerances to organophosphorus (OP) insecticides, the local and regional histories of total OP use were the independent variables. For regressions involving tolerances to carbaryl, the corresponding histories of carbamate insecticide use were used. Insecticides of the same class (OP or carbamate) were combined in this manner in an attempt to include the possible effects of cross-resistance.

An index of overall resistance of each colony was calculated relative to that of colony 1. Each colony's  $LC_{50}$  for an insecticide was divided by that of colony 1. A similar ratio was calculated for each of the insecticides for which the colony was tested, and the ratios were totaled and averaged for each colony. The resulting overall average relative resistance values were then regressed against the total number of insecticide treatments applied

to the grove from which the colony came, against the county-wide pesticide use, and against the combined level of in-grove and county-wide pesticide use.

Finally, for each chemical tested, we determined whether the slope values of the concentration/mortality regressions were linearly related to 1) the associated  $LC_{50}$ 's, 2) the selective pressure experienced by the population (as estimated by the sum total of the in-grove and county-wide pesticide use histories), or 3) the degree to which the level of in-grove pesticide use diverged from that of the county (as estimated by subtracting the in-grove pesticide use value from the county-wide pesticide use value).

### Results

For each of the five insecticides tested, the concentration/mortality regressions varied significantly among the *A. melinus* colonies (Table 1). The hypothesis of equality was rejected for most population comparisons for each chemical.

The collection site characteristics and the in-grove and county-wide pesticide use histories for the 13 colonies tested are shown in Table 2. All but two of the collection sites were commercial groves. The species of *Citrus* chosen was generally the one that was predominantly grown in the region being sampled. The overall amounts of insecticides used varied widely, both regionally among counties (0.099–4.67 kg [AI]/ha per year), and locally among groves (0–15 applications from 1980 to 1984). No significant relationship between the total (carbamate plus OP) in-grove and county-wide insecticide use was found ( $r^2 = 0.0012$ ;  $P > 0.25$ ). Since *A. melinus* colonies were collected from groves that were chosen for their varied histories of pesticide use, a lack of correlation was expected.

The results of the regression analyses of the  $LC_{50}$ 's (Table 1) on the levels of past pesticide use (Table 2) are shown in Table 3. The slopes of the significant regressions were all positive, indicating a positive correlation between  $LC_{50}$ 's and levels of past pesticide use. Due in part to the small sample size (7–11), the levels of significance associated with the single independent variable correlations were often inadequate to draw strong conclusions. (For the eight nonsignificant regressions,  $0.14 \leq P \leq 0.44$ .) However, when the influences of the two factors, the in-grove and county-wide pesticide use histories, were combined, the resulting regressions were strong enough to be significant for three of the five chemicals tested ( $P \leq 0.10$ ). (Due to the small sample sizes employed,  $\alpha = 0.10$  was used. Additional studies with larger sample sizes will be necessary to confirm these results and reduce the overall risk of type I error.) This suggests that both the in-grove and county-wide pesticide use are important in accurately describing a population's history of selection pressures.

**Table 1.** Variation in concentration/mortality regressions of *A. melinus* colonies collected from California exposed to residues of five insecticides

Insecticide	Colony	n <sup>a</sup>	Slope $\pm$ SEM	LC <sub>50</sub> (95% CL) in mg (AI)/liter
Carbaryl (suggested field rate <sup>b</sup> : 960 mg [AI]/liter)				
	7	1,182	3.22 $\pm$ 0.20	15.4 (13.3–17.5)a
	8	1,061	3.42 $\pm$ 0.22	17.9 (15.8–20.1)b
	5	1,101	3.91 $\pm$ 0.24	19.9 (18.5–21.4)c
	11	1,153	3.74 $\pm$ 0.21	19.9 (18.1–21.8)cd
	4	983	4.27 $\pm$ 0.25	23.5 (21.5–25.9)e
	3	498	3.10 $\pm$ 0.30	24.0 (20.3–28.2)f
	1	648	4.28 $\pm$ 0.42	27.9 (24.4–31.2)g
Chlorpyrifos (suggested field rate: 450 mg [AI]/liter)				
	7	427	2.70 $\pm$ 0.26	0.78 (0.58–0.97)a
	11	1,337	3.31 $\pm$ 0.16	0.84 (0.76–0.92)ab
	3	1,443	2.55 $\pm$ 0.14	0.93 (0.81–1.06)c
	4	381	3.40 $\pm$ 0.32	1.02 (0.87–1.20)d
	5	1,035	3.59 $\pm$ 0.27	1.11 (0.98–1.23)de
	8	614	5.69 $\pm$ 0.78	1.24 (1.01–1.40)f
	1	1,172	4.60 $\pm$ 0.51	1.37 (1.18–1.50)g
	2	255	2.42 $\pm$ 0.33	1.43 (1.06–2.07)h
Dimethoate (suggested field rate: 1,200 mg [AI]/liter)				
	9	325	3.06 $\pm$ 0.47	2.18 (1.26–2.85)a
	7	1,444	2.69 $\pm$ 0.15	2.58 (2.19–2.96)b
	13	410	3.09 $\pm$ 0.29	2.73 (2.25–3.20)bc
	10	1,168	2.28 $\pm$ 0.13	2.73 (2.33–3.15)d
	3	1,143	2.63 $\pm$ 0.15	3.55 (3.02–4.13)e
	2	438	2.65 $\pm$ 0.29	3.62 (2.64–4.62)ef
	8	1,464	2.55 $\pm$ 0.13	3.91 (3.40–4.46)ef
	12	196	2.70 $\pm$ 0.37	3.95 (2.82–5.79)efg
	6	910	2.71 $\pm$ 0.17	4.16 (3.57–4.79)fg
	4	1,244	2.72 $\pm$ 0.17	4.46 (3.75–5.25)g
	1	435	2.67 $\pm$ 0.49	6.36 (3.49–8.71)h
Malathion (suggested field rate: 720 mg [AI]/liter)				
	9	929	2.65 $\pm$ 0.15	0.54 (0.45–0.63)a
	13	460	3.15 $\pm$ 0.24	0.78 (0.57–0.99)b
	7	1,436	2.78 $\pm$ 0.13	1.23 (1.09–1.37)c
	10	1,521	3.20 $\pm$ 0.19	1.27 (1.10–1.42)cd
	6	1,369	2.50 $\pm$ 0.14	1.73 (1.50–1.98)e
	12	1,226	4.52 $\pm$ 0.35	1.94 (1.72–2.15)f
	3	1,644	3.09 $\pm$ 0.20	2.07 (1.84–2.28)g
	8	1,459	3.56 $\pm$ 0.25	2.29 (1.99–2.57)h <sup>c</sup>
	11	869	4.43 $\pm$ 0.31	2.36 (2.07–2.67)h
	2	1,294	2.98 $\pm$ 0.24	2.37 (2.02–2.70)h
	4	1,272	3.04 $\pm$ 0.15	3.11 (2.69–3.58)i
	1	553	2.53 $\pm$ 0.31	4.22 (3.33–5.16)j
Methidathion (suggested field rate: 300 mg [AI]/liter)				
	9	1,102	2.38 $\pm$ 0.14	0.44 (0.37–0.51)a
	6	1,093	2.43 $\pm$ 0.16	2.07 (1.79–2.37)b
	8	1,121	2.45 $\pm$ 0.15	2.30 (2.03–2.60)bc
	1	957	2.90 $\pm$ 0.22	2.46 (2.13–2.81)cd
	5	776	2.80 $\pm$ 0.24	2.54 (2.19–2.97)cd
	2	1,231	2.08 $\pm$ 0.17	2.68 (2.16–3.23)e <sup>d</sup>
	3	1,055	2.57 $\pm$ 0.17	2.71 (2.32–3.15)de
	4	654	2.30 $\pm$ 0.20	3.00 (2.54–3.58)e
	12	286	1.99 $\pm$ 0.36	3.36 (1.76–7.90)e

Concentration/mortality regressions followed by the same letter are not significantly different ( $\alpha = 0.05$ ; likelihood-ratio test [Savin et al. 1977]).

<sup>a</sup> Natural mortality ranged from 0 to  $8.8 \pm 1.9\%$  and averaged 2.2%.

<sup>b</sup> Recommendations of the University of California Cooperative Extension citrus treatment guide (Morse & Bailey 1984).

<sup>c</sup> The regression for colony 8 is not significantly different ( $\alpha = 0.05$ ) from the regressions of colonies 11 or 2. However, the regression of colony 11 is significantly different from that of colony 2.

<sup>d</sup> The regression for colony 1 is not significantly different ( $\alpha = 0.05$ ) from the regressions of colonies 5 or 3; likewise, the regression for colony 3 is not significantly different from that of colony 2. However, the regression for colony 2 is significantly different from those of colonies 1 and 5.

**Table 2. Collection-site characteristics and in-grove<sup>a</sup> and county-wide<sup>b</sup> pesticide use histories for populations of *A. melinus* sampled from California citrus**

Colony no.	County of origin, (number of founders), and orchard type	Total no. of in-grove pesticide applications, 1980-84 <sup>c</sup>										Avg yearly county-wide pesticide use, 1980-84, in kg (AI)/ha <sup>c</sup>									
		Car	Chl	Dim	Mal	Met	All Carb	All OP	Carb + OP	Car	Chl	Dim	Mal	Met	All Carb	All OP	Carb + OP				
1	Tulare (600) Commercial navel orange	2	1	3	0	0	5	5	8	1.09	0.20	1.14	0.10	0.81	1.71	2.96	4.67				
2	Tulare (214) Commercial navel orange	3	0	4	0	0	6	5	9	1.09	0.20	1.14	0.10	0.81	1.71	2.96	4.67				
3	Tulare (735) Commercial navel orange	1	0.5	4	0	0.5	3.5	6	8	1.09	0.20	1.14	0.10	0.81	1.71	2.96	4.67				
4	Tulare (90)	1	1	1	0	0	1	2	3	1.09	0.20	1.14	0.10	0.81	1.71	2.96	4.67				
5	Tulare (552) Small residential planting; mixed <i>Citrus</i> spp.	0	1	0	0	0	0	1	1	1.09	0.20	1.14	0.10	0.81	1.71	2.96	4.67				
6	Riverside (134) University of California Riverside experimental grove; mixed <i>Citrus</i> spp.	0	0	0	0	0	0	0	0	0.08	0.08	0.37	0.03	0.10	0.20	0.75	0.95				
7	Riverside (213) Commercial lemon	0	0	0	0	0	0	0	0	0.08	0.08	0.37	0.03	0.10	0.20	0.75	0.95				
8	Riverside (42) Commercial grapefruit	0	0	6	0	1	0	10	10	0.08	0.08	0.37	0.03	0.10	0.20	0.75	0.95				
9	Orange (44) Commercial Valencia orange	0	0	0	0	0	0	2	2	0	0	0	0	0.03	0.02	0.08	0.10				
10	Ventura (—) Commercial lemon	0	0	0	0	0	0	0	0	0.41	0.26	0.39	0.01	0.28	0.47	1.69	2.15				
11	Madera (1,560) Commercial lemon	2.5	0	3	0	0	2.5	4	6.5	2.38	0.01	1.12	0.02	0.13	2.46	1.78	4.24				
12	Butte (24) Commercial navel orange	0	0	7	6	0	1	14	15	0	0	0.12	0	0.01	0.01	0.14	0.14				
13	Laboratory colony <sup>e</sup> (—)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				

<sup>a</sup> Applications made to part of a grove were scored as 0.5 applications. Mixtures of pesticides were scored as a single application for each constituent chemical, but only as a single application under the Carb + OP column. Hence, the Carb + OP column is not a summation of previous columns.  
<sup>b</sup> Calculated as the total amount of active ingredient (AI) in kilograms applied to citrus per county (as reported by the California Department of Food and Agriculture, Division of Pest Management) divided by the total number of hectares planted in citrus in that county (as reported by the California Department of Agriculture [1980-84]). The accuracy of the figures in the county-wide pesticide use reports issued by the California Department of Agriculture is dependent upon whether the pesticide is a restricted-use compound or not. Applications of nonrestricted insecticides (e.g., chlorpyrifos, dimethoate, malathion) by growers are not required to be reported, so the corresponding figures represent only a fraction of the total use. The rules for reporting applications are uniform throughout California.

<sup>c</sup> Car, carbaryl; Chl, chlorpyrifos; Dim, dimethoate; Mal, malathion; Met, methidathion; Carb, carbamates; OP, organophosphates.  
<sup>d</sup> Number of colony founders unknown. Emerging parasitoids were placed in direct contact with fresh host material, so manual transfer and counting of emerged individuals were bypassed.  
<sup>e</sup> Long-established laboratory colony obtained April 1985 from the Division of Biological Control, University of California, Riverside.

Regressions were also performed using the history of use of the specific chemical for which the concentration/mortality regression was generated. The results of these regressions (data not shown) were similar (two of the five correlations were significant and had positive slopes) to those described below for the regional analyses, but were not significant for the local, in-grove analyses ( $P > 0.10$ ). The in-grove analyses were hampered by the small number of local pesticide applications made during the 5-year survey (Table 2). For example, neither chlorpyrifos nor methidathion were applied more than once in any of the groves during the 5 years (Table 2).

The index of overall average relative tolerance values was significantly correlated ( $\alpha = 0.10$ ) to the in-grove, county-wide, and combined total pesticide use histories (Table 3). The results of both  $F$  tests to remove each independent variable from the multiple regression ( $P \leq 0.05$ ) indicated that both the local and regional pesticide use histories individually contributed significantly to the overall regression (Fig. 3). Partial correlation analysis indicated that the relative importances of the in-grove and county-wide pesticide use histories were not significantly different. Partial correlation coefficients were 0.51 and 0.58, respectively ( $\alpha = 0.20$ ,  $P > 0.10$ ). Fig. 3C shows the expected values of each colony's overall average relative tolerance, as predicted by the multiple regression equation, versus the observed values of the same.

Linear regression analysis showed that the slopes of the concentration/mortality regressions were not correlated with either the associated  $LC_{50}$ 's, the total pesticide use history, or the difference between the in-grove and county-wide pesticide use histories. The slopes of the regression lines varied from positive to negative for different chemicals, and the correlations were generally not significant (data not shown). The fact that the slopes of the probit regressions were not positively correlated with their  $LC_{50}$ 's indicates that the intrapopulation variation in resistance (i.e., change in response per unit increase of concentration) has not decreased as the  $LC_{50}$ 's have increased in response to selection pressures. This suggests that additional responses to selection could occur.

### Discussion

Our investigation has shown substantial variability in the levels of resistance exhibited by field populations of *A. melinus* to five commonly used insecticides. The history of pesticide use on both the local and regional scale appears to have contributed to the observed levels of resistance. These results led us to consider the roles of food limitation, migration, and host distribution in directing the observed evolutionary pathways of *A. melinus* populations.

**Variation in Resistance Levels.** *A. melinus* was colonized throughout southern California from

Table 3. Regression analyses of in-grove and county-wide pesticide use histories on the  $LC_{50}$ 's for five insecticides and an overall relative tolerance level of *A. melinus*

Independent variable(s)	Regression parameters					Dependent variables <sup>a</sup> are the $LC_{50}$ 's obtained for:					Overall relative tolerance level <sup>b</sup>
	$r^2$	Slope $\pm$ SEM	Carbaryl	Chlorpyrifos	Dimethoate	Malathion	Methidathion				
A) In-grove pesticide use history <sup>c</sup>			0.70	0.22	0.08	0.07	0.20	0.28			
			1.76 $\pm$ 0.51 <sup>d</sup>	0.035 $\pm$ 0.027	0.071 $\pm$ 0.086	0.057 $\pm$ 0.072	0.082 $\pm$ 0.061	0.025 $\pm$ 0.013 <sup>e</sup>			
B) County-wide pesticide use history			0.35	0.14	0.25	0.47	0.19	0.36			
			2.91 $\pm$ 1.76	0.088 $\pm$ 0.088	0.467 $\pm$ 0.290	0.573 $\pm$ 0.202 <sup>d</sup>	0.263 $\pm$ 0.208	0.068 $\pm$ 0.028 <sup>d</sup>			
C) A and B/			0.73 <sup>f</sup>	0.43	0.37	0.60 <sup>f</sup>	0.59 <sup>f</sup>	0.62 <sup>d</sup>			
			1.55 $\pm$ 0.67 <sup>e</sup>	0.041 $\pm$ 0.026	0.091 $\pm$ 0.076	0.081 $\pm$ 0.050	0.122 $\pm$ 0.045 <sup>e</sup>	0.024 $\pm$ 0.010 <sup>d</sup>			
			0.884 $\pm$ 1.55	0.110 $\pm$ 0.80	0.519 $\pm$ 0.286	0.618 $\pm$ 0.188 <sup>d</sup>	0.404 $\pm$ 0.170 <sup>e</sup>	0.066 $\pm$ 0.023 <sup>d</sup>			

<sup>a</sup> All  $LC_{50}$ 's used in the analysis were expressed in mg (AI)/liter (Table 1).

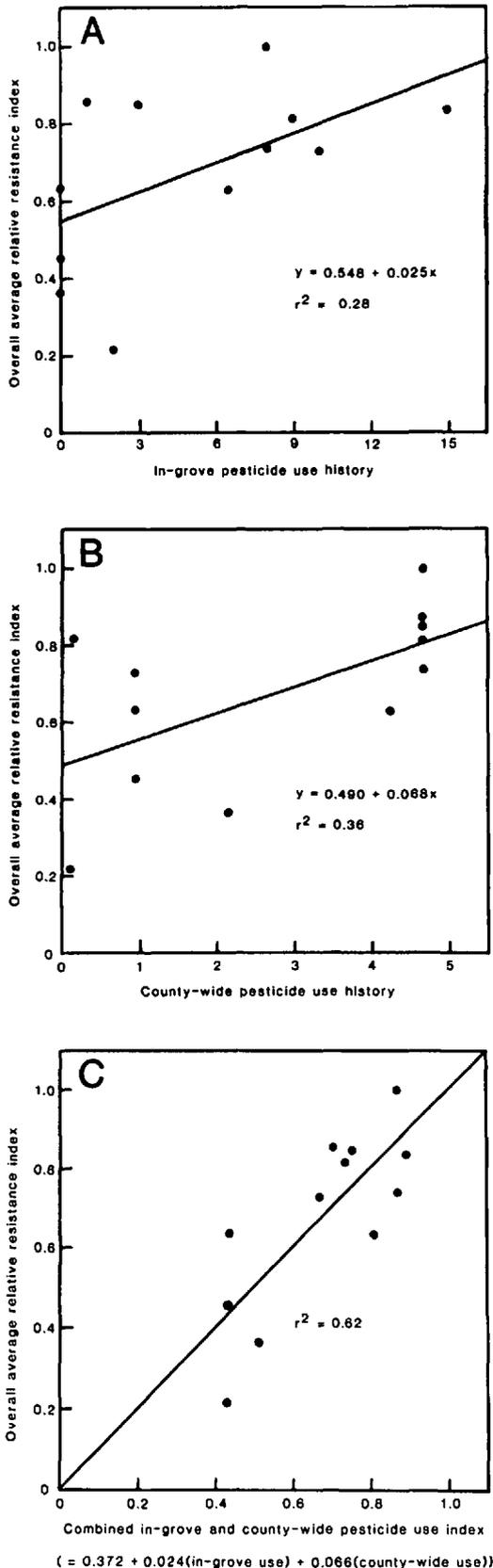
<sup>b</sup> Calculated for each colony as the arithmetic mean of the  $LC_{50}$ 's to each of the chemicals for which it was tested relative to the corresponding value for colony 1 (Tables 1 and 2).

<sup>c</sup> For regressions of carbaryl  $LC_{50}$ 's the total in-grove and county-wide histories of carbamate insecticide use were used as the independent variables (Table 2). Similarly, for each of the chlorpyrifos, dimethoate, malathion, and methidathion  $LC_{50}$  regressions, the history of total organophosphate insecticide use was employed. For the regression of overall relative tolerance level, the history of combined carbamate plus organophosphate insecticide use was employed.

<sup>d</sup> Significant result ( $P \leq 0.05$ ).

<sup>e</sup> Significant result ( $P \leq 0.10$ ).

<sup>f</sup> Superscripts beside the  $r^2$  values indicate the significance level of the overall regression. Superscripts beside slope values indicate the significance of the contribution made by that independent variable, as determined by an  $F$  test to remove that variable.



1957 to 1960. Approximately 2.5 million insectary-reared parasitoids were released into 200 citrus plots; these parasitoids originated from a single culture consisting of a mixture of four small collections made in Pakistan and India (DeBach 1959, DeBach & Landi 1959, DeBach & Sundby 1963). Since these initial introductions, the degree of resistance of *A. melinus* populations to the insecticides tested appears to have diverged. The most susceptible colony tested, colony 9, originated from a population that has been exposed to insecticides only minimally. This colony's in-grove pesticide use history is known for the 21-year period, 1964-84, during which only four applications of broad-spectrum OP insecticides were made. In addition, this population is relatively isolated from other extensive citrus plantings, suggesting that migration of individuals from heavily sprayed areas into this population may have been relatively low. The most resistant colonies had  $LC_{50}$ 's up to 7.8-fold greater than that of this colony (Table 1). The evolution of increased resistance to pesticides by *A. melinus* appears to represent an example of post release adaptation in a species introduced for classical biological control.

The evolutionary divergence of *A. melinus* populations also reflects evolutionary flexibility in populations that might have been expected to be relatively low in genetic variability. *A. melinus* is a member of the order Hymenoptera, which has been found to display the least amount of electrophoretically detected genetic variation of any of the insect orders (Graur 1985). Although within the Hymenoptera solitary wasps show a greater level of average heterozygosity than do the social species, they still exhibit less variation than non-hymenopteran orders (Graur 1985). Furthermore, *A. melinus* is a recently introduced species. The genetic variability of species introduced to a new area as part of a biological control program may be reduced by the limited numbers of individuals collected from the indigenous area, genetic drift and inadvertent selection during the processes of quarantine and mass-rearing, and additional bottlenecks occurring during the release and colonization of the new habitat (Messenger & van den Bosch 1971, Hoy 1985b). It is unknown to what extent, if any, the process of importing *A. melinus* has reduced the evolutionary flexibility of its California populations.

Previous investigations of the evolution of resistance to insecticides among field populations of parasitoids have yielded mixed results. Adams & Cross (1967) reported no significant difference be-

←  
**Fig. 3.** Regression analyses of each colony's overall average relative tolerance on (A) its in-grove pesticide use history, (B) its county-wide pesticide use history, and (C) both A and B simultaneously (multiple regression). See Table 2 for values plotted.

tween the tolerances of three field and one laboratory colony of *Bracon mellitor* Say to five insecticides used on cotton. Similarly, Krukier et al. (1975) failed to detect differences between the responses to oxydemeton-methyl of two populations of *Trichogramma evanescens* Westwood or two populations of *T. minutum* Riley. Strawn (1978) was unable to detect significant variation in the responses of either adults of four field populations of *Comperiella bifasciata* Howard or pupae of six populations of *A. melinus* to four OP compounds. Likewise, no significant differences in levels of resistance to malathion were exhibited by seven field populations of *Aphytis holoxanthus* DeBach (Havron 1983).

In contrast, Schoonees & Giliomee (1982) found a 5.7- and a 65.6-fold difference in levels of resistance to methidathion in two field populations of *Aphytis africanus* Quednau and two field populations of *C. bifasciata*, respectively. Strawn (1978) found significant interpopulation variation in response to parathion by *C. bifasciata* pupae and in the tolerances of *A. melinus* adults to dimethoate, methidathion, and parathion. Unfortunately, because Strawn's tests were performed using single-dose bioassays and because probit regressions were not generated, further comparison of these results with ours is difficult. Finally, Hsieh (1984) found a ca. 2-fold difference between the tolerances exhibited by two field populations of *Diaeretiella rapae* (M'Intosh) to methomyl. Thus the increase in resistance levels exhibited by parasitoids produced by natural selection in the field has varied from undetectable to substantial. In none of these studies, in which explicit comparisons of different populations were made, was the increased level of resistance adequate to enable adult parasitoids to survive field application rates of insecticides. The pattern of evolution of resistance among parasitoids clearly does not resemble the explosive emergence of resistance in pest species (Georghiou & Mellon 1983).

Several possible explanations for this apparent relative inability of parasitoids to develop resistance have been proposed. The evolution of resistance in field populations of parasitoids may often go unnoticed (Croft & Brown 1975). The differential exposures of parasitoids relative to their hosts due to morphological, behavioral, or ecological differences may lead to a greater effective toxicity (Georghiou 1972). Because parasitoids are often initially less tolerant of pesticides than their hosts, a larger increase in tolerance may be required to achieve effective levels of resistance (J.A.R., unpublished data). The lack of genetic flexibility among the highly ecologically specialized parasitoids may restrict the evolution of resistance (Hufaker 1971, Georghiou 1972). The relatively low activity of preadapting detoxifying enzyme systems may limit the potential for resistance (Croft & Strickler 1983). Finally, the reliance of parasitoid populations upon the host population for sur-

vival after pesticide applications may effectively select against resistant individuals (Croft & Morse 1979, Hoy 1979, Tabashnik & Croft 1985). The last hypothesis, the food limitation hypothesis, has received increased attention recently (Morse & Croft 1981, Tabashnik & Croft 1982, 1985, Tabashnik 1986).

A corollary of the food limitation hypothesis (Georghiou 1972, Morse & Croft 1981, Croft & Strickler 1983, Tabashnik & Croft 1985) is that resistance is unlikely to develop in a natural enemy species until after its host or prey (henceforth called host) has become resistant. Consideration of the patterns of pesticide use in citrus and the resulting impact on *A. melinus* and its host scale populations leads to the conclusion, however, that this corollary may have been described in an overly restrictive form. Generally, chemicals being discussed in this regard are not directed against the host population. Rather, they are being used to control other key pests in the agroecosystem. Therefore, resistance in the host population is not always necessary; any means by which this population can survive the pesticide application and thereby continue to serve as hosts to the natural enemy species will circumvent the problem of food limitation.

There are two general means by which a non-resistant host population can survive an insecticide application (Fig. 1). First, a non-resistant host population will be relatively unaffected by a chemical application that it does not physically contact. For example, dimethoate applied to control citrus thrips is applied only to the periphery of the tree (Table 4). The dimethoate will, therefore, contact only a fraction of the total California red scale population, which is distributed throughout the tree (Ebeling 1959). The same is true for chlorpyrifos, which is applied to the outside of the tree for control of various orangeworms (Table 4). The application of pesticides to a restricted portion of the tree may be described as a form of ecological selectivity towards the host population. Second, a natural tolerance relative to the tolerance of the target pest species will also enable the host population to survive treatment. Thus, California red scale, while potentially controllable with dimethoate (which is registered for control of *A. aurantii*), largely appears to be tolerant of the lower concentrations applied for control of citrus thrips.

The results of our study may be considered with regard to this broadened concept of the food limitation hypothesis and its corollary. The absence of a substantial host population should, according to the hypothesis, retard the evolution of resistance in *A. melinus* to carbaryl, malathion, and methidathion (used to control scales) but not to dimethoate (applied for thrips control). The evolution of resistance to chlorpyrifos (applied for control of either scales or orangeworms) should fall somewhere between these two extremes.

Are these predictions reflected in the ranges of

**Table 4. Recommendations<sup>a</sup> of the University of California Cooperative Extension for the treatment of citrus with carbaryl, chlorpyrifos, dimethoate, malathion, and methidathion**

Pesticide and formulation	Target species	Earliest recommendation			1984-86 recommendation	
		Year	Concn	Coverage type <sup>b</sup>	Concn	Coverage type
Carbaryl 80 WP	<i>A. aurantii</i>	1966-67	1.20-1.44 g/liter (1-1.2 lb/100 gal)	TC	Same as 1966-67	
Chlorpyrifos 0.48 kg/liter E (4 lb/gal)	Several Lepidopteran pests	1984-86	1.24-12.4 ml/liter/ha (1-2 qt/100-500 gal/acre)	OC	Same	
	<i>A. aurantii</i>	1984-86	0.94 ml/liter (0.75 pt/100 gal)	TDC	Same	
Dimethoate 0.32 kg/liter E (2.67 lb/gal)	<i>S. citri</i>	1966-67	3.75 ml/liter (3 pts/100 gal)	MS	4.64-18.5 ml/liter 3-6 pts/100-200 gal/acre <sup>c</sup>	OC
Malathion 25 WP	<i>A. aurantii</i>	1957 <sup>d</sup>	3.00-4.20 g/liter (2.5-3.5 lb/100 gal)	TC	2.88-4.07 g/liter (2.4-3.4 lb/100 gal)	TC
Methidathion 0.24 kg/liter E (2 lb/gal)	<i>A. aurantii</i>	1976-78	1.25 ml/liter (1 pt/100 gal)	TDC	Same as 1976-78	

<sup>a</sup> Recommendations published in the irregularly issued citrus treatment guides (e.g., Morse & Bailey 1984).

<sup>b</sup> Abbreviations used are taken verbatim from treatment guides (e.g., Morse & Bailey 1984). MS, mist spray; low literage, 936-2,807 liters per hectare (100-300 gallons per acre), applications without droplet size restrictions achieving limited droplet depositions on tree surfaces; OC, outside coverage; median literage, not more than 4,676 liters per hectare (500 gallons per acre), applications achieving thorough distribution to outside or peripheral parts of the tree only; TC, thorough coverage: high literage, 114-132 liters per tree (30-35 gallons per tree), applications achieving thorough film wetting of all interior and exterior parts of the tree; TDC, thorough distribution coverage: median literage, 76-114 liters per tree (20-30 gallons per tree), applications achieving thorough distribution to all interior and exterior parts of tree without necessity of obtaining film wetting.

<sup>c</sup> For the first time, in the 1984-86 Treatment Guide, this recommendation was accompanied by a footnote reading "Non-resistant thrips only."

<sup>d</sup> Because *A. melinus* was introduced in 1957, this is the earliest relevant recommendation.

resistance values observed in the populations tested with each of these insecticides? Because not all colonies were tested with all of the chemicals, the ranges of resistance values must be considered with caution. To compare the ranges of resistance values for two chemicals, only those colonies tested with both chemicals should be considered (e.g., to compare the ranges of LC<sub>50</sub>'s of carbaryl with dimethoate, only colonies 1, 3, 4, 7, and 8 should be included). The LC<sub>50</sub>'s for these colonies (Table 1) indicate that the maximum ranges of resistance are 27.9/15.4 = 1.8-fold for carbaryl and 6.36/2.58 = 2.5-fold for dimethoate. The ranges of resistance values observed for malathion and dimethoate were 7.8- and 2.9-fold, and methidathion and dimethoate were 7.7- and 2.9-fold, respectively. Analogous figures for the three scalcides, carbaryl, malathion, and methidathion, compared with chlorpyrifos are 1.8- and 1.7-fold, 3.4- and 1.8-fold, and 1.3- and 1.5-fold, respectively. The food limitation hypothesis predicts that the second value of each of these paired numbers, representing the extent of evolved resistance to chemicals not impacting the host population, should be greater than the first value, representing the extent of evolved resistance to chemicals that reduce the host population. However, our observations suggest the reverse. This is a limited test of the hypothesis; many other factors, including the length of time and the degree to which the chemicals have been used (Tables 2 and 4) and the possible

existence of cross-resistance patterns, will affect the outcome. We conclude that, at least in this system, food limitation does not appear to be the key factor in determining the extent to which resistance has developed.

**The Role of Past Selection Pressures.** One of our two initial goals was to evaluate the role of past selection pressures in any observed interpopulation patterns of variation. To measure selection pressures, spatial delimitation of a population was required. Two important considerations were the geographic distribution of potential hosts and the dispersal ability of the parasitoid.

The distribution of hosts of *A. melinus* proved to be difficult to describe. Although the distribution of commercially cultured citrus in California is known to consist of several fairly discrete regions in widely separated valleys bounded by substantial mountain ranges (Fig. 1), we concluded that commercial citrus did not represent the entire host plant pool. Substantial scale populations also exist in common, small, dooryard citrus plantings (DeBach 1965). *A. aurantii* also infests many other host plants. McKenzie (1956) considered the species to be one of California's 22 omnivorous armored scales, and the total number of plant hosts may be large (Quayle 1911, McKenzie 1946). Compounding this situation in California, *A. melinus* develops not only on *A. aurantii* but also on nine other species of armored scales (Rosen & DeBach 1979; S. C. Warner, personal communication). Most of

these additional hosts are also omnivorous; McKenzie (1956) listed over 80 plant genera, including many commercially cultivated species, as "only a few of the more preferred hosts."

The significance of these alternate hosts is difficult to assess, and their collective contribution to the total host pool may be less than that of *A. aurantii* (R. F. Luck, personal communication). *A. melinus* is not the dominant parasite of any of the alternate hosts (R. F. Luck & S. C. Warner, personal communications), and some of the scales are relatively rare. However, alternate host scale/host plant combinations may provide refugia and avenues for population movement both within and between the major citrus-growing areas.

Although the dispersal ability of *A. melinus* has not been investigated specifically, reports of the parasitoid's spread following introduction into new areas provides a crude estimate of its mobility. From studying the spread of *A. melinus* in Greece following its introduction there, DeBach & Argyriou (1967) concluded that its effective rate of dispersal was 75–100 km per year, even across relatively barren land cultivated only in scattered areas. DeBach & Sundby (1963) noted that *A. melinus* could cross a 15-km barrier of barren high hills separating California's San Fernando and Simi Valleys. On a smaller scale, the ability of the parasitoid to spread throughout an Australian citrus grove within 10 months of its release was documented by Campbell (1976). Thus, *A. melinus* appears to have a well-developed ability to disperse.

The diffuse nature of the host pool of *A. melinus*, coupled with the parasitoid's mobility, indicated that an investigation of both the history of pesticide use in the grove where the population sample was collected and some measure of pesticide use in the surrounding areas would be necessary to adequately describe the historical selection pressures experienced by the population. Although not precise and not explaining all of the variability in  $LC_{50}$ 's of the populations sampled, the combined influences of these two factors appear to provide a means of explaining the essential features of the observed patterns. Part of the unexplained variability may also be attributable to the artificial movement of *A. melinus* populations resulting from the activities of commercial insectaries and biological control workers.

Unfortunately, it is difficult to compare these conclusions with those drawn from other studies dealing with either beneficial or pest arthropods. Many authors have hypothesized that either the local history of pesticide use (e.g., Georghiou 1966, Herne 1971, Penman et al. 1976, Strawn 1978, Hoy & Knop 1979, Schoonees & Giliomee 1982, Mansour 1984, Quisenberry et al. 1984, Robertson & Stock 1985, Schmidt et al. 1985, Georghiou 1986), regional history of pesticide use (e.g., Georghiou 1972, Grafton-Cardwell & Hoy 1985), or some combination of the two (e.g., Follett et al. 1985) have generated patterns of variable resis-

tance observed. Although suggestive data are presented in these studies, we are not aware of any instance in which these hypotheses have been tested statistically. Hopefully, future studies will include analyses of 1) past selection pressures, and 2) the roles of migration and host distribution in affecting the relative importance of local and regional patterns of pesticide use. Progress in the nascent field of resistance management, which attempts to prolong the useful life of pesticides by slowing or halting the evolution of resistance, is dependent upon the ability to implement specific pesticide use strategies. The results of the studies we propose should be relevant to determining the geographic scale over which such resistance management programs should be implemented for optimal effect.

**Implications for IPM.** The results of this study are encouraging because the observed trend towards increased resistance levels indicate a possible means of increasing the effectiveness of *A. melinus* within an IPM framework. However, for at least three reasons, these results are not yet cause for complacency. First, levels of resistance exhibited by the populations sampled were not sufficient to enable them to survive field application rates of commonly used insecticides. This conclusion is in accordance with other recent studies performed both in the laboratory (Bellows et al. 1985, Morse & Bellows 1986) and the field (Griffiths et al. 1985). Second, we have no way to predict whether or not the observed trend towards increased resistance will continue in the future. Finally, *A. melinus* does not exist in an ecological vacuum. While populations of this parasitoid may be evolving increased tolerance levels, the pest populations against which the chemicals are being applied may simultaneously be evolving increased resistance. Growers may be forced, thereby, to increase their insecticide application rates or shift to the use of new insecticides. These responses represent an intensification of old selection pressures and the creation of new selective pressures upon the parasitoid population. Thus, the parasitoid may be engaged in an evolutionary race to resistance with the key pests of citrus.

Since the introduction of *A. melinus* in 1957, California citrus growers have had relatively few problems with resistant insect pests. For all the insecticides tested, the recommended application rates have remained essentially unchanged from 1957 to the present (Table 4). A warning regarding resistant populations does, however, accompany the recommended application rates for dimethoate for thrips control. The citrus thrips is the only key insect pest of citrus that has recently developed resistance to an insecticide widely used for its control (Morse & Brawner 1986). The declining effectiveness of dimethoate is reflected in the increasing application rates used by some growers. Fig. 4 provides one such example taken from a grove in Butte County. Within the last few years, many growers have begun using insecticides other than dimeth-

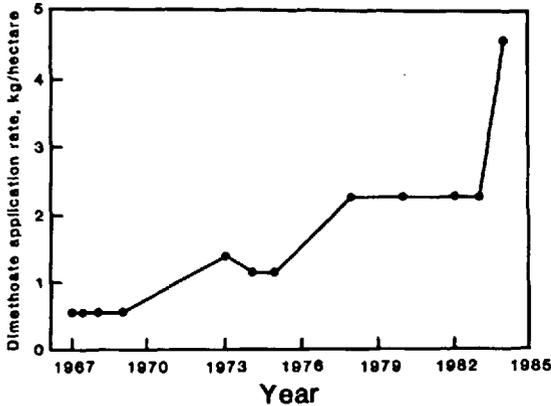


Fig. 4. Dimethoate application rates for citrus thrips control in a Butte County navel orange grove, 1967–84.

oate for thrips control. Thus, *A. melinus* may already have missed the opportunity to become resistant to dimethoate. The other insecticides tested in this study continue to be used, but further increases in the resistance levels exhibited by *A. melinus* will be necessary before they can be used selectively.

One means to obtain these additional increases in resistance is through a program of artificial selection in the laboratory. Artificial selection has been successful in producing populations of predator mites (Roush & Hoy 1981, Hoy 1985a) and a predaceous insect (Grafton-Cardwell & Hoy 1986) that are able to survive exposure to field concentrations of insecticides. Thus far, selection of insect parasitoids has been unsuccessful. Based upon our observations of increased resistance levels in populations of *A. melinus* and the retention of intrapopulation variation in resistance levels in these populations, *A. melinus* appears to be an appropriate subject for a program of artificial selection.

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