Genetic Improvement of a Parasitoid Biological Control Agent: Artificial Selection for Insecticide Resistance in *Aphytis melinus* (Hymenoptera: Aphelinidae)

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ABSTRACT Populations of *Aphytis melinus* DeBach, a parasitoid of California red scale, *Aonidiella aurantii* (Maskell), in citrus, were artificially selected for increased tolerance to five insecticides (carbaryl, chlorpyrifos, dimethoate, malathion, and methidathion) commonly used in citrus IPM. Selection produced gradual, small to moderate increases in resistance, accompanied in 3 of 12 selected lines with apparent decreases in genetic variation for pesticide tolerance. LC_{50}'s for carbaryl increased to >5 times that of the original field population and approximately 20 times that of a susceptible natural population. Major resistance phenotypes were not detected in large field samples of *A. melinus*, suggesting that such phenotypes are either present at low frequencies ($<5.2 \times 10^{-4}$ for dimethoate resistance) or are absent from the sampled populations. Two bioassay techniques, using one or two treated leaf surfaces, were compared as means of assessing toxicity of field-weathered pesticide residues to parasitoid colonies selected for pesticide resistance. The two-leaf bioassays of carbaryl residues revealed >50% survival of the carbaryl-selected strain as early as 18 d after treatment, whereas the unselected colony continued to suffer >86% mortality on 75-d-old residues (the oldest tested). Because larval *A. melinus* are protected from insecticides within their scale insect hosts, this observed increase in carbaryl tolerance of adults may enable the carbaryl-resistant *A. melinus* populations to persist in groves treated with carbaryl.

KEY WORDS Insecta, genetic improvement, integrated pest management, pesticide resistance

SUCCESSFUL INTEGRATION of biological and chemical control strategies in insect pest control programs is a major challenge facing applied entomologists. The frequent incompatibility of these two keystones of integrated pest management (IPM) has resulted in widespread target-pest resurgences, secondary pest outbreaks, and accelerated evolution of pesticide resistance, thereby incurring significant economic and environmental costs (Metcalf 1986). Several approaches to this problem have been pursued, including the use of ecologically or physiologically selective insecticides (Hull & Beers 1985, Mullin & Croft 1985) and naturally or artificially selected natural enemies resistant to insecticides (Croft 1982; Hoy 1985, 1987).

Artificial selection for pesticide resistance has been successful with several predatory phytoseiid mites (Avella et al. 1985, Hoy 1985, Markwick 1986, Huang et al. 1987) and the predatory green lacewing, *Chrysoperla carnea* (Stephens) (Grafton-Cardwell & Hoy 1986). Several attempts have been made to artificially generate resistant parasitoid biological control agents (Pielou & Glasser 1952, Robertson 1957, Adams & Cross 1967, Abdelrahman 1973, Havron 1983, Delorme et al. 1984, Hsieh 1984), but no selection response has been demonstrated to be adequate to incorporate the selected strain into an IPM program.

How might recent insights into the host–parasitoid–pesticide interaction and the ecological genetics of resistance evolution be incorporated into a redesigned program of artificial selection? Previous parasitoid selection programs have, with few exceptions (Adams & Cross 1967, Havron 1983), been initiated with either long-term laboratory colonies or a single field collection, thereby probably failing to sample naturally existing genetic variation effectively. As reviewed by Whitten & McKenzie (1982), Keiding (1986), and Roush & McKenzie (1987), gradual laboratory selection of small colonies often produces low-level, polygenically determined resistances, whereas intense natural selection in the field can exploit novel, rare genetic variation to produce single, major gene resistances. This suggested designing an artificial selection project to include large numbers of field-collected parasitoids, colony management practices designed to reduce the severity of genetic bottlenecks that may occur during establishment of laboratory colonies, and the use of a selecting dose that is high relative to the susceptible population's distribution of pesticide tolerances. Also, as proposed by Roush & McKenzie (1987), laboratory selection is more likely to generate single major gene resistances when laboratory colonies are

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founded from populations that have experienced strong pesticide pressures in the field. Thus, we surveyed geographical patterns of pesticide use and naturally occurring variation in pesticide tolerance to identify potential foci of incipient resistance in *Aphytis melinus* DeBach (Rosenheim & Hoy 1986).

Previous artificial selection studies with parasitoids also failed to evaluate selected lines under field or simulated field conditions. Instead, most studies have attempted to predict parasitoid performance from assessments of the adult's ability to survive on fresh pesticide residues in the laboratory. However, recent studies have highlighted one of the dominant features of the host–parasitoid–pesticide interaction—the existence of protected, immature parasitoid stages (see reviews by Hassan et al. 1987, Theiling & Croft in press). Host-protected immature parasitoids can often survive field applications and thereby continue to develop successfully to the more pesticide-susceptible adult stage. Upon emergence from the host, the adult parasitoid may then encounter pesticide residues that have decreased in toxicity for intervals up to the duration of the parasitoid's developmental period. Therefore, a critical factor for predicting the effect of pesticides on a parasitoid population is the interaction between differential susceptibility of parasitoid developmental stages, parasitoid development rates, and pesticide residue degradation rates.

Aphytis melinus DeBach is the dominant biological control agent of California red scale, *Aonidiella aurantii* (Maskell), a key pest of citrus in California and other citrus-growing regions of the world (Rosen & DeBach 1979, Luck et al. 1986). Although the degree of control exerted by *A. melinus* varies across California's citrus-growing regions, its effectiveness is consistently impeded by the application of broad-spectrum insecticides for control of key citrus pests—California red scale, citrus thrips, *Scirtothrips citri* (Moulton), and several lepidopteran species (Griffiths et al. 1985). Attempts to identify physiologically selective insecticides for use in citrus IPM have been only partially successful (Bellows et al. 1985, Morse & Bellows 1986, Rosenheim & Hoy 1986, Bellows & Morse 1988), and effective ecologically selective application techniques are currently unknown. A survey of 13 field populations of *A. melinus* also failed to reveal fully resistant natural populations, although considerable levels of variability in response were found (1.8- to 7.8-fold differences at LC_{50}) (Rosenheim & Hoy 1986). However, immature stages of *Aphytis* spp. have been found to be largely tolerant of a broad range of pesticides applied at field rates; these chemicals include dimethoate, malathion, and methidathion (Davies & McLaren 1977, Strawn 1978, Cohen et al. 1987).

In this study we investigated the potential of artificial selection of extensively sampled *A. melinus* field populations to generate significantly increased levels of pesticide resistance. To determine if larval *A. melinus* protected from insecticides by their hosts would be able to survive on aged residues upon eclosing as adults, we also evaluated adult survival of the resistant and susceptible strains on field-weathered insecticide residues.

Materials and Methods

Colony Collection and Maintenance. Two series of field collections were made (Fig. 1). The first collection, a survey of 13 populations from across California in October 1984, has been described (Rosenheim & Hoy 1986). Three populations exhibiting the greatest levels of pesticide tolerance (the Stutsman, Moisi, and Pendery colonies described in Rosenheim & Hoy [1986] as colonies 1, 2, and 4, respectively) were retained for artificial selection during 1985–1986. These populations were collected in groves with moderate to high pesticide application frequencies in Tulare County, the California county with the greatest per-hectare insecticide use (1980–1984) on citrus (Rosenheim & Hoy 1986).

The second series of collections was made in October 1985 and included four commercial citrus groves and two smaller residential plantings in Tulare County and one commercial grove in Madera County. Citrus fruits bearing parasitized California red scale were collected and held in the laboratory for *A. melinus* emergence. Emerging parasitoids were either combined to form the aggregation colony (founded with 541 individuals, derived in approximately equal numbers from the seven sites); or exposed to insecticides to isolate the relatively more tolerant individuals, which were then used to establish "preselected" colonies (Fig. 1, and see below). All three colonies established in 1985 were initiated by caging field-collected *A. melinus* directly on excess host material (*Aspidiotus nerii* Bouché), using 30-ml plastic cups (Anchor Hocking Plastics, St. Paul, Minn.) held in place with Mortite caulking cord (Mortell Company, Kankakee, Ill.). In a pilot study, this procedure yielded successful oviposition by each of 29 individually confined field-collected female *A. melinus* (unpublished data), thereby minimizing genetic bottlenecks associated with adaptation to laboratory conditions. Field-collected *A. melinus* not confined with *A. nerii* had previously been observed to exhibit variable, and in some cases very low (<10% of normal), oviposition rates (unpublished data).

All parasitoid colonies, including the selected lines and the unselected colonies from which they were derived, were maintained in the laboratory at 27 ± 2°C and a photoperiod of 16:8 (L:D). Uniparental oleander scale, *A. nerii*, grown under constant darkness at 24 ± 1°C on pink banana squash, *Cucurbita maxima* Duchesne, were provided as hosts. Honey was provided in all colony cages.

Artificial Selection. Adult parasitoids of both sexes (0–6 d old) were collected for selection by placing squash bearing parasitized *A. nerii* in an emergence cage. The emergence cage was light-

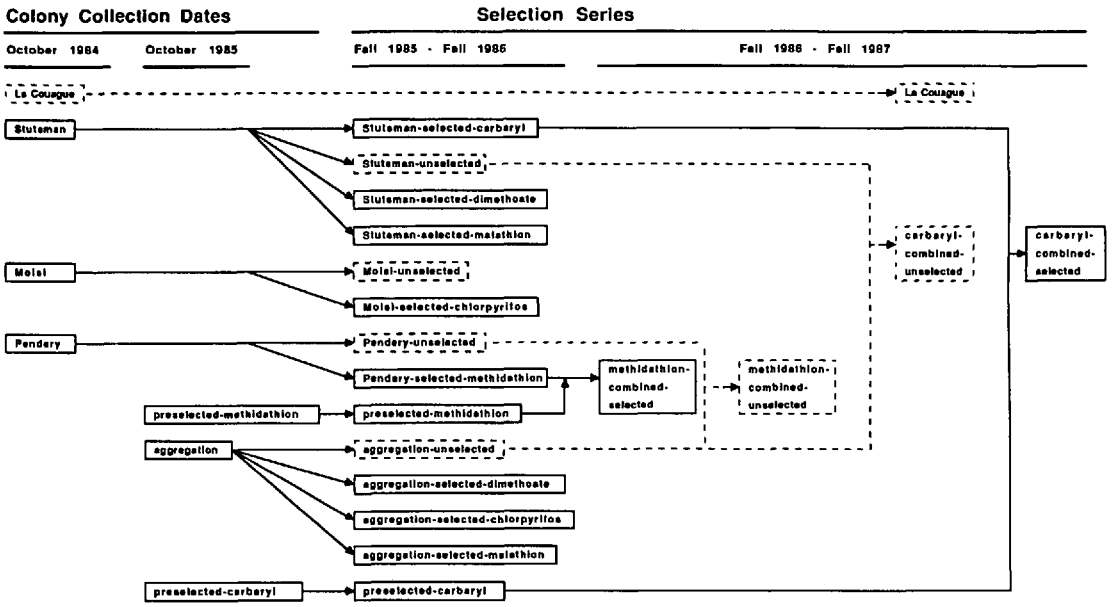

Fig. 1. Colony collections and establishment of selected and unselected colonies of *A. melinus* for artificial selection of insecticide resistance. Two series of field collections (October 1984 and October 1985) and two series of artificial selections (Fall 1985–Fall 1986 and Fall 1986–Fall 1987) were performed. Solid boxes, selected colonies; dashed boxes, unselected colonies or standard susceptible colony (La Couague). The preselected colonies were founded directly from field-collected *A. melinus* and therefore do not have true unselected colonies. However, because the aggregation base colony was established with parasitoids collected simultaneously from the same sites, it was used for comparison.

proof except for 36 removable glass test tubes inserted at the cage top, each streaked with honey. Parasitoids congregated in the test tubes because of their positive phototropism and negative geotropism. Emergence cages, which were held in greenhouses, were effective only when the weather was at least partially sunny; parasitoids were collected with a manual aspirator if emergence was insufficient on cloudy days. Disposable plastic cups (30 ml) and polyester gauze (Poly Puff brand, P & B Fabrics, San Francisco) were treated by dipping them for 5 s in commercial-grade insecticide solutions formulated in distilled water with a spreader (0.025% Triton AG-98; Rohm & Haas Company, Philadelphia). The cups were drained onto paper toweling, the gauze was pressed to remove excess solution, and both were air-dried in a hood. The cups capped with the gauze were then used as exposure vials. Honey was provided on an untreated strip of black vinyl electrician's tape (Manco, Cleveland) (5 by 18 mm) affixed to the gauze cap.

Selection was done with three insecticides widely used for California red scale control—carbaryl (Sevin 80S [sprayable]; Union Carbide Chemical Company, Research Triangle Park, N.C.), malathion (Malathion 25S; American Cyanamid Company, Wayne, N.J.), and methidathion (Supracide 2EC [emulsifiable concentrate]; CIBA-GEIGY Company, Basel, Switzerland); one insecticide used for citrus thrips control (dimethoate; Cygon 400; American Cyanamid Company, Wayne, N.J.); and one insecticide used for control of California red scale and several lepidopteran pests (chlorpyrifos; Lorsban 4EC; Dow Chemical Company, Midland, Mich.). Parasitoids were also exposed to a control of water plus spreader during each selection. Approximately 25 *A. melinus* (both sexes) were tapped into each vial and held for 24 h at 26 ± 0.5°C and 74% RH under constant light. A sample of test vials was scored by counting the number of dead parasitoids (i.e., those unable to maintain a normal posture or walk at a rate >1 mm/s), then anaesthetizing the parasitoids with carbon dioxide (10 s) and counting the total number present. The sex of parasitoids in a sample of vials was also determined to monitor sex ratio during the course of the selection program. Parasitoids were then transferred into a new colony cage with fresh host material for oviposition. Mortality at 24 h was maintained at about 50% by increasing insecticide concentrations by about 50% as required.

The protocol used to establish the two preselected colonies from field-collected *A. melinus* was slightly modified; i.e., the age distribution of parasitoids was not controlled and insecticide concentrations were chosen based upon preliminary tests to generate >80% mortality. The preselected-carbaryl and preselected-methidathion colonies were founded with 249 and 207 *A. melinus* (both sexes), respectively.

To assess responses to selection, concentration–mortality data were generated for each colony be-

Table 1. Histories of pesticide use for citrus foliage collection sites (Tulare County, Calif.) for insecticide residue bioassays with *A. melinus*

Grove no.	Citrus variety	Residue age (d)	1987 collection date	1987 pesticide application[a]	
				Date	Material and rate (kg [AI]/ha)
			Carbaryl residues		
1	Valencia orange	3	25 Aug.	22 Aug.	Carbaryl 13.4 + 35 liters oil in 7,015 liters water
		18	9 Sept.	4 May	Formetanate HCl 1.3 in 2,338 liters water
				10 Apr.	Methomyl 0.9 in 4,677 liters water
2	Navel, Valencia orange	Background[b]	17 Aug.	18 Aug.	Carbaryl 13.4 + 35 liters oil in 7,015 liters water
		7	25 Aug.	1 May	Formetanate HCl 1.3 in 2,338 liters water
		22	9 Sept.	14 Apr.	Methomyl 0.9 in 140 liters water (by air)
3	Navel orange	8	9 Sept.	1 Sept.	Carbaryl 13.4 + 46.8 liters oil in an unknown volume of water
				30 Apr.	Formetanate HCl 1.3 in 935 liters water
4	Navel orange	14	9 Sept.	26 Aug.	Carbaryl 13.4 + 23.4 liters oil in 4,677 liters water
				2 June	Dimethoate 2.25 in 935 liters water
				30 Apr.	Formetanate HCl 1.0 + dibrom 2.24 in 935 liters water
5	Bonanza orange (small trees)	32	25 Aug.	24 July	Carbaryl 3.4 + 14 liters oil in 2,806 liters water
				1 May	Avermectin, unknown rate; experimental treatment
6	Valencia orange	38	17 Aug.	10 July	Carbaryl 13.4 + 42.1 liters oil in 7,015 liters water
				1 May	Formetanate HCl 1.3 in 2,338 liters water
7	Lemon	42	25 Aug.	14 July	Carbaryl 13.4 + 35 liters oil in 7,015 liters water
				1 May	Formetanate HCl 1.3 in 2,338 liters water
				13 Apr.	Methomyl 0.9 in 140 liters water (by air)
8	Navel orange	48	17 Aug.	30 June	Carbaryl 13.4 + 35 liters oil in 7,015 liters water
				1 May	Formetanate HCl 1.3 in 2,338 liters water
				7 Apr.	Methomyl 0.68 in 2,338 liters water
9	Navel orange	75	17 Aug.	3 June	Carbaryl 13.4 + 35 liters oil in 7,015 liters water
				30 Apr.	Formetanate HCl 1.3 + methomyl 0.68 in 935 liters water
			Methidathion residues		
10	Navel orange	48	17 Aug.	30 June	Methidathion 4.2 in 7,015 liters water
				30 Apr.	Formetanate HCl 1.3 in 2,338 liters water
				7 Apr.	Methomyl 0.68 in 2,338 liters water
11	Navel orange	48	17 Aug.	30 June	Methidathion 3.37 in 2,338 liters water
				1 May	Formetanate HCl 1.3 in 2,338 liters water
				7 Apr.	Methomyl 0.68 in 2,338 liters water

[a] Carbaryl 80 wettable powder; Dibrom 8 emulsifiable concentrate; Dimethoate 267 emulsifiable concentrate; formetanate HCl, Carzol 92 sprayable; Methidathion 2 emulsifiable concentrate; Methomyl 90 sprayable; oil, narrow-range 415 oil.

[b] This foliage was bioassayed to assess the toxicity of background residues of formetanate HCl and methomyl to *A. melinus*.

fore selection and for both the unselected and selected lines at the conclusion of the selection regimes. For comparison, a concentration–mortality regression for carbaryl also was generated for a susceptible *A. melinus* colony collected in Orange County (La Couague population; colony 9 of Rosenheim & Hoy [1986]) (Fig. 1). The testing protocol was identical to that described above for selections except that parasitoids were 0–2 d old, between 10 and 20 parasitoids (both sexes) were confined per vial, and at least five concentrations and a water-spreader control were used. Unselected and selected lines were always tested simultaneously. Tests were repeated for 3–5 d for a total of 8–23 replicates per concentration (n = 469–1,667).

The three colonies established in 1984 and the three colonies established in 1985 were selected for approximately 1 yr (1985–1986) and then evaluated for their selection responses (Fig. 1). Some colonies were then discarded and some were combined—the aggregation and Stutsman colony lines selected with carbaryl were combined (then called "carbaryl-combined"), as were the aggregation and Pendery colony lines that were being selected with methidathion (subsequently called "methidathion-combined") (Fig. 1). The respective unselected colonies were also combined. Colonies were combined by placing 120–150 mixed-sex *A. melinus* from each of the two parent lines into a new colony cage with fresh host material; the exact relative contribution of each parent line to the ensuing generations is unknown. Selection with carbaryl or methidathion on the combined colonies was continued for a second year (1986–1987). For logistical reasons rather than in response to perceived differ-

ences in the selection responses to the different insecticides, selection with chlorpyrifos, dimethoate, and malathion was discontinued.

Residue Bioassays. To relate selection results to the survival of *A. melinus* under field conditions, laboratory bioassays were done with foliage collected from carbaryl- or methidathion-treated citrus groves. Collections were made during August–September 1987 in Tulare County; 1987 insecticide applications for the foliage collection sites are summarized in Table 1. Leaves from 10 trees (replicates) were collected from the SE and SW tree quadrants at a height of approximately 2 m and were refrigerated for up to 6 d in plastic bags until used. All groves treated with carbaryl were sprayed with 13.4 kg (AI)/ha except for grove 5, which, because of its small trees and interplanting with olive, received only 3.4 kg (AI)/ha (Table 1). Methidathion was applied at 3.37 or 4.2 kg (AI)/ha (Table 1). Untreated (control) citrus leaves were collected in Berkeley, Calif. Bioassays comparing the responses of different parasitoid colonies to foliage from a given citrus grove were done simultaneously.

Bioassay units were constructed with two citrus leaves, both oriented with the upper surface upright, forming the chamber's floor and ceiling; parasitoids were therefore exposed to both the upper and lower leaf surface, approximately the natural condition in the field. Two thin sections of rigid acrylic plastic pipe (top piece height = 10 mm, lower piece height = 5 mm; inside diameter = 32 mm, outside diameter = 38 mm), each affixed to one leaf by dipping the joint surface in molten paraffin, were used to form the chamber walls. The two halves of the unit were held together with hair clips (Goody Products, New York). The two sections of plastic pipe were finely sanded to ensure a close fit without the need for glue. Two mesh-covered holes (5 mm diameter) in the upper section of plastic pipe allowed for passive air flow. A strip of paper toweling (10 by 2 mm) soaked in honey was affixed to the wall of each unit. Parasitoids (approximately 25 per unit) were held in the bioassay units for 24 h at 26 ± 1°C and 74% RH under constant light.

Bioassay Methodology Comparison. Two experiments were done to explain the quantitative differences between the results of our two-leaf bioassays and the one-leaf bioassay results reported by Bellows & Morse (1988). The first experiment incorporated leaves from grove 2 (collected 9 September 1987; Table 1) into three bioassays: (1) standard two-leaf bioassay described above; (2) as above, but with air drawn through each bioassay unit (flow rate, 1.7 ml/s) to eliminate possible fumigation effects; and (3) as in (1) but with the upper treated leaf replaced by an untreated leaf to simulate the one-leaf bioassay condition.

A second experiment was done to determine the proportion of time spent by *A. melinus* on each of the three bioassay unit surfaces (floor, ceiling, and walls). Ten bioassay units (replicates) with untreated leaves, each with approximately five *A. melinus* placed on the unit's floor following carbon dioxide anaesthesia (10 s), were assembled and held under standard conditions for 24 h. Units were quickly opened and numbers of *A. melinus* on each surface were counted at 0.4, 4, 9, 19, and 24 h; counts were summed across readings to provide a single score for each replicate.

Statistical Analysis. Concentration–mortality data were analyzed by probit analysis (POLO; Russell et al. 1977), which tests hypotheses of parallelism (equal slopes) and equality (equal slopes and intercepts) with likelihood-ratio tests (Savin et al. 1977). Mortality estimates for each selected generation were corrected for control mortality with Abbott's (1925) formula and are presented as described by Elston (1969) as confidence intervals for the quotient of normal variates. Field residue bioassay data and bioassay methodology comparisons were analyzed with pairwise contrasts using Bonferroni's inequality to maintain an overall $\alpha < 0.05$ (Dixon 1985); separate variance t tests (Welch model) were used in the BMDP computer statistical package, program P7D (Dixon 1985).

Results

Preselection Screenings. Colonies preselected with a single treatment of 19.2 mg (AI)/liter carbaryl or 3.0 mg (AI)/liter methidathion were established successfully. These initial selections generated 78.9% (n = 2,005) and 89.5% (n = 2,825) mean corrected mortality, respectively. In contrast, survivors of screenings with 7.2 and 8.4 mg (AI)/liter dimethoate, which generated 90.4% (n = 2,929) and 98.5% (n = 2,886) mean corrected acute mortality after 24 h, were unable to reproduce because of sublethal insecticide effects, and the colony could not be established. Because the dimethoate prescreening dosages were equivalent to only about 4 times the acute LC_{50} for these populations (see below), major dimethoate resistance phenotypes are apparently rare or absent from the sampled populations.

Artificial Selection. Responses to artificial selection for insecticide resistance during the first year (1985–1986) were consistently positive but small to moderate (Table 2). LC_{50}'s for the 10 selected lines had increases of 1.5–2.6 times the corresponding unselected colonies. Three of the 10 selected lines (Stutsman-selected-carbaryl, Moisi-selected-chlorpyrifos, and preselected-methidathion) also showed significantly increased slopes for the concentration–mortality regressions (increases of 1.6–1.7 times relative to the unselected colonies; Table 2), suggesting that decreases in intracolony genetic variation for pesticide tolerance had occurred. Selection responses obtained for the aggregation and preselection lines initiated in 1985 (increases of 1.5–2.0 times in LC_{50}'s) were of magnitude similar to those obtained for the single-population lines

Table 2. Results of artificial selection for insecticide resistance in *A. melinus*

Insecticide Colony name	Colony type	No. selections	Total no. generations[a]	Mean approx. no. parasitoids per selection ($\bar{x}$ ± SD)[b]	Mean % mortality ($\bar{x}$ ± SD) per selection after 24 h[c]	Concentration/mortality regressions				
						Bioassays conducted before or after selections	*n*	Slope ± SEM[d]	LC_{50} (95% CL) in mg (AI)/liter	Resistance ratio (LC_{50} selected line/LC_{50} unselected line)
Carbaryl (suggested field rate[e]: 960 mg [AI]/liter)										
Stutsman	Unselected	0	—	—	—	Before	870	3.17 ± 0.25	6.81 (6.09–7.55)	0.90
Stutsman	Unselected	0	—	—	—	After	730	2.97 ± 0.25	7.59 (6.50–8.81)	1.00
Stutsman	Selected	10	15	1,079 ± 1,055	57.9 ± 13.7	After	907	4.59 ± 0.38*	19.96 (17.41–22.37)*	2.63
Aggregation	Unselected	0	—	—	—	Before	469	3.81 ± 0.36	5.20 (4.39–5.98)	0.91
Aggregation	Unselected	0	—	—	—	After	1,449	3.49 ± 0.19	5.70 (4.39–5.98)	1.00
Preselected	Selected	9	11	689 ± 647	49.1 ± 17.2	After	1,149	2.98 ± 0.21	11.50 (10.46–12.64)*	2.02
La Couague	Standard susceptible[f]	0	—	—	—	After	925	2.58 ± 0.25	1.55 (1.28–1.80)*	0.26
Carbaryl-combined	Unselected	0	—	—	—	After	1,250	2.92 ± 0.31	5.97 (4.87–7.00)	1.00
Carbaryl-combined	Selected	12	15	656 ± 293	70.9 ± 18.1	After	593	2.90 ± 0.22	30.60 (26.13–35.12)*	5.13
Chlorpyrifos (suggested field rate: 450 mg [AI]/liter)										
Moisi	Unselected	0	—	—	—	Before	830	4.06 ± 0.34	0.29 (0.25–0.32)*	1.71
Moisi	Unselected	0	—	—	—	After	623	3.78 ± 0.28	0.17 (0.14–0.20)	1.00
Moisi	Selected	8	14	792 ± 469	43.4 ± 17.8	After	764	5.88 ± 0.46*	0.38 (0.35–0.41)*	2.24
Aggregation	Unselected	0	—	—	—	Before	858	4.48 ± 0.34	0.22 (0.19–0.24)*	1.57
Aggregation	Unselected	0	—	—	—	After	1,220	4.49 ± 0.32	0.14 (0.13–0.16)	1.00
Aggregation	Selected	8	14	1,311 ± 553	45.9 ± 20.9	After	911	5.13 ± 0.35	0.24 (0.23–0.27)*	1.71
Dimethoate (suggested field rate: 1,200 mg [AI]/liter)										
Stutsman	Unselected	0	—	—	—	Before	764	2.93 ± 0.25	2.34 (1.88–2.77)	1.00
Stutsman	Unselected	0	—	—	—	After	1,030	3.45 ± 0.22	2.33 (2.05–2.58)	1.00
Stutsman	Selected	8	13	612 ± 525	45.3 ± 13.9	After	799	2.93 ± 0.21	4.02 (3.05–4.58)*	1.73
Aggregation	Unselected	0	—	—	—	Before	1,349	3.37 ± 0.22	2.12 (1.86–2.36)	1.01
Aggregation	Unselected	0	—	—	—	After	1,121	3.52 ± 0.18	2.10 (1.92–2.29)	1.00
Aggregation	Selected	8	14	923 ± 630	46.8 ± 15.2	After	812	3.90 ± 0.35	3.48 (2.99–4.04)*	1.66
Malathion (suggested field rate: 720 mg [AI]/liter)										
Stutsman	Unselected	0	—	—	—	Before	1,667	3.46 ± 0.18*	0.91 (0.81–1.00)*	0.91
Stutsman	Unselected	0	—	—	—	After	1,235	4.23 ± 0.30	1.00 (0.89–1.11)	1.00
Stutsman	Selected	10	15	840 ± 581	59.3 ± 19.1	After	1,090	4.42 ± 0.34	2.58 (2.38–2.79)*	2.58
Aggregation	Unselected	0	—	—	—	Before	975	5.03 ± 0.33	1.19 (1.09–1.28)*	1.51
Aggregation	Unselected	0	—	—	—	After	1,036	4.88 ± 0.28	0.79 (0.74–0.84)	1.00
Aggregation	Selected	8	14	714 ± 402	43.8 ± 18.8	After	1,469	5.24 ± 0.29	1.32 (1.22–1.41)*	1.67
Methidathion (suggested field rate: 300 mg [AI]/liter)										
Pendery	Unselected	0	—	—	—	Before	1,124	3.30 ± 0.26*	1.21 (1.07–1.34)*	0.83
Pendery	Unselected	0	—	—	—	After	494	4.53 ± 0.53	1.13 (0.95–1.27)	1.00

Table 2. Continued

Insecticide Colony name	Colony type	No. selections	Total no. generations[a]	Mean approx. no. parasitoids per selection ($\bar{x} \pm$ SD)[b]	Mean % mortality ($\bar{x} \pm$ SD) per selection after 24 h[c]	Concentration/mortality regressions: Bioassays conducted before or after selections	*n*	Slope ± SEM[d]	LC_{50} (95% CL) in mg (AI)/liter	Resistance ratio (LC_{50} selected line/LC_{50} unselected line)
Pendery	Selected	8	12	1,092 ± 1,241	50.9 ± 18.3	After	641	4.27 ± 0.56	2.79 (2.23–3.23)*	2.47
Aggregation	Unselected	0	—	—	—	Before	895	3.97 ± 0.23	1.22 (1.13–1.32)*	1.33
Aggregation	Unselected	0	—	—	—	After	1,175	3.89 ± 0.23	0.92 (0.85–0.98)	1.00
Preselected	Selected	8	16	1,781 ± 1,569	71.4 ± 19.0	After	940	6.51 ± 0.56*	1.39 (1.28–1.49)*	1.51
Methidathion-combined	Unselected	0	—	—	—	After	999	4.39 ± 0.27	0.86 (0.78–0.93)	1.00
Methidathion-combined	Selected	9	14	1,201 ± 707	74.4 ± 13.3	After	765	4.64 ± 0.41	1.67 (1.38–1.90)*	1.94

[a] Because selection was omitted when *A. melinus* populations fell below about 250 individuals, the total number of generations exceeds the number of selections.
[b] Average sex ratio was about 2.5 females to 1 male.
[c] Mean mortalities are corrected for control mortality. Because of significant sublethal effects of organophosphorus insecticide exposure on *A. melinus*, the actual intensity of selection pressures was greater than indicated by these values (see discussion).
[d] Within each grouping of two to three colonies, slope and LC_{50}'s are compared with the final (after selection) concentration/mortality regression of the unselected colony; *, $P < 0.05$.
[e] Recommendations of the University of California Cooperative Extension citrus treatment guide (Morse & Bailey 1984). Rates cited for carbaryl, chlorpyrifos, malathion, and methidathion are for dilute applications for California red scale control. Rate cited for dimethoate is for dilute applications for citrus thrips control.
[f] The La Couague colony, collected in Orange County, Calif., was used for comparative purposes as a standard susceptible colony.

Fig. 2. Mean percentage of mortality at 24 h of *A. melinus* selected with carbaryl. Colonies selected were Stutsman (selection rounds 1–12) and carbaryl-combined (selection rounds 13–24). C, first selection of the combined colony; I, carbaryl concentration increased. Concentrations used (in mg [AI]/liter) were 9.6 (rounds 1–4), 14.4 (rounds 5–7), 19.2 (rounds 8–10), 24.0 (rounds 11–20), and 33.6 (rounds 21–24). All values are corrected for control mortality; 95% confidence intervals are presented when ≥3 control replicates were tested.

initiated in 1984 (increases of 1.7–2.6 times; Table 1).

The response to a second year of artificial selection (1986–1987) was positive for the carbaryl-combined line, whose resistance ratio increased to 5.1 relative to its unselected colony. However, no selection response occurred in the methidathion-combined line, whose resistance ratio remained near 2.0 (Table 1).

The greatest selection responses were evident in the lines selected with carbaryl; carbaryl mortality appeared to decrease at a consistent, gradual rate, with a single increase occurring following the mixing of the Stutsman and aggregation lines (Fig. 2). Concentration–mortality regressions for the carbaryl-combined-unselected and carbaryl-combined-selected lines and the relatively susceptible La Couague colony are shown in Fig. 3. The carbaryl-combined-selected line reached levels of carbaryl resistance approximately 20 times greater than that exhibited by the La Couague colony (Table 2, Fig. 3).

Parasitoid sex ratios were stable throughout the selection programs in all of the 12 selected lines (data not shown).

Residue Bioassays. Most of the citrus foliage sampled had received not only an insecticide application for California red scale control (carbaryl or methidathion, 3 June–1 September 1987) but also had received earlier applications for citrus thrips (usually formetanate hydrochloride, 30 April–4 May 1987) and lepidopteran pests (usually methomyl, 7 April–30 April 1987) (Table 1). The impact of background residues of formetanate hydrochloride (108 d old) and methomyl (125 d old) were assessed by sampling leaves from grove 2 on 17

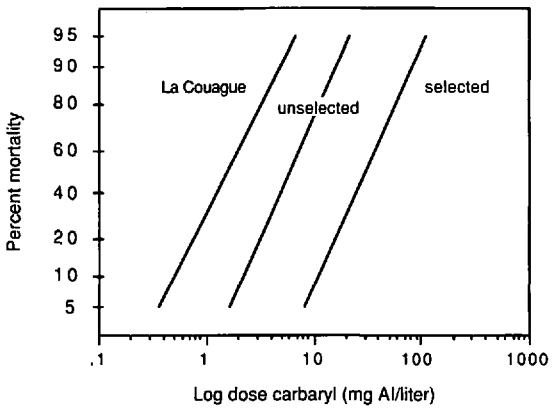

Fig. 3. Concentration–mortality regressions for three colonies of *A. melinus* tested with carbaryl. La Couague, susceptible population; unselected, carbaryl-combined-unselected colony; selected, carbaryl-combined colony, selected with carbaryl.

August 1987, 1 d before application of carbaryl. Mortalities at 24 h ($\bar{x} \pm$ SD) were (1) 1.0 ± 1.6%, (2) 3.0 ± 5.7%, and (3) 12.7 ± 9.8% for the carbaryl-combined-unselected, carbaryl-combined-selected (11 rounds), and La Couague colonies, respectively (the only significant difference was [1] versus [3]: $t = -3.73$; df = 9; $P = 0.0043$). Thus, background residues in this citrus grove made a small but perceptible contribution to the mortality estimate for the most susceptible colony (La Couague) and induced negligible mortality in the other colonies tested.

Mortality induced by carbaryl residues field-weathered for 3–75 d are illustrated in Fig. 4; mortality estimates are not corrected for mean control mortality, which was <0.75% for each of the three colonies tested. All residues tested generated ≥86.3% mortality in the carbaryl-combined-unselected colony and ≥98.7% mortality in the more susceptible La Couague colony. However, the carbaryl-combined-selected line exhibited less than 50% mortality as early as 18 d after spray. Mortality rates <50% were consistently observed beginning 32 d after spray.

Leaves sampled from two groves with 48-d-old methidathion residues caused different mortality, but in neither case was the difference between the selected and base colonies substantial. Leaves from grove 10 caused 60.4 ± 25.0% ($\bar{x} \pm$ SD) and 75.3 ± 28.6% mortality in the methidathion-combined-selected and methidathion-combined-unselected colonies, respectively. Grove 11 leaves produced 1.5 ± 2.5%, 7.6 ± 6.0%, and 58.2 ± 27.2% mortalities in the methidathion-combined-selected, methidathion-combined-unselected, and La Couague colonies, respectively. These figures are not corrected for control mortalities, which averaged 5.9 ± 0.5%, 10.5 ± 14.9%, and 0.0 for these three colonies, respectively.

Bioassay Methodology Comparison. Our conclusion that carbaryl residues remain highly toxic to unselected *A. melinus* colonies for at least 75 d after spray differs substantially from previous reports that residues degrade to levels inducing <30% mortality within 3 d after treatment (Bellows & Morse 1988). Therefore, we compared bioassay techniques. Mortality ($\bar{x} \pm$ SD) in (1) two-leaf (79 ± 4%), (2) two-leaf with forced air-flow (75 ± 10%), and (3) one-leaf (bottom) bioassay units (10 ± 2%) suggested that fumigation effects were insignificant ([1] versus [2]: $t = -0.36$; df = 11; $P = 0.73$), whereas the effect of reducing the pesticide-treated surface area was pronounced ([1] versus [3]: $t = 14.55$; df = 13; $P < 0.0001$) (Fig. 5). In the two-leaf bioassay unit, 51.6% of the interior area was treated. In the one-leaf unit, only one-half of this area (25.8% of the total interior area) was treated. The refuge effect was amplified by the preference of *A. melinus* for the underside of the upper leaf (Fig. 6). Thus, parasitoids spent 84.4% of their time on leaf surfaces in the two-leaf bioassay but only 15.6% of their time on the single leaf surface in the one-leaf bioassay. Although the possibility of residue repellency, which could potentially affect the time spent by *A. melinus* on treated leaf surfaces, was not specifically investigated, such effects were not suggested by extensive observations made while scoring bioassays.

Discussion

Artificial selection caused low-level increases (1.51–2.58 times) in the tolerance of *A. melinus* to four organophorphorus (OP) insecticides—chlorpyrifos, dimethoate, malathion, and methidathion. Parasitoid lines selected with chlorpyrifos, dimethoate, and malathion were subjected to artificial selection for only 1 yr (eight generations); additional selection responses similar to those observed for carbaryl might have occurred had selection been continued. Although we did not detect major OP-resistant phenotypes (see below), we cannot exclude the possibility that long-term selection with chlorpyrifos, dimethoate, or malathion might produce levels of pesticide resistance enabling survival in the field. Moderate increases (2.0–5.1 times) in the tolerance to carbaryl (a carbamate) occurred without an increase in the concentration–mortality regression slope value for the most resistant line (carbaryl-combined; Table 2). However, in 3 of the 12 selected lines (Stutsman-selected-carbaryl, Moisi-selected-chlorpyrifos, and preselected-methidathion), the increase in resistance was accompanied by an apparent loss of genetic variability for insecticide tolerance as reflected by the increasing slopes of the concentration–mortality regressions (Table 2). Thus, additional selection responses were not expected.

Aphytis melinus selected with carbaryl (carbaryl-combined line) were able to survive in substantial numbers on citrus foliage with field-weathered

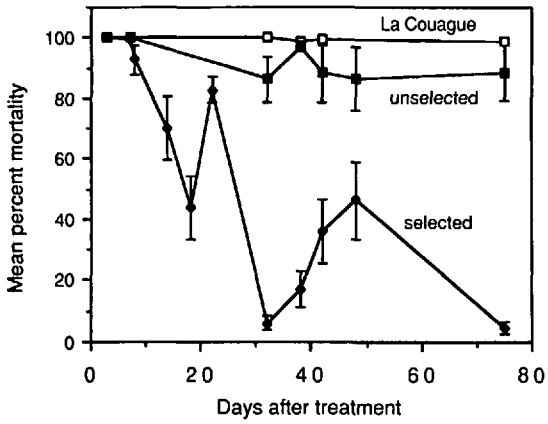

Fig. 4. Mean percentage of mortality of *A. melinus* exposed to citrus foliage bearing field-weathered carbaryl residues. For collection site characteristics see Table 1, groves 1–9. La Couague, susceptible population; unselected, carbaryl-combined-unselected colony; selected, carbaryl-combined colony, selected with carbaryl 11 times (with residues 3, 7, 32, 38, 42, 48, and 75 d old) or 12 times (with residues aged 8, 14, 18, and 22 d). Differences between La Couague and unselected colonies are nonsignificant in all cases ($P > 0.05$); differences between selected and either unselected or La Couague colonies are significant ($P < 0.05$) for all residues except those 3 and 7 d old.

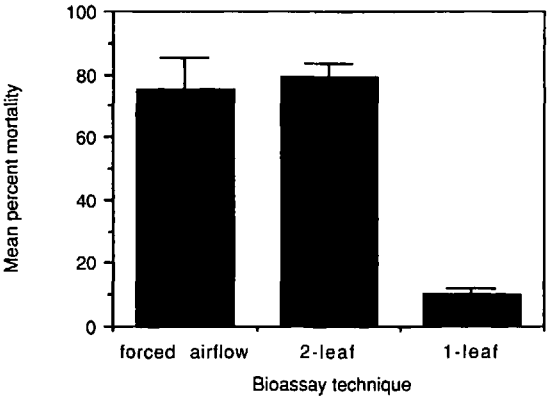

Fig. 5. Comparison of mean percentage of mortality at 24 h of *A. melinus* (carbaryl-combined colony) exposed to 22-d-old carbaryl residues (Table 1, grove 2) in three bioassays: left, two-leaf bioassay with forced air flow; center, two-leaf bioassay with passive air flow only; and right, one-leaf bioassay with passive air flow.

carbaryl residues beginning the third week after spray, compared with the nearly complete mortality of the unselected strain 11 wk after spray. Quantitative differences between our estimates of the duration of residue toxicity and those from a one-leaf bioassay design were caused at least in part by differences in the area and location of surfaces treated with pesticide. These differences were compounded by the preference of *A. melinus* for the ceiling of bioassay units, which are treated in the two-leaf bioassay but untreated in the one-leaf bioassay unit. Other differences between our 2-leaf bioassay and the 1-leaf bioassay technique of Bellows & Morse (1988), including the carbaryl application rate (1-leaf, 6.7 kg [AI]/ha; 2-leaf, mainly 13.4 kg [AI]/ha), bioassay duration (1-leaf, 48 h; 2-leaf, 24 h), weather conditions in the field during the period of residue degradation, and intrinsic differences in the carbaryl tolerance of the *A. melinus* colonies tested, may also have contributed to the observed differences.

A problem commonly encountered during long-term culturing of many parasitoids in the laboratory (the breakdown of normal sex ratios) was not observed at any time during our study. This result may be explained by the fact that *A. melinus* is facultatively gregarious and has no premating period after eclosion; thus, *A. melinus* may commonly inbreed and have evolved a tolerance to inbreeding.

Resistance Detection. Ideas developed for the detection and monitoring of insecticide resistance in pest species as part of resistance management programs (e.g., Brent 1986, Roush & Miller 1986) are equally applicable to the search for resistance in beneficial organisms. We were unable to detect any major resistance phenotypes in populations sampled during the study. (We use the term resistance "phenotypes" rather than "genotypes" because we have not investigated the genetic basis for the observed variation in pesticide tolerance and because our bioassays measure phenotypic expressions of resistance traits.) What then can we say about the possible frequency of such phenotypes? Rearranging equation (1) of Roush & Miller (1986), we obtained an expression for the maximum frequency, f_{max}, of a resistance phenotype that would remain undetected with probability $(1 - P)$, given a sample of n individuals,

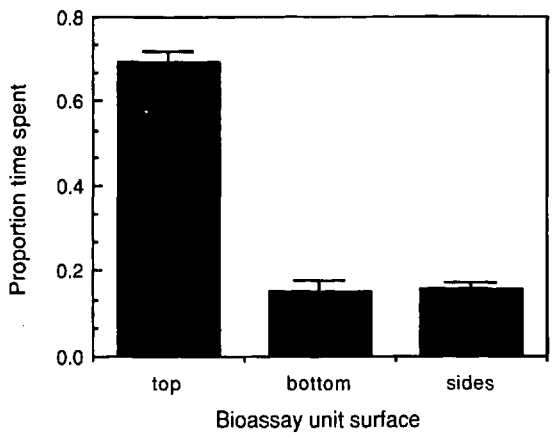

Fig. 6. Distribution of *A. melinus* over the three internal surfaces of the untreated two-leaf bioassay unit averaged over 24 h.

$$f_{max} = 1 - e^{[\ln(1-P)/n]}, \quad (1)$$

assuming that the bioassay is perfectly diagnostic. (f_{max} may also be thought of as the minimum frequency of a resistance phenotype that would have been detected with probability P.) With $P = 0.95$, data from our line preselected with dimethoate, in which $n = 5{,}754$ parasitoids were tested with high discriminating doses (i.e., doses killing all susceptible individuals), yield an estimate of f_{max} for dimethoate of 5.2×10^{-4}, assuming that individuals with major dimethoate resistance phenotypes would have survived. If we assume that any *A. melinus* with major resistances to carbaryl or methidathion would have been identified during the >20 rounds of artificial selection with the lines preselected with carbaryl ($n = 2{,}005$) and methidathion ($n = 2{,}825$), we estimate f_{max} values of 1.49×10^{-3} and 1.06×10^{-3} for carbaryl and methidathion, respectively. Therefore, major resistance phenotypes for these insecticides are either present at frequencies lower than these or are absent altogether from the sampled populations.

Artificial Selection. Selection responses observed were generally small and gradual (Table 2, Fig. 2). A decreasing rate of response to selection was evident in the methidathion-combined line (Table 2) and may be inferred from the increased slopes of the concentration–mortality regressions for the preselected-methidathion and Moisi-selected-methidathion lines. No decrease in the rate of selection response or increase in slope was evident for the carbaryl-combined line, however (Table 2, Fig. 2), suggesting that a potential for further increases in levels of carbaryl resistance remains.

The mean mortality figures presented in Table 2 imply that we used a relatively modest selection intensity. However, these figures are misleading because they fail to incorporate the significant delayed mortality and depressed rates of progeny production observed in *A. melinus* exposed to OP insecticides (Rosenheim & Hoy 1988). Exposure to OP insecticides used in this study at rates that generated approximately 50% acute mortality after 24 h actually reduces total *A. melinus* progeny production by approximately 90–95% (Rosenheim & Hoy 1988). In contrast, sublethal effects of carbaryl exposure are not detectable (Rosenheim & Hoy 1988). Thus, parasitoid lines selected with OP insecticides probably experienced average effective selection intensities significantly greater than those experienced by the carbaryl-selected lines.

One possible reason for the slow selection response observed may be that our selection regime acted effectively only on female phenotypes. *Aphytis* spp. females mate only once and do not have premating periods after eclosion (Rosen & DeBach 1979). Under our high-density rearing conditions, females were often mated within seconds of emergence (unpublished data). Thus, mating generally preceded selection, and male genomes were probably transmitted randomly with respect to pesticide resistance throughout the selection regime. The slow selection responses also suggest that the resistances generated have a polygenic basis.

Implications for Citrus IPM. The carbaryl-resistant *A. melinus* strain holds promise for successful incorporation into citrus IPM programs. Data shown in Fig. 4 show that substantial survival occurred on foliage bearing carbaryl residues 2–3 wk old. Our experimental design was chosen to provide some estimate of the intergrove variability of residue deposits, which were substantial (Fig. 4); however, on average, our estimates of carbaryl residue toxicity may be conservative. We sampled groves sprayed with relatively high carbaryl concentrations (13.4 kg [AI]/ha in 7,015 liters of water instead of the more typical 14,030–23,380 liters of water carrier; Morse & Bailey 1984). Reducing the volume of water carrier results in greater foliar residues and increased duration of residue toxicity to *A. melinus* (Bellows & Morse 1988). In addition, sampled foliage generally bore heavy deposits of dust and dirt, which sorb pesticides and may prolong their residual activity (Adams et al. 1976). Sorption also may have contributed to the differences between the residue toxicities observed by us and Bellows & Morse (1988), who sampled a grove with very clean leaves.

Increased carbaryl tolerance in *A. melinus* should enable augmentative releases of commercial insectary-reared parasitoids to be made sooner after an application of carbaryl. Carbaryl is currently recommended for control of several armored and soft scales, a complex of lepidopteran pests (during nonbloom periods only), and is effective against the Fuller rose beetle, *Pantomorus cervinus* (Boheman) (Morse & Bailey 1984, Haney et al. 1987). Rates recommended for several of these pests are substantially lower than those used for control of California red scale (Morse & Bailey 1984). Perhaps more significantly, increased carbaryl tolerance may allow *A. melinus* populations resistant to carbaryl to persist in sprayed groves once established, reducing or eliminating the need for augmentative releases. During the spring, summer, and fall months (1 April–1 October), when most pesticide applications are made, egg-to-adult development of *A. melinus* in the field is estimated to require 14–50 d depending on exact local temperature regimes (Yu & Luck 1988; D. S. Yu, University of California, Riverside, personal communication). Thus, carbaryl-resistant immature *A. melinus* present at the time of application, after developing to adults and eclosing, may encounter carbaryl residues that have degraded below toxic levels. Because carbaryl controls such a broad range of important insect pests of citrus, including all but the citrus thrips (which may be suppressed nondisruptively with the bait-formulated botanical insecticide sabadilla [Morse & Bailey 1984]), effective biological control of California red scale may be possible while outbreaks of other pests are controlled with carbaryl. Additional studies of the fitness, genetic basis for

resistance, and field performance of the carbaryl-resistant strain will be required to assess this possibility more fully.

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