The tiny wasp *Aphytus melinus* is an effective biological control of red scale in coastal and southern California citrus districts. Improving its tolerance of insecticides may add to its effectiveness in the San Joaquin Valley.

Selecting for insecticide resistance in a California red scale parasitoid

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Natural insecticide resistance of a biological control agent of California red scale was augmented in the laboratory, suggesting that selected strains of the parasitoid,* Aphytis melinus, *may be able to survive in citrus groves sprayed with carbaryl.

A serious impediment to successful integrated pest management (IPM) is the frequent incompatibility of biological and chemical control tactics. The disruption of biological control by broad-spectrum insecticides may encourage resurgence of target pests and outbreaks of secondary pests, creating a need for more pesticide applications. Greater pesticide use results in higher direct and indirect costs such as environmental pollution and faster buildup of pesticide resistance in insects.

One solution to the incompatibility problem is to modify the biological control agent itself. This approach involves either identifying and distributing field-evolved pesticide-resistant strains of the agent or artificially generating resistant strains.

The goal of our study was to produce a pesticide-resistant strain of a tiny wasp, the parasitoid *Aphytis melinus* DeBach, that could control California red scale, *Aonidiella aurantii* (Maskell), in pesticide-treated citrus groves.

Although several potentially serious citrus pests—soft scales, armored scales, mealybugs, and whiteflies—are under biological control in some California citrus-growing areas, insecticides are still used against citrus thrips, *Scirtothrips citri* (Moulton), citrus red mite, *Panonychus citri* (McGregor), and a number of lepidopteran species. Biological control of California red scale by *A. melinus* varies in different citrus-growing regions of California, but in all regions it is hampered by the use of broad-spectrum insecticides. We identified natural populations of *A. melinus* with elevated pesticide tolerances and have increased these tolerances through artificial selection in the laboratory.

Variation in resistance

To learn if natural selection in the field had built higher levels of insecticide tolerance in wild populations of *A. melinus*, we collected 13 parasitoid populations from citrus groves in the northern Sacramento Valley, the San Joaquin Valley, and coastal and interior southern California. Pesticide use information was obtained for each collection site for 1980 to 1984 at two levels: local, or in-grove use, gathered from grower or pest control advisor records, and regional, or county-wide use, gathered from unpublished records of the California Department of Food and Agriculture, Division of Pest Management.

Aphytis melinus colonies were established in the laboratory and tested for sensitivity to five insecticides commonly used in citrus: carbaryl (Sevin, 80% S), chlorpyrifos (Lorsban 4EC), dimethoate (Cygon 400), malathion (25% WP), and methidathion (Supracide 2EC). Sensitivity tests consisted of placing 15 parasitoids of mixed sex, 0 to 2 days old, in 30 ml plastic cups containing dried insecticide residues. Honey was added to cups, which were then capped with untreated polyester gauze.

These tests revealed significant variation among colonies in resistance to all five insecticides. LC_{50} values ranged from 1.8-fold for carbaryl and for chlorpyrifos, to 2.9-fold for dimethoate, 7.8-fold for malathion, and 7.6-fold for methidathion. Previous carbamate plus organophosphate insecticide use, measured at the local and regional geographical scales, explained 62% of the variation. In-grove and county-wide pesticide use histories each made significant and approximately equal contributions.

These results suggest that, while individual growers influence the levels of resistance shown by their local *A. melinus*, the insecticide use patterns of neighboring growers may also play an important role. The high mobility of *A. melinus* appears to restrict the independent evolution of parasitoids living in neighboring citrus groves.

The geographical variability survey revealed an encouraging trend toward naturally evolved insecticide resistance in *A. melinus*. None of the surveyed populations, however, appeared to have developed sufficient resistance to survive field application rates of the insecticides tested. Whether additional resistance was likely to occur through natural selection in the field was hard to predict. Because insect pests of citrus may also be developing increased pesticide tolerance, *A. melinus* may be in an evolutionary race to resistance. A resistant strain of *A. melinus* will be useful only if it can be identified before the key pests of citrus become resistant to the insecticide in question. With this in mind, we attempted to augment resistance in *A. melinus* through artificial selection.

Artificial selection

We chose three *A. melinus* colonies collected during the 1984 survey that showed the highest levels of insecticide resistance. During 1985, six collections from the San Joaquin Valley were pooled to form an additional colony for artificial selection. The different parasitoid lines were separately selected for resistance to carbaryl, chlorpyrifos, dimethoate, malathion, and methidathion.

Parasitoids of mixed sex, 0 to 6 days old, were kept for 24 hours in insecticide-treated plastic cups capped with insecticide-treated polyester gauze. Insecticide concentrations were adjusted to provide 45% to 75% mortality within 24 hours. Actual selection intensities, however, were much greater (>90% effective mortality) for the organophosphorus insecticides (chlorpyrifos, dimethoate, malathion, and methidathion) because of their negative effect on surviving parasitoid longevity, reproductiveness, and progeny sex ratio. We detected none of these sublethal effects from carbaryl.

Parasitoids surviving insecticide exposure were provided with excess scale insect hosts (oleander scale, *Aspidiotus nerii* Bouché, grown on pink banana squash) for reproduction. During 1986, two parasitoid lines were selected 8 to 12 times with each of the five insecticides. For logistical reasons only, selection during 1987 was continued with only two lines, one selected for carbaryl resistance and the other for methidathion resistance.

The first year of artificial selection (1986) generated consistent low to moderate increases in insecticide resistance in *A. melinus.* Resistance ratios, calculated as the LC_{50} of the selected line divided by the LC_{50} of the corresponding base colony, increased to 2.02 and 2.63 for the two lines selected with carbaryl, 1.71 and 2.24 for chlorpyrifos, 1.66 and 1.73 for dimethoate, 1.67 and 2.58 for malathion, and 1.51 and 2.47 for methidathion. In the second year, the buildup of resistance halted in the methidathion-selected line (final resistance ratio of 1.94), but continued in the carbaryl-selected line, reaching a final resistance ratio of 5.13. This is equivalent to a 19.7-fold increase over the tolerance of the most susceptible colony detected in the 1984 survey (fig. 1).

The response to carbaryl selection was strong enough to suggest evaluating parasitoid survival under semi-field conditions. We designed our test to reflect a dominant feature of the host-parasitoid-pesticide relationship: although adult *A. melinus* are highly susceptible to fresh pesticide residues, immature forms are shielded from pesticides by the bodies of their scale insect hosts and have been found to be equally or more tolerant of pesticides than those hosts. Thus, immature *A. melinus* may survive pesticide applications and, on emerging later as adults, encounter pesticide residues that have lost some of their toxicity. A critical point in predicting the effect of pesticides on *A. melinus* is therefore the relationship between the rates of residue breakdown and parasitoid development.

Leaf bioassays

To evaluate the ability of the carbaryl-selected line to survive exposure to field-weathered carbaryl residues on citrus foliage, we tested leaves from commercial citrus groves in which carbaryl had been used to control California red scale. Parasitoids spent 84% of their time on leaf surfaces in the two-leaf test units designed to approximate field conditions.

These leaf bioassays revealed large differences in the ability of the carbaryl-selected line, the corresponding base colony, and

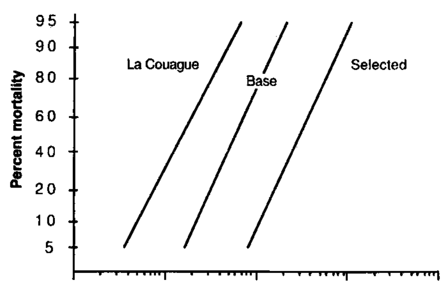

Fig. 1. Carbaryl concentration/mortality relationships for *A. melinus* parasitoids from the carbaryl-selected line, the corresponding base colony, and the relatively susceptible LaCouague colony. The carbaryl dose is presented on a logarithmic scale; major divisions on the bottom line represent 10-fold increases in dose.

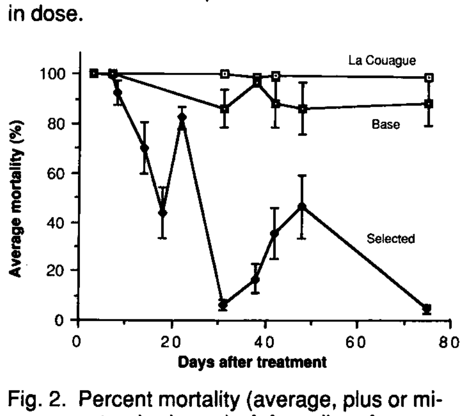

Fig. 2. Percent mortality (average, plus or minus one standard error) of *A. melinus* from three colonies on citrus foliage bearing field-weathered carbaryl residues.

the LaCouague colony (the most susceptible colony identified during the 1984 survey) to survive contact with field-weathered carbaryl residues (fig. 2). The oldest carbaryl residues tested (75 days old) continued to cause more than 86% mortality in the base colony and 98% mortality in the LaCouague colony. In contrast, the selected line showed more than 50% percent survival as early as 18 days after treatment. The fluctuations in mortality caused by residues of different ages probably are due to variation in how the carbaryl was originally applied and other differences between groves. Between April 1 and October 1, when pesticides are usually applied in citrus, egg-to-adult development of *A. melinus* requires 14 to 50 days, depending on local temperature conditions. Our results suggest that some carbaryl-selected parasitoids may be able to survive in groves sprayed with carbaryl.

Conclusions

Our studies have revealed significant variation in pesticide resistance among field populations of the parasitoid *A. melinus.* Resistance was influenced by past selection pressures at both local and regional scales. Artificial selection in the laboratory improved resistance, generating a carbaryl-resistant line with an LC_{50} 5.13 times as great as the corresponding base colony and 19.7 times that of a relatively susceptible natural population. Leaf bioassays revealed that carbaryl-resistant *A. melinus* adults survived in significant numbers on citrus foliage bearing 2- to 3-week-old field-weathered carbaryl residues.

Carbaryl-resistant *A. melinus* may be profitably incorporated into California citrus IPM programs in the coastal and southern interior citrus districts, where the parasitoid is very effective. It also has potential value in the San Joaquin Valley, where a program of inundative releases is currently being developed.

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