

Project No. 87-B11 - Navel Orangeworm Mite and Insect  
Research

**Control of Mites on Almonds**

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## INTERPRETIVE SUMMARY

Omite resistance in the Pacific spider mite (Tetranychus pacificus) was studied in a colony collected from an almond orchard in Kern County. The results of the analysis indicate that Omite resistance is determined by a major semi-recessive gene. (We can't exclude the possibility that modifying genes are also involved.) The resistance was also evaluated in populations held in the greenhouse without selection with Omite for 5 months. We compared the stability of the resistance in two colonies of the resistant strain (not selected), a resistant strain which continued to receive Omite selections, four colonies derived from the reciprocal F<sub>1</sub> progeny of crosses between the resistant and susceptible colonies, and two colonies of a susceptible colony. The results suggest that Omite resistance is stable; the unselected resistant colonies were highly resistant at the end of the experiment. The four colonies derived from crossing resistant and susceptible strains were also just as resistant as when they started out 5 months previously. This suggests that Omite resistance is unlikely to be lost rapidly once well established in San Joaquin Valley populations.

The implications for integrated mite management are important. Plictran/Vendex resistance was also found in T. pacificus, particularly in the Kern county region (work described in last year's report). Thus, in Kern County, populations exist that are resistant to both Vendex and Omite. Because Plictran is no longer registered for use in California, the number of available acaricides is dangerously low, particularly in the southern San Joaquin Valley. The speed with which new acaricides become registered for use in almonds is critical. Of the new products being considered for registration, our laboratory trials indicate that Apollo and Savey (both ovicides) will be the easiest to integrate into the almond system since they have no apparent toxicity to the western predatory mite, Metaseiulus occidentalis; their registration status is unknown at this time. Other new acaricide possibilities include Thuringiensin and Abamectin. Both are somewhat selective to the western predatory mite, but at higher rates may cause substantial mortality. Thus, we will have to learn how to use them; their registration status is also unknown at this time. This leaves us with oils, which require considerable care to

apply without causing phytotoxicity, and biological control by the western predatory mite.

Long term preservation of acaricides is enhanced by relying primarily on predators to control your mites. Use acaricides only if monitoring indicates a treatment is truly required; spot treat; treat early in hot spots when monitoring indicates an imbalance in predator-prey ratios; use the lowest rate of acaricide possible, and, if you lack predators, consider releasing M.occidentalis in your orchards. Even if new acaricides become available, they will be lost to resistance if they are overused; integrated mite management is a long term approach to achieving better mite control and delaying resistance in the spider mite pests.

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## I. Introduction and Objectives

During 1987-1988, our goals were to 1) Conduct mode of inheritance tests with Omite on resistant strains of the Pacific spider mite, Tetranychus pacificus. 2) Determine how stable Omite resistance is by initiating colonies with gene frequencies of 0.5 for the resistance gene (assuming it is caused by a single gene) and determine the fate of resistance over succeeding generations in the absence of selection.

The above objectives were met and the results are described in Chapter II. In addition, we conducted tests on spider mites submitted to us by growers or pest control advisors who were having difficulty in controlling spider mites in their almond orchards. The results of these tests are summarized in Chapter III.

Finally, we provide a list of papers published during the past year (or in press) that relate to our work with spider mite control in almond orchards. In addition, we include xerox copies of these papers or manuscripts.

During the next few months, we intend to test a few additional acaricides for their selectivity to the western predatory mite. Because new acaricides are urgently needed for the integrated mite management program, we will continue to evaluate new products as they become available; our laboratory tests will provide useful information about the ease with which such compounds can be used without disrupting biological control of spider mites by Metaseiulus occidentalis.

## II. Genetic Analysis of Omite Resistance in T. pacificus

The following manuscript has been submitted to the Journal of Economic Entomology. A final version will be sent after peer review has occurred.

Journal of Economic Entomology

Insecticide Resistance & Resistance  
Management

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Propargite Resistance in Pacific Spider Mite  
(Acari: Tetranychidae): Stability and Mode of Inheritance

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ABSTRACT A colony of the Pacific spider mite, Tetranychus pacificus McGregor, collected from California almond orchards during 1984, was found to be resistant (R) to propargite. This propargite-resistant colony was selected 25 times with propargite for 25 months to ensure a pure colony for mode of inheritance tests; the results were consistent with a model in which cyhexatin resistance in this colony is primarily determined by a major semi-recessive gene. Greenhouse populations held for five months without selection with propargite retained their original resistance levels, as indicated by the % survival of colonies initiated from RR, SR and RS females. Negative fitness did not appear to be associated with the resistance allele under these conditions.

Key words: Tetranychus pacificus, propargite resistance, mode of inheritance, stability

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TETRANYCHUS PACIFICUS McGregor is a key pest of almonds in California. T. pacificus has become resistant to nearly all registered acaricides (Andres & Reynolds 1958, Andres & Prout 1960, Jeppson & Jesser 1962, Dennehy & Granett 1984, Keena & Granett 1985, Cranham & Helle 1985, Hoy et al. 1987). T. pacificus, as well as other spider mite species occurring in almonds, has been a target of an integrated mite management program (Hoy 1984, 1985, Hoy et al. 1985), in which the western predatory mite, Metaseiulus (= Galendromus or Typhlodromus) occidentalis (Nesbitt), selective insecticides, and low rates of selective acaricides are key components. Cyhexatin (Plictran), propargite (Omite), and fenbutatin-oxide (Vendex) have been applied at low rates to assist M. occidentalis in controlling spider mites whenever monitoring indicated that the predators needed assistance in maintaining the prey population below the treatment threshold. The selective acaricides are thus valuable resources, and the development of resistance to them by T. pacificus could disrupt the highly cost-effective integrated mite management program (Headley & Hoy 1987).

During 1984 and 1985 we surveyed colonies of T. pacificus collected from almond orchards in California and discovered variability in responses of T. pacificus to propargite, cyhexatin, and fenbutatin-oxide (Hoy et al. 1987; unpublished). We then selected the most propargite-tolerant colony with propargite to ensure the colony was pure for mode of inheritance tests. In addition, we investigated the stability of the propargite resistance by monitoring populations derived from the resistant strains (RR) and reciprocal F<sub>1</sub> females (RS, SR) that were held in the greenhouse for five months without selection with propargite. Susceptible (S) colonies and a resistant colony that continued to receive propargite applications were also evaluated for their propargite resistance levels.

## Materials and Methods

Colony Sources. The Bidart I. pacificus colony was collected from almonds in Kern County, California in June 1984. The LC<sub>50</sub> for propargite was 271 (95% CL = 228 to 307) ppm based on an assay using a wettable powder (WP) formulation and a slide dip technique (M.A.H. unpubl.). The LC<sub>90</sub> was 570 (95% CL = 481 to 768) ppm and the slope was  $4.0 \pm 0.55$ ;  $n = 600$ .

To assure that we had a pure colony so that we could determine the mode of inheritance to propargite, we reared this colony on pinto beans, Phaseolus vulgaris L., in a University of California at Berkeley greenhouse and selected it with propargite 25 times during a 25-month period. (Such long term selection was probably not necessary to purify the colony, but because we were working with the cyhexatin and fenbutatin-oxide resistant strains, time constraints prevented conducting the tests sooner.) The first three selections were performed by placing an average of 500 females on pinto bean leaf disks (50 females per disk) and spraying them with 360 ppm propargite (30 WP) with a Crown Spra-Tool<sup>®</sup>. Survivors (mites that could walk when touched with a fine camel's-hair brush) were placed on clean leaf disks, and the leaf disks were placed on clean plants in cages in the greenhouse. The survivors then colonized the plants and multiplied. Subsequent selections with propargite were done on all life stages on bean plants in the greenhouse. Bean plants that were either infested or uninfested with mites were sprayed. If uninfested, propargite-treated plants were subsequently infested by cutting old foliage and placing it on the clean new plants and allowing the mites to walk off. Propargite concentrations used ranged from 36 to 1080 ppm (30 WP) for subsequent selections.

The Chapla *T. pacificus* colony was collected from an almond orchard in Glenn County, California in June 1984. The initial LC<sub>50</sub> obtained with the slide dip technique was 195 ppm (95% CL = 114-251); the LC<sub>90</sub> was 521 (400-951) ppm, and the slope was  $3.01 \pm 0.54$  ( $n = 400$ ). This colony was also maintained on pinto bean plants in cages in the greenhouse, but no selections with propargite were done.

Concentration/Mortality Tests. Concentration/mortality lines for adult females were obtained in the mode of inheritance test and stability of resistance test using a leaf spray technique. For mode of inheritance tests, leaf spray tests were done on four different days with six to eleven concentrations, including distilled water controls. Solutions were made fresh each test date using wettable powder (30 WP) propargite and distilled water. About 25-30 females were aspirated using a vacuum pump system and tapped onto pinto bean leaf disks (3.0 cm in diam) resting on moist cotton in plastic trays. Between 50 and 300 females were tested per concentration. Leaf disks with spider mites were then sprayed for five seconds with a Crown Spra-Tool held 23 cm away from the disks, and were held at 25-29<sup>o</sup> under a 24 h daylength. Scoring of dead vs. alive was done after 48 h. Live mites were those that could walk when touched with a fine camel's-hair brush. All others were scored as dead, including those that ran off the disk. Data were analyzed using the probit option of the POLO program (Russell et al. 1977). Concentration/mortality lines were compared using the likelihood ratio test (Savin et al. 1977), which tests for equal slopes and intercepts of the regressions.

Mode of Inheritance. The Chapla (susceptible = S) and Bidart-25 (resistant = R) colonies were crossed to produce reciprocal F<sub>1</sub> female (RS or SR) progeny. Crosses were done in the laboratory with 200 virgin females and 200 males for R X S and S X R crosses, and 140 virgin females and 140 males for R X R and S X S crosses. Virgins were obtained by isolating quiescent deutonymphal females. Ten quiescent females and ten adult males were placed on each of fourteen (or twenty) pinto bean leaf disks for each cross: Bidart-25 X Bidart-25 (R X R), Chapla X Chapla (S X S), Bidart-25 females X Chapla males (R X S), Chapla females X Bidart-25 males (S X R). Once parental females had emerged and mated, the mated females were moved to clean bean leaf disks (four per disk) each day for eight days. The eggs that these parental females deposited were held for about 10-11 days at 25-29°C under constant light until the F<sub>1</sub> adults appeared. F<sub>1</sub> females were tested within one to three days after becoming adults; the leaf spray technique was used to obtain concentration/mortality lines.

To obtain F<sub>2</sub> females, reciprocal F<sub>1</sub> females (RS or SR) and males (R or S) were allowed to mate. (Because males are haploid and inherit their resistance genes from their mothers, F<sub>2</sub> female progeny from such crosses are genetically equivalent to backcrosses.) Mated F<sub>1</sub> females were then transferred (as described above) to clean leaf disks to produce reciprocal F<sub>2</sub> female progeny, which were tested with propargite using the leaf spray technique to obtain concentration/mortality lines.

Concentration/mortality lines from the parental, reciprocal F<sub>1</sub>, and reciprocal F<sub>2</sub> females were analyzed using the probit option of POLO (Russell et al. 1977). The degree of dominance was estimated for the reciprocal F<sub>1</sub> females using the formula  $D = \frac{2X_2 - X_1 - X_3}{X_1 - X_3}$ , where X<sub>1</sub> = logarithm to



the base 10 of the LC<sub>50</sub> of the resistant colony (RR),  $X_2 = \log_{10}$  of the LC<sub>50</sub> of the heterozygous colony (RS), and  $X_3 = \log_{10}$  of the LC<sub>50</sub> of the susceptible colony (SS) (Georghiou 1969). Data were also analyzed to determine whether the concentration/mortality lines obtained with females fit a model of a single major gene. Since *I. pacificus* is an arrhenotokous species, and F<sub>2</sub> females are genetically equivalent to backcross females, the expected responses for females at each concentration were calculated by the formulae for a backcross to the S males:  $X_y = W_{(SR)} 0.50 + W_{(SS)} 0.50$ , where  $X$  = expected response at a given concentration  $y$ ,  $W$  = observed response of SS and SR genotypes at concentration  $y$ , obtained from the respective regression lines. The expected responses of the females derived from the backcross to the R strain was calculated by  $X_y = W_{(RS)} 0.50 + W_{(RR)} 0.50$  (Georghiou 1969). The relationships between observed and expected concentration/responses were analyzed by a Chi Square goodness of fit test ( $P < 0.05$ ).

Stability of Propargite Resistance. To determine the rate with which colonies of *I. pacificus* having different resistance gene frequencies would lose their resistance if held without propargite selection, we conducted tests with mites of known genotypes in cages in the greenhouse. Greenhouse colonies were initiated using F<sub>1</sub> females from laboratory crosses (Chapla X Chapla, Bidart-25 X Chapla, Chapla X Bidart-25, and Bidart-25 X Bidart-25). Leaf discs containing F<sub>1</sub> females and their progeny were placed on pinto bean plants in cages in the greenhouse. Two colonies were started for each cross with at least 240 F<sub>1</sub> females per colony. Each colony was placed in a cage and cages were placed on two benches in a randomized block design; cages were ca. 60 cm apart.

Cages rested on water moats to minimize mite movement from cage to cage. Each cage contained two pots of beans, one of which was replaced with a fresh pot each week. Ca. three generations of mites developed in these cages each month from April 1987 to September 1987. During this period, the temperature ranged from 20-31<sup>0</sup>C. Mites were held under continuous light. In addition, we selected a cage of the Bidart-25 colony with propargite three times during this interval (Bidart-28) to use as a comparison with the Bidart-25 colonies that received no additional propargite selections.

After the colonies were established in April 1987, each colony, and the selected Bidart-25 colony, was screened with 36 ppm propargite every four weeks beginning in May 1987 until September 1987. For each test, ca. 250 females from each cage were tested, and ca. 50 females were used as water controls. Females were tested with the leaf spray technique, as described for the mode of inheritance tests.

A regression of % mortality over bioassay date was conducted with each colony to determine whether the slope differed significantly from zero. A significant decline in slope through time (5 months) would suggest that selection against propargite resistance allele(s) occurred in the absence of propargite selection.

A complete concentration/mortality test was conducted for all the colonies at the end of the experiment: selected Bidart-28 (one colony), Bidart-25 (2 colonies), RS (2 colonies), SR (2) and the susceptible Chapla (2) colonies. In addition, a concentration/mortality test was conducted on the original (unselected) Bidart base colony and compared to that of the selected Bidart-28 colony.

## Results and Discussion

Mode of Inheritance. The reciprocal  $F_1$  females yielded lines which were intermediate between the susceptible Chapla and resistant Bidart-25 colonies' lines (Table 1A, Fig. 1). The  $LC_{50}$  for the S colony was 19 ppm, the  $LC_{50}$  for the R colony was 270 ppm, and the values for the RS and SR  $F_1$  females were 45 and 48 ppm, respectively (Table 1A). This indicated that propargite resistance is not completely dominant or recessive (Tsukamoto 1963). The lines for the reciprocal  $F_1$  females are not different (Savin et al. 1977). The estimate of dominance (D) for the  $F_1$  females from the Bidart-25 female X Chapla male cross was -0.356. For the reciprocal cross, D was -0.315. Both values indicate that propargite resistance is incompletely recessive (Georghiou 1969).

The concentration/mortality lines for the reciprocal  $F_2$  females were significantly different, as expected (Fig. 2, Table 1B). The  $LC_{50}$  for the Bidart-25 colony was 235 ppm; for the susceptible Chapla colony's  $LC_{50}$  it was 15 ppm. The  $F_2$  females derived from the RS X R cross had an  $LC_{50}$  of 78 ppm; the  $F_2$  females derived from the SR X S cross had an  $LC_{50}$  of 22 ppm (Table 1B).

The dotted lines in Fig. 2 give the values expected for  $F_2$  females if we assume that propargite resistance is determined by a single major semirecessive gene. The visual fit of the observed data to these lines appears good. However, Chi Square analysis of the goodness of fit of the observed mortality to that expected, assuming the probit model, was significantly different on 5 of 11 propargite concentrations, ( $P < 0.05$ ) for the SR X S cross. For the RS X R cross, 6 of 13 propargite concentrations yielded significant departures from expected ( $P < 0.05$ ). In the case of the RS X R cross, the departures were

primarily at the lower propargite concentrations, with more mortality than expected. With the reciprocal cross, departures from expected occurred primarily at the higher propargite concentrations, again more mites died than expected.

The data for the F<sub>2</sub> females were examined to determine whether they fit a polygenic model, in which multiple alleles contribute equally and additively to the resistance. According to this model, concentration/mortality lines for the F<sub>1</sub> females should have been approximately equidistant between the R and S lines. This did not occur (Fig. 1). Nor were the lines for the reciprocal F<sub>2</sub> females equidistant between the F<sub>1</sub> lines and the R and S lines, respectively (Fig. 2). The simplest model providing the best fit to the data appears to be the semirecessive gene model, although other models involving modifying genes cannot be excluded.

Stability of Propargite Resistance. Fig. 3 shows that survival of females held in the greenhouse without propargite selection from May 1987 through September 1987 varied from sample date to sample date. However, the slope of regression lines estimated for each replicate differed significantly from zero in only one case. One of the Bidart-25 lines had a negative slope, suggesting that propargite resistance levels could have declined over the five month interval evaluated. The decline, if real and not a sampling error, involved a change in survival from 100% to 89.3% at the single propargite concentration tested

These data support the hypothesis that propargite resistance in this colony of *I. pacificus* is stable under these conditions; the failure of the RS or SR colonies to develop a negative slope (Fig. 3) suggests that there was no selection against the propargite resistance allele(s) in the absence of

propargite selection. If substantial reductions in fitness were associated with the propargite resistance allele(s), the slopes should have declined over the five months of the project, during which a minimum of 15 generations (3 generations per month x 5 months) could develop.

At the end of the greenhouse persistence test complete concentration/mortality lines were obtained for these colonies (Fig. 4). For comparison, we tested the Bidart colony maintained under regular selection (Bidart-28) and the original Bidart Base colony held without selection after collection from the field. The susceptible Chapla colonies (C1,2) remained susceptible (Table 2, Fig. 4) during the course of the experiment. The Bidart-25 colonies (B1,2), maintained without selection for five months, remained resistant, with LC<sub>50</sub> values of 159 and 190 ppm, respectively. These lines are not the same, although they are parallel. The Bidart-28 colony, selected an additional three times, had an LC<sub>50</sub> of 214 ppm propargite. The Bidart-28 and B2 lines are the same, but the B1 line is not the same as the B28 line (Fig. 4, Table 2). The Bidart Base colony, never selected with propargite, had an LC<sub>50</sub> of 177 ppm. This line is not different from the B1 or B2 lines, but is different from the B-28 line. Thus we conclude that the selection with propargite increased the LC<sub>50</sub> value of the original field-collected Bidart colony a relatively small amount (Table 2). The colonies derived from the Chapla female X Bidart-25 male crosses (CB1,2) had LC<sub>50</sub> values of 35 and 39 ppm, respectively, at the end of five months; these lines are not different. The two colonies derived from the reciprocal cross (Bidart-25 female X Chapla male or BC1,2) yielded LC<sub>50</sub> values of 87 and 71 ppm. These lines were also not different. The complete concentration/mortality lines confirm the conclusions suggested by the single

dose tests (Figure 3): resistance levels did not decline nor did they differ between the replicates. Also, because the resistance levels of the B1 and B2 lines did not differ significantly from that of the B28 line, which was periodically selected with propargite, we conclude the propargite resistance is stable (Figures 3, 4).

### Discussion

I. pacificus from almond orchards in the southern San Joaquin Valley of California has now been found to have a high level of cyhexatin and fenbutatin-oxide resistances (Hoy et al. 1987). The data presented here show that propargite resistance is also present and stable in one of these colonies. The data on mode of inheritance of propargite resistance suggest the propargite resistance is incompletely recessive ( $D = -0.356$  and  $-0.315$ ), as it is for cyhexatin and fenbutatin-oxide (Hoy et al. 1987). We did not, however, ascertain whether cyhexatin/fenbutatin-oxide resistance and propargite resistance are determined by the same locus. The propargite concentration/mortality lines of the reciprocal  $F_2$  females are consistent with the model involving a single semirecessive gene, although we cannot eliminate the possibility that modifier genes are also involved (Tsukamoto 1963). Stability of the propargite resistance in the replicated cage populations initiated with known genotypes (RR, RS, SR, or SS females) suggests that little contamination occurred between the cages, and that the propargite resistance is stable under these test conditions. The stability of the resistance suggests that the fitness of the propargite resistance allele(s) is(are) similar to the wild type allele in this population of I. pacificus. The RS and SR cages were most

likely to have shown a decline in resistance allele frequency, and this did not occur. These data suggest that, once populations of *I. pacificus* become homozygous for propargite resistance, reversion to propargite susceptibility is unlikely to occur rapidly. The loss of propargite, cyhexatin and fenbutatin-oxide to resistance or registration problems leaves some growers with few options for controlling spider mites in California almond orchards.

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## Figure Legends

Figure 1. Mode of inheritance of propargite resistance in I. pacificus; concentration/mortality lines of the reciprocal F<sub>1</sub> females (leaf spray technique and the 30 WP propargite formulation).

Figure 2. Concentration/mortality data for reciprocal F<sub>2</sub> females derived from crossing Bidart-25 females with Chapla males and Chapla females with Bidart-25 males. Dashed lines represent responses expected if propargite resistance in I. pacificus were determined by a single major semirecessive gene.

Figure 3. Stability of propargite resistance in colonies of I. pacificus derived from known genotypes that were untreated with propargite. Lines C1, C2 are of the susceptible Chapla colony; lines CB1 and CB2 are of a colony derived from F<sub>1</sub> females from the cross (Chapla female X Bidart-25 male); lines BC1, BC2 are from the reciprocal F<sub>1</sub> cross (Bidart-25 female X Chapla male); B1, B2 are of the resistant (Bidart-25) colony without additional propargite selection, while the B28 (Bidart-28) colony received 3 additional propargite selections. Survival of 250 adult females was assessed using 36 ppm propargite with a leaf spray technique.

Figure 4. Concentration/mortality data for colonies of I. pacificus maintained in the greenhouse five months without selection (C1,2; BC1,2; CB1,2; B1,2) compared to Bidart colonies selected an additional three times (B-28) and not selected (B-Base) with propargite subsequent to field collection.

Table 1. Mode of inheritance of propargite resistance in *I. pacificus* using a leaf spray technique

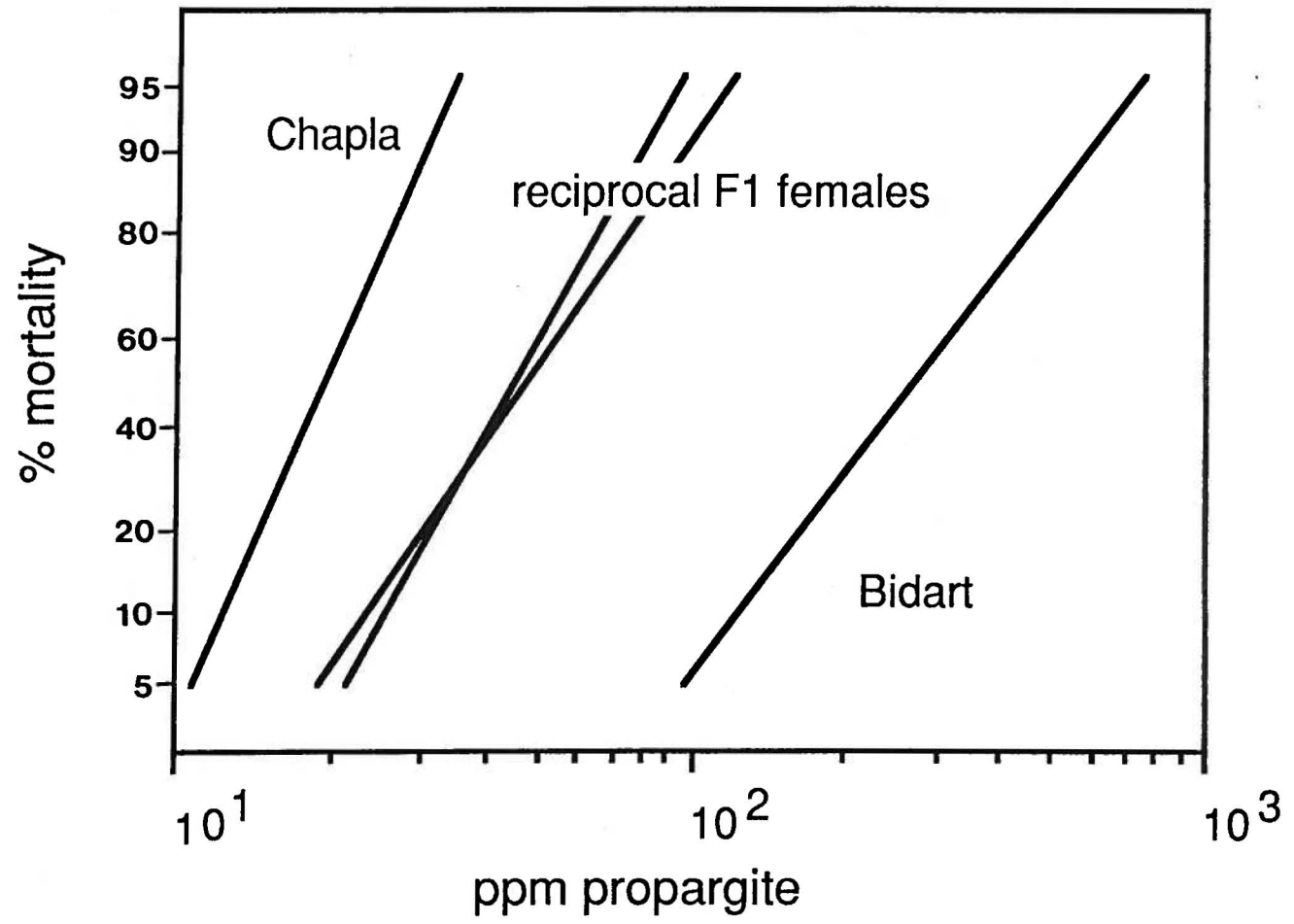
| Cross   | Total no. tested | LC <sub>50</sub> ppm | 95% CL      | LC <sub>90</sub> ppm | 95% CL      | Slope±S.D. |
|---|------------------|----------------------|-------------|----------------------|-------------|------------|
| A) Tests of reciprocal F <sub>1</sub> females |                  |                      |             |                      |             |            |
| S (Chapla)                                    | 1223             | 19.4                 | 17.6- 21.2  | 31.0                 | 28.8- 34.6  | 6.38±0.48  |
| R (Bidart-25)                                 | 1559             | 269.6                | 241.9-296.6 | 602.3                | 538.6-691.2 | 3.67±0.19  |
| F <sub>1</sub> SR (S♀ X R♂)                   | 2107             | 47.9                 | 42.8- 52.9  | 98.6                 | 87.5-115.9  | 4.09±0.21  |
| F <sub>1</sub> RS (R♀ X S♂)                   | 2158             | 45.4                 | 42.5- 48.2  | 82.1                 | 76.3- 89.6  | 4.99±0.26  |
| B) Tests of reciprocal F <sub>2</sub> females |                  |                      |             |                      |             |            |
| S (Chapla)                                    | 1786             | 14.8                 | 13.7- 15.5  | 28.1                 | 25.9- 30.6  | 4.58±0.25  |
| R (Bidart-25)                                 | 1623             | 235.4                | 211.3-258.8 | 591.1                | 520.9-693.4 | 3.20±0.18  |
| F <sub>2</sub> (SR♀ X S♂)                     | 1852             | 21.6                 | 19.4- 23.4  | 46.4                 | 41.8- 52.9  | 3.87±0.23  |
| F <sub>2</sub> (RS♀ X R♂)                     | R309             | 77.8                 | 69.1- 87.1  | 368.6                | 301.0-477.0 | 1.90±0.11  |

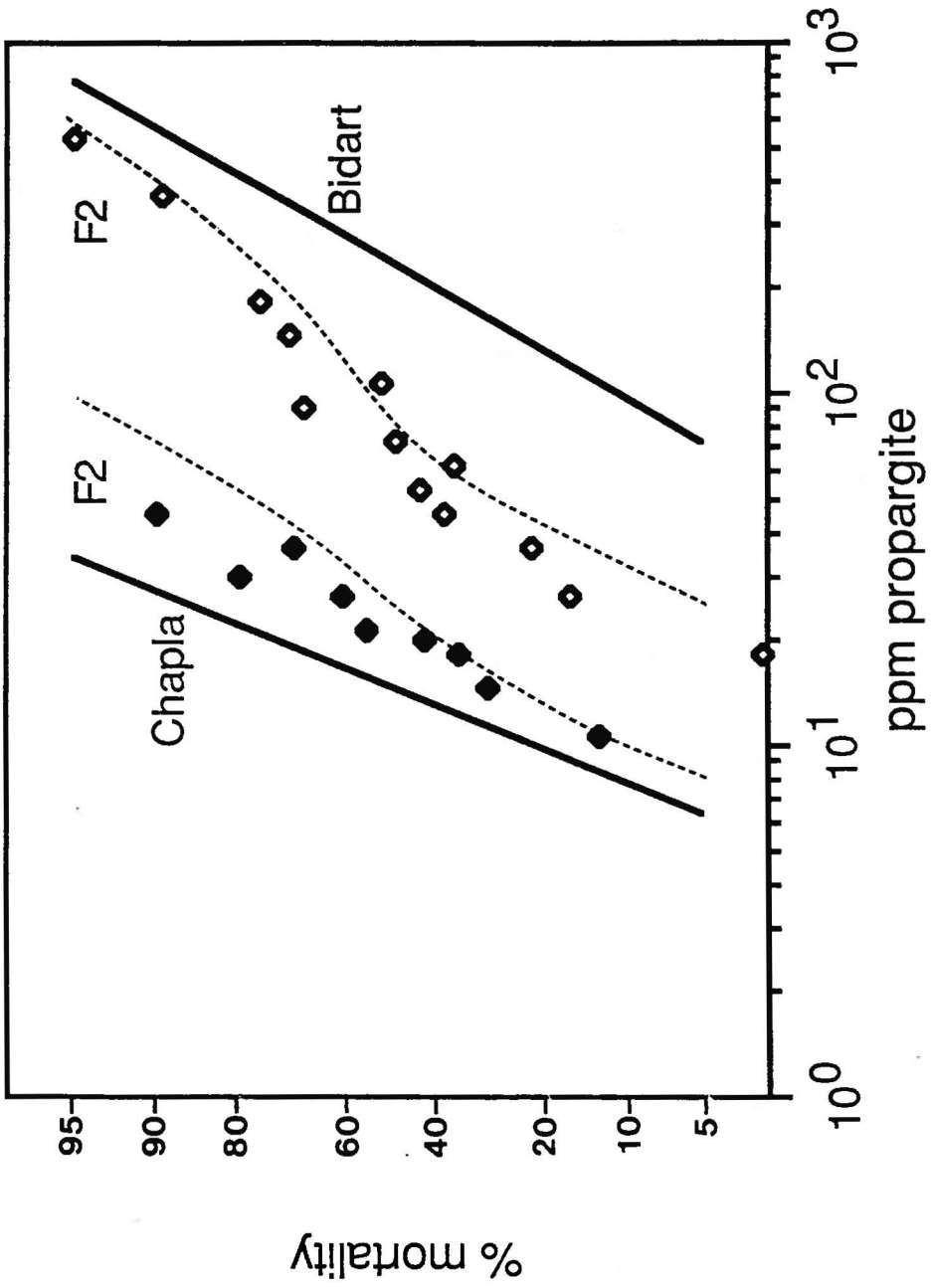
Table 2. Concentration/mortality data for colonies of *I. pacificus* held in the greenhouse without selection with propargite for five months

| Colony                                     | Total no. tested | LC <sub>50</sub> ppm | 95% CL      | LC <sub>90</sub> ppm | 95% CL      | Slope±S.D. |
|--|------------------|----------------------|-------------|----------------------|-------------|------------|
| Chapla-1                                   | 526              | 21.2                 | 17.3- 24.1  | 34.2                 | 29.9- 43.9  | 6.25±.82   |
| Chapla-2                                   | 532              | 19.1                 | 14.4- 21.6  | 31.3                 | 26.6- 41.8  | 5.94±.67   |
| CB-1 (SR♀ X S♂)                            | 631              | 34.9                 | 24.5- 44.3  | 127.1                | 86.0-332.6  | 2.28±.36   |
| CB-2 (SR♀ X S♂)                            | 645              | 38.9                 | 31.7- 45.0  | 97.6                 | 76.7-156.2  | 3.20±.49   |
| BC-1 (RS♀ X R♂)                            | 631              | 88.6                 | 65.9-111.6  | 298.8                | 228.6-446.8 | 2.43±.26   |
| BC-2 (RS♀ X R♂)                            | 641              | 70.9                 | 52.2- 90.7  | 267.8                | 201.6-403.2 | 2.22±.22   |
| Bidart-25-1                                | 623              | 158.8                | 128.5-183.2 | 344.2                | 297.4-427.3 | 3.81±.46   |
| Bidart-25-2                                | 632              | 190.4                | 149.4-226.1 | 398.9                | 238.7-538.9 | 4.00±.42   |
| Bidart-28 <sup>a</sup> /<br>(Selected)     | 625              | 213.5                | 153.0-265.3 | 514.4                | 401.8-813.6 | 3.36±.41   |
| Bidart Base <sup>b</sup> /<br>(Unselected) | 642              | 176.8                | 136.4-208.8 | 363.6                | 307.8-472.3 | 4.10±.51   |

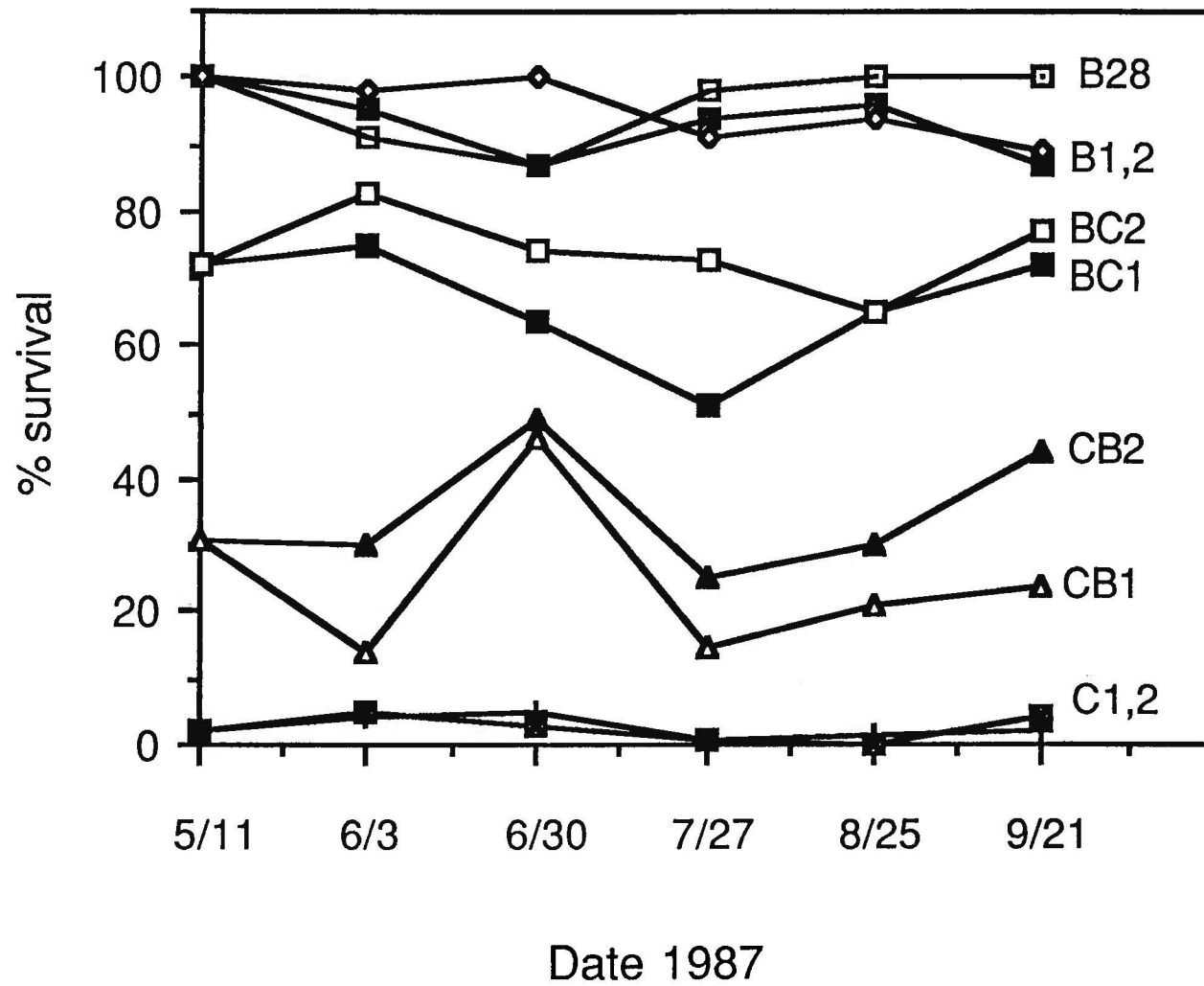
<sup>a</sup>/ This colony was held in the greenhouse and selected an additional three times with propargite during the experiment.

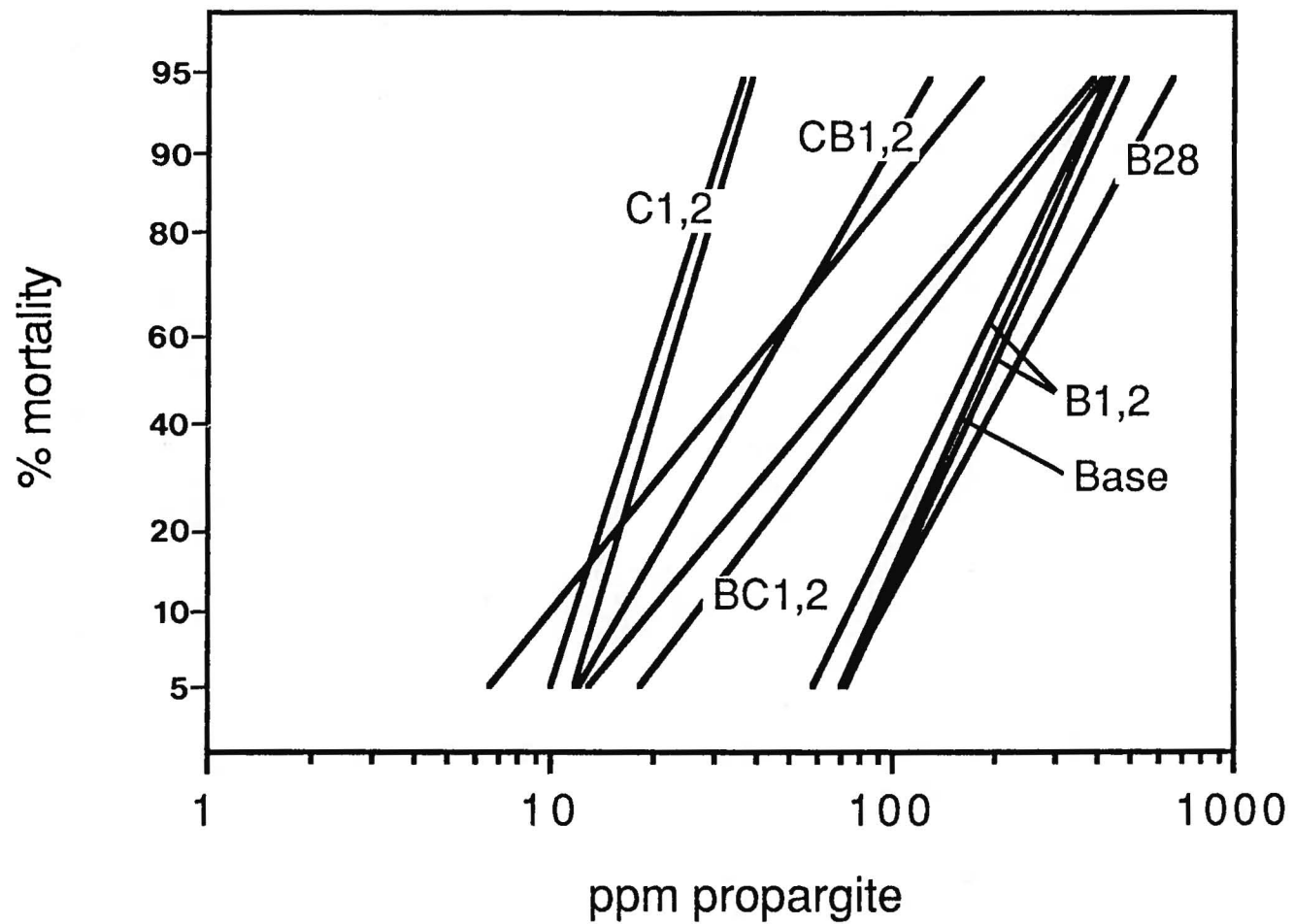
<sup>b</sup>/ This unselected base colony was tested at the end of the experiment to compare the amount of response to selection achieved.











### III. Assays for Omite Resistance

During the 1987 growing season, we received two requests to assay spider mites for their resistance levels to Omite. Colonies were initiated and allowed to build up for several generations. Adult females were then tested on bean leaf discs using the leaf spray technique. We also tested our Chapla and Bidart colonies of T. pacificus as Omite-susceptible and -resistant colonies. In addition, we tested females from all colonies with water.

The colonies were received as follows:

I. McFarlane Ranch, submitted by Mark Freeman, Farm Advisor, Fresno County, June 24, 1987. Sample collected June 18 from Block 1-11, Barstow & Temp NE. Sprayed 5/17/86 every other row with 2 lbs 30W Omite/acre; 5/23/86 with Omite (30W 4 lbs/acre) south part of field every other row; 5/24/86, Omite 6E 2.5 pts/acre, north part of field every other row; 6/24 every row 3 lbs Omite/acre; 8/8 Plictran 0.4 lb/acre. In 1987, the orchard was treated with Omite 30W (2 lb/acre) every other row; 4/10, south part of field 50W Plictran (1.25 lb/acre); 5/8, east half of orchard with 2lbs Omite 30W; 5/30, sprayed from row 20 north, solid, 3 lbs Omite 30W/acre; 6/10, west half, row 14--north, 6 lbs Omite/acre; 6/18/87, picked up missed spots with 2.5 lbs Plictran/5 acres.

II. Cinco-Huron almond orchard, Cinco Farms, 5 miles north of Huron, Fresno County. Collected 22 July 1987 by Larry Orsak, pest control advisor. Treated in 1987 with 1.5 lb 30W Omite/100 gallon/acre on June 23; treated with 3 pints 6E Omite/20 gallon by air.

### RESULTS

I. The McFarlane Ranch mites were T. pacificus. We tested with with 0.1 lb 30 WP Omite/100 gallons water using the leaf spray technique. Two hundred adult females were tested and they exhibited only 2% survival at this rate. In contrast, the resistant (Bidart) colony had 72% survival and the susceptible (Chapla) colony had 0% survival. We conclude that these mites are not resistant to Omite, based on this test method. We don't know why the survival

rates are so poor; items to investigate are poor coverage, poor predator population ratios, and improper timing of sprays.

II. Cinco farms mites were T. urticae and T. pacificus. These mites were tested with 0.1 lb 30WP Omite/100 gallons of water. Survival of the Cinco-Huron Pacific mites was 79%; survival of the two-spotted spider mite was 0%. The resistant (Bidart) and susceptible (Chapla) colonies of Pacific mite were also tested. Our resistant lab colonies had 86% and 92% survival rates; our susceptible strain had a 0.6% survival rate. The results indicate that the Pacific mite is resistant to Omite while the two-spotted mite is not in this orchard. This is the first time we have found Omite resistance in a colony of Pacific mite from western Fresno County.

## Conclusions

The Omite resistant colony from the Cinco-Huron orchard suggests that Omite resistance is not confined to the southern part of the San Joaquin Valley. Thus, it is very important that integrated mite management be practiced as widely as possible so as to delay the further development of resistance to Omite and Vendex.

#### IV. Publications List

- Hoy, M.A., J. Conley, & W. Robinson. 1987. Cyhexatin and fenbutatin-oxide resistance in Pacific spider mite (Acari: Tetranychidae): Stability and mode of inheritance. J. Econ. Entomol. 81: (in press).
- Hoy, M.A. and J. Conley. 1987. Selection for abamectin resistance in Tetranychus urticae and T. pacificus (Acari: Tetranychidae). J. Econ. Entomol. 80:221-225.
- Headley, J.C. & M.A. Hoy. 1987. Benefit/cost analysis of an integrated mite management program for almonds. J. Econ. Entomol. 80:555-559.
- Hoy, M.A. & J. Conley. 1987. Toxicity of pesticides to western predatory mite. California Agriculture, July-August, 41 (7&8): 12-13.
- Hoy, M.A. & J. Conley. Propargite resistance in Pacific spider mite (Acari: Tetranychidae): stability and mode of inheritance. (submitted).

RH: HOY ET AL.: *T. pacificus* RESISTANT TO CYHEXATIN/FENBUTATIN-OXIDE

## Cyhexatin and Fenbutatin-Oxide Resistance in Pacific Spider Mite (Acari: Tetranychidae): Stability and Mode of Inheritance

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J. Econ. Entomol. 81(0): 000-000(1988)

**ABSTRACT** The Pacific spider mite, *Tetranychus pacificus* McGregor, collected from California almond orchards during 1984 and 1985, was tested with cyhexatin and fenbutatin-oxide to determine whether resistance (R) to these acaricides was present. A cyhexatin-resistant colony was selected in the greenhouse with cyhexatin for 15 mo to obtain a pure colony for genetic analysis. Mode-of-inheritance data were consistent with a model in which cyhexatin resistance in this colony is primarily determined by a major semirecessive gene. A flowable formulation of cyhexatin was more toxic to both R and susceptible (S) strains than was the wettable powder formulation. Larvae of the R and S strains exhibited survival rates similar to those of adults. Concentration/mortality lines for both fenbutatin-oxide and cyhexatin were obtained for both R and S strains; results were consistent with the hypothesis that cyhexatin resistance confers fenbutatin-oxide resistance in this population. Greenhouse populations held for 9 mo without selection with cyhexatin retained their original resistance levels, as indicated by the percent survival of colonies initiated from RR, SR, RS, and SS females. Negative fitness did not appear to be associated with the resistance allele under these conditions.

**KEY WORDS** ~~insecta~~, *Tetranychus pacificus*, resistance, mode of inheritance

*Tetranychus pacificus* McGregor is a key pest of almonds in California. *T. pacificus* has become resistant to various pesticides, including parathion, ethion, Aramite (2-(p-t-butyl phenoxy)-1-methyl-ethyl isopropyl-2-chloroethyl sulfite), dicofol, and propargite (Andres & Reynolds 1958; Andres & Prout 1960; Jeppson & Jessor 1962; Dennehy & Granett 1984; Cranham & Helle 1985; Keena & Granett 1985; M.A.H. & J.C., unpublished). Recently, *T. pacificus*, as well as other spider mite species occurring in almonds, has been a target of an integrated mite management program (Hoy 1984, 1985, Hoy et al. 1985), in which the western predatory mite, *Metaseiulus* (= *Galendromus* or *Typhlodromus*) *occidentalis* (Nesbitt), selective insecticides, and low rates of selective acaricides are key components. Cyhexatin (Plictran), propargite (Omite), and fenbutatin-oxide (Vendex) are applied at low rates to assist *M. occidentalis* in controlling spider mites when monitoring indicates that the predators need assistance in maintaining the prey population below the treatment threshold. Thus, these three selective acaricides are valuable resources, and the development of resistance to them by *T. pacificus* could disrupt the highly cost-effective integrated mite management program (Headley & Hoy 1986, 1987).

During 1984 and 1985, we surveyed colonies of *T. pacificus* collected from almond orchards in California and discovered variability in responses of *T. pacificus* to cyhexatin and fenbutatin-oxide. We then selected a colony with cyhexatin. Once an apparently pure resistant (R) colony was obtained, we compared the concentration/mortality lines of R and susceptible (S) colonies to cyhexatin and fenbutatin-oxide, compared the toxicity of two formulations of cyhexatin to R and S populations, and conducted mode-of-inheritance tests. In addition, we investigated the stability of the cyhexatin resistance by monitoring populations of *T. pacificus* derived from RR, SS, SR, and RS females that were held in the greenhouse for 9 mo without selection with cyhexatin or fenbutatin-oxide.

### Materials and Methods

**Colony Sources.** The Wasco *T. pacificus* colony was collected from almonds in Kern County, Calif., in June 1984. The initial LC<sub>50</sub> for cyhexatin was 1,830 ppm based on the wettable powder (WP) formulation and a slide-dip technique (M.A.H., unpublished). The LC<sub>90</sub> was 4,254 ppm and the slope was 3.5 ± 0.84. No confidence limits were calculated due to the variability ( $n = 580$ ). While it was the most cyhexatin-tolerant colony collected, the variability and flat slope suggested the colony was not sufficiently so pure that we could determine

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the mode of inheritance of cyhexatin resistance. We therefore reared this colony on pinto beans, *Phaseolus vulgaris* L., in a Univ. Calif. Berkeley greenhouse and selected it with cyhexatin 20 times during a 15-mo period. The first three selections were performed by placing an average of 500 females on pinto bean leaf disks (50 females per disk) and spraying them with 2,400 ppm cyhexatin (50 WP) with a Crown Spra-Tool. Survivors (mites that could walk when touched with a fine camel's-hair brush) were placed on clean leaf disks, and the leaf disks were placed on clean plants in cages in the greenhouse. The survivors then colonized the plants and multiplied. Subsequent selections with cyhexatin were conducted on all life stages on bean plants in the greenhouse. Bean plants that were either infested or uninfested with mites were sprayed. If uninfested, cyhexatin-treated plants were subsequently infested by cutting old foliage and placing it on the clean new plants and allowing the mites to walk off. Cyhexatin concentrations used ranged from 120 to 9,600 ppm (50 WP) for selections four through eighteen, and from 60 to 120 ppm (flowable formulation) for selections 19 through 23.

The Chapla *T. pacificus* colony was collected from an almond orchard in Glenn County, Calif., in June 1984. The initial  $LC_{50}$  was 293 ppm (95% CL = 172–415), the  $LC_{90}$  was 1,351 (937–2,437) ppm, and the slope was  $1.93 \pm 0.19$  for the 50 WP cyhexatin formulation tested with a slide-dip technique ( $n = 540$ ). This colony was also maintained on pinto bean plants in cages in the Univ. Calif. Berkeley greenhouse, but no selections with cyhexatin were made.

**Concentration/Mortality Tests.** Concentration/mortality lines for adult females and larvae were obtained with a leaf-spray technique for the Wasco base colony and colonies selected 15 (Wasco-15) and 18 (Wasco-18) times with cyhexatin. The susceptible Chapla colony was used for comparison. In the mode of inheritance and stability tests, the leaf-spray test was also used to test adult females and males. Leaf-spray tests were done over 2–4 d with six to nine concentrations, including distilled water controls. Solutions were made fresh each test date using flowable (Dow XRM-4868) or wettable powder (50 WP) cyhexatin and distilled water. Five females or five larvae were placed on pinto bean leaf disks (1.75 cm diameter) resting on moist cotton in plastic trays. At least 100 females and at least 50 males or larvae were tested per concentration. When males were tested, two females as well as five males were placed on each leaf disk, as this reduced runoff by males. Leaf disks with spider mites were then sprayed for 5 s with a Crown Spra-Tool held 23 cm away from the disks. Scoring of dead vs alive was done after 48 h at 25–29°C with a photoperiod of 18:6 (L:D) for females and larvae and after 24 h for males. Live mites were those that could walk when touched with a fine camel's-hair brush. All others were scored as dead, including those that ran off the disk. Data were analyzed

using the probit option of the POLO program (Russell et al. 1977). Concentration/mortality lines were compared using the likelihood ratio test (Savin et al. 1977), which tests for equal slopes and intercepts of the regressions.

**Comparison of Flowable and Wettable Powder Formulations.** Because concentration/mortality lines of the Wasco and Wasco-15 colonies were flat, we compared the 50 WP and flowable formulations of cyhexatin to determine if the lines obtained with flowable formulation gave more discrimination between R and S colonies. Tests were done with adult females and the leaf-spray technique using the Wasco-18, Chapla, and Wasco base colonies.

**Relationship between Cyhexatin and Fenbutatin-Oxide Resistance.** The Chapla and Wasco-18 colonies were tested simultaneously with flowable formulations of cyhexatin and fenbutatin-oxide (4 L) to determine whether tolerances of these colonies for the two acaricides were correlated. Adult females were tested with the leaf-spray technique as described above.

**Mode of Inheritance.** The Chapla (susceptible, S) and Wasco-18 (resistant, R) colonies were crossed to produce reciprocal  $F_1$  female (RS or SR) and male (S or R) progeny. Crosses were done in the laboratory with 72 virgin females and 72 males for each cross; virgins were obtained by isolating quiescent deutonymphal females. Eight quiescent females and eight adult males were placed on each of nine pinto bean leaf disks for each cross: Wasco-18  $\times$  Wasco-18 (R  $\times$  R), Chapla  $\times$  Chapla (S  $\times$  S), Wasco-18 females  $\times$  Chapla males (R  $\times$  S), Chapla females  $\times$  Wasco-18 males (S  $\times$  R). Once parental females had emerged and mated, the mated females were moved to clean bean leaf disks (three per disk) each day for 7 d. The eggs that these parental females deposited were held for ca. 10–11 d at 25–29°C under constant light until the  $F_1$  adults appeared.  $F_1$  progeny were then tested within 1–3 d after becoming adults; the leaf-spray technique was used to obtain concentration/mortality lines. After 24 (males) or 48 h (females), mites were scored as dead or alive.

To obtain  $F_2$  females and males, the above cross was repeated with the Wasco-20 colony and the Chapla colony, and the reciprocal  $F_1$  females (RS or SR) and males (R or S) were allowed to mate. Mated  $F_1$  females were then transferred (as described above) to clean leaf disks to produce reciprocal  $F_2$  progeny, which were tested with the flowable formulation of cyhexatin and the leaf-spray technique to obtain concentration/mortality lines.

Concentration/mortality lines from the parental, reciprocal  $F_1$ , and reciprocal  $F_2$  progeny were analyzed using the probit option of POLO (Russell et al. 1977). The degree of dominance was estimated for the reciprocal  $F_1$  female progeny using the formula

$$D = \frac{2X_2 - X_1 - X_3}{X_1 - X_3}$$



where  $X_1$  is the logarithm to the base 10 of the  $LC_{50}$  of the resistant colony (RR),  $X_2$  is the logarithm<sub>10</sub> of the  $LC_{50}$  of the heterozygous colony (RS), and  $X_3$  is the logarithm<sub>10</sub> of the  $LC_{50}$  of the susceptible colony (SS) (Georghiou 1969). Data were also analyzed to determine whether the concentration/mortality lines obtained with the  $F_2$  males and females fit a model of a single major gene. Because *T. pacificus* is an arrhenotokous species,  $F_2$  females are genetically equivalent to backcross females;  $F_2$  males would respond in a 1:1 ratio of R and S phenotypes if resistance fits a single major gene model and males inherit their resistance genes from their mothers. The expected responses for females at each concentration were calculated by the formula for a backcross to the S males  $X_y = W_{(SR)}0.50 + W_{(SS)}0.50$  where  $X$  is the expected response at a given concentration  $y$  and  $W$  is the observed response of SS and SR genotypes at concentration  $Y$ , obtained from the respective regression lines. The expected responses of the females derived from the backcross to the R strain were calculated by  $X_y = W_{(RS)}0.50 + W_{(RR)}0.50$  (Georghiou 1969). The relationships between observed and expected concentration-responses were analyzed by a  $\chi^2$  test ( $P < 0.05$ ).

**Stability of Cyhexatin Resistance.** To determine the rate at which colonies of *T. pacificus* having different resistance gene frequencies would lose their resistance to cyhexatin if held without cyhexatin selection, we conducted tests with mites of known genotypes in cages in the Univ. Calif. Berkeley greenhouse. Each cross (Chapla  $\times$  Chapla, Wasco-18  $\times$  Wasco-18, Wasco-18 females  $\times$  Chapla males, and Chapla females  $\times$  Wasco-18 males) was made with 200 pairs of spider mites. Female deutonymphs and adult males were placed on cut leaves on moist cotton in plastic trays and held in the laboratory at 25–29°C under continuous light. After 2 d, females had mated and they were moved to clean leaves every 2–3 d to deposit eggs. When these eggs hatched, the leaves containing larvae were placed on bean plants in cages in the greenhouse. Two colonies were started for each cross with at least 4,000 individuals per colony. Each colony was placed in a cage, and cages were placed on two benches in a randomized block design; cages were ca. 60 cm apart.

Cages rested on water moats to minimize mite movement from cage to cage. Each cage contained two pots of beans, one of which was replaced with a fresh pot each week. About three generations of mites developed in these cages each month from May 1986 to February 1987. From May to October, the temperature ranged from 23 to 31°C; from October to February, temperatures ranged from 21 to 26°C. Mites were held under continuous light. In addition, we selected one cage of the Wasco-18 colony with cyhexatin periodically to use as a comparison with the Wasco-18 colonies that received no additional cyhexatin selections. All colonies were treated twice with 1.2 g (AI) of carbaryl per liter

of water to eliminate the phytoseiid predator *Amblyseius californicus* (McGregor), which was discovered in one cage.

After the colonies were established, each colony, and the selected Wasco-18 colony, was screened with 45 ppm flowable cyhexatin every 2–6 wk from June 1986 until February 1987. For each test, ca. 200 adult females from each cage were tested, and ca. 50 females were used as water controls. Females were tested with a leaf-spray technique, but instead of transferring females individually with a fine camel's-hair brush, females were transferred using a vacuum pump aspirator system. About 25–30 females were aspirated, then tapped onto leaf disks (3.0 cm diameter) that rested on moist cotton in plastic trays. The trays were sprayed with 45 ppm cyhexatin or water, held for 48 h at 25–29°C, and females were scored as dead or alive.

The results from the single-concentration assays were confirmed by complete concentration/mortality lines after 6 mo, or ca. 15 generations. Concentration/mortality lines at the start of the experiment had been conducted as part of the mode-of-inheritance test. The test done after 6 mo differed from the first only in that mites were placed on the leaf disks using the vacuum aspirator system.

A regression of % mortality over bioassay date was conducted with each colony to determine whether the slope differed significantly from zero. A significant decline in slope through time (9 mo) would suggest that selection against cyhexatin resistance allele(s) occurred in the absence of cyhexatin selection.

## Results

**Concentration/Mortality Lines for Adult Females.** Fifteen selections of the Wasco colony increased tolerance to cyhexatin. The  $LC_{50}$  of the Wasco-15 colony was 27-fold greater than that of the susceptible Chapla colony and 6-fold greater than that of the Wasco base colony. The  $LC_{50}$  of the Wasco base colony was 966 (95% CL = 492–1,500) ppm and the  $LC_{90}$  was 18,804 (9,294–76,872) ppm ( $n = 889$ ). The  $LC_{50}$  of the Wasco-15 colony was 5,809 (3,516–12,012) ppm and the  $LC_{90}$  was 250,098 (66,360–5,437,380) ppm ( $n = 950$ ). However, because the slopes for the Wasco base and Wasco-15 colonies were flat ( $0.99 \pm 0.12$  and  $0.78 \pm 0.12$  [mean  $\pm$  SE], respectively) with this assay, which used the wettable powder formulation, the tests were repeated with adult females of the Wasco-18 and Chapla colonies to determine whether the flowable formulation might give a steeper slope. In both the Chapla and Wasco-18 colonies, the flowable formulation resulted in steeper slopes (see below). The reasons for this are unknown, but it is likely that particle size influences the toxicity of these formulations. Particle sizes for the flowable formulation are smaller than for the wettable powder formulation.



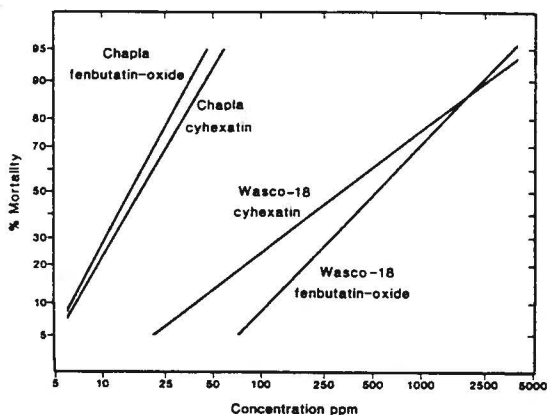


Fig. 1. Concentration/mortality lines comparing responses of the susceptible Chapla and resistant Wasco-18 colonies of *T. pacificus* to cyhexatin and fenbutatin-oxide (leaf-spray technique and flowable formulations).

**Concentration/Mortality of Resistant and Susceptible Larvae.** Larvae from the Wasco base and Wasco-15 colonies were resistant to cyhexatin, while the Chapla larvae were susceptible. However, resistance ratios could not be calculated because we did not obtain a line for the resistant Wasco larvae due to a plateau in their response. The Wasco base colony larvae ( $n = 250$ ) were tested with water, 300, 600, 1,200, or 2,400 ppm (50 WP) cyhexatin, and survival after 48 h was 98, 84, 80, 90, and 62%, respectively. Wasco-15 larvae ( $n = 250$ ), treated with water, 1,200, 2,400, 3,600, or 4,800 ppm cyhexatin, had survival rates of 100, 94, 92, 98, and 90%, respectively. Concentration/mortality lines could not be calculated. The Wasco-15 larvae, like the adult females, are more resistant to cyhexatin than were the Wasco base colony larvae, indicating that selection was effective on both stadia. The concentration/mortality line for the Chapla larvae

( $n = 250$ ) was calculated— $LC_{50}$  was 295 ppm (212–395), the  $LC_{90}$  was 1,252 ppm (841–2,428), and the slope was  $2.04 (\pm 0.25)$ . Survival on water, 60, 300, 600, or 1,200 ppm was 100, 82, 62, 34, and 0%, respectively.

**Relationship Between Cyhexatin and Fenbutatin-Oxide Resistance.** Concentration/mortality lines obtained with flowable formulations of cyhexatin and fenbutatin-oxide were compatible with the hypothesis that fenbutatin-oxide and cyhexatin resistances are related in *T. pacificus* (Fig. 1). Thus, the cyhexatin-susceptible Chapla colony was equally susceptible to fenbutatin-oxide, and the cyhexatin-resistant Wasco-18 colony was resistant to fenbutatin-oxide.  $LC_{50}$  (95% CL),  $LC_{90}$  (95% CL), and slopes ( $\pm SE$ ) for the susceptible Chapla colony were as follows: fenbutatin-oxide ( $n = 675$ ), 15 (13–17) ppm, 36 (31–44) ppm, and  $3.43 (\pm 0.29)$ ; cyhexatin ( $n = 675$ ), 17 (14–21) ppm, 45 (36–65) ppm, and  $3.11 (\pm 0.3)$ . The lines and intercepts were equal (Savin et al. 1977).

$LC_{50}$  (95% CL),  $LC_{90}$ 's, and slopes ( $\pm SE$ ) for the resistant Wasco-18 colony were as follows: fenbutatin-oxide ( $n = 675$ ), 527 (357–738) ppm, 2,443 (1,495–6,885) ppm, and  $1.92 (\pm 0.24)$ ; cyhexatin ( $n = 675$ ), ~~they were~~ 327 (233–443) ppm, 2,638 (1,645–5,590) ppm, and  $1.41 (\pm 0.16)$ . Slopes of these lines and intercepts were not equal, nor were lines parallel (i.e., equal slopes; Savin et al. [1977]). Thus, the Wasco colony selected with cyhexatin was actually more resistant to fenbutatin-oxide than to cyhexatin.

**Mode of Inheritance.** The reciprocal  $F_1$  females yielded lines which were intermediate between the lines of the susceptible Chapla and resistant Wasco-18 colonies (Table 1A; Fig. 2) the  $LC_{50}$  for the S colony was 18 ppm, the  $LC_{50}$  for the R colony was 176 ppm, and the values for the RS and SR  $F_1$  females were 39 and 50, respectively (Table 1A). This indicated that cyhexatin resistance is not com-

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Table 1. Mode of inheritance of cyhexatin resistance in *T. pacificus* using a leaf-spray technique and a flowable formulation (DOW XRM-4868)

| Cross                                | Total no. tested | $LC_{50}$ ppm | 95% CL  | $LC_{90}$ ppm | 95% CL    | Slope $\pm SE$  |
|--------------------------------------|------------------|---------------|---------|---------------|-----------|-----------------|
| A. Tests of reciprocal $F_1$ progeny |                  |               |         |               |           |                 |
| S (Chapla) ♀♀                        | 750              | 17.6          | 16–19   | 31.0          | 27–38     | $5.25 \pm 0.58$ |
| R (Wasco-18) ♀♀                      | 960              | 175.5         | 145–209 | 728.1         | 577–985   | $2.07 \pm 0.16$ |
| $F_1$ RS ♀♀ (R ♀ × S ♂)              | 765              | 39.0          | 33–45   | 98.5          | 84–121    | $3.18 \pm 0.27$ |
| $F_1$ SR ♀♀ (S ♀ × R ♂)              | 780              | 50.3          | 39–60   | 156.6         | 123–232   | $2.60 \pm 0.32$ |
| $F_1$ ♂♂ (R ♀ × S ♂)                 | 280              | 79.7          | 28–150  | 758.6         | 391–2,566 | $1.31 \pm 0.22$ |
| $F_1$ ♂♂ (S ♀ × R ♂)                 | 350              | 21.5          | 15–27   | 55.5          | 42–99     | $3.12 \pm 0.59$ |
| S (Chapla) ♂♂                        | 360              | 25.6          | 20–30   | 43.5          | 36–64     | $5.57 \pm 1.02$ |
| R (Wasco-18) ♂♂                      | 316              | 121.4         | 32–236  | 1,116.3       | 574–5,608 | $1.31 \pm 0.27$ |
| B. Tests of reciprocal $F_2$ progeny |                  |               |         |               |           |                 |
| S (Chapla) ♀♀                        | 1,335            | 15.2          | 14–16   | 24.4          | 23–27     | $6.29 \pm 0.44$ |
| R (Wasco-20) ♀♀                      | 1,335            | 200.8         | 171–232 | 861.2         | 713–1,090 | $2.03 \pm 0.12$ |
| $F_2$ (RS × R) ♀♀                    | 1,525            | 87.1          | 75–100  | 412.8         | 334–542   | $1.90 \pm 0.11$ |
| $F_2$ (SR × S) ♀♀                    | 1,475            | 22.9          | 20–26   | 75.9          | 66–91     | $2.47 \pm 0.14$ |
| $F_2$ ♂♂ (RS × R)                    | 325              | 35.2          | 20–55   | 230.8         | 140–501   | $1.57 \pm 0.20$ |
| $F_2$ ♂♂ (SR × S)                    | 430              | 20.0          | 11–31   | 199.5         | 124–427   | $1.28 \pm 0.16$ |
| S (Chapla) ♂♂                        | 320              | 10.5          | 5–14    | 30.9          | 24–52     | $2.74 \pm 0.53$ |
| R (Wasco-20) ♂♂                      | 340              | 289.1         | 161–419 | 1,151.0       | 752–2,687 | $2.14 \pm 0.36$ |

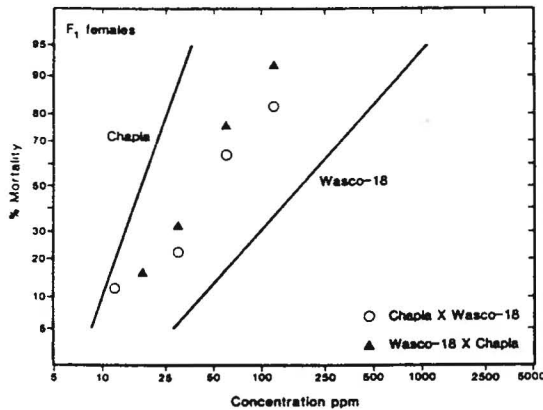


Fig. 2. Mode of inheritance of cyhexatin resistance in *T. pacificus*; concentration/mortality lines of the reciprocal  $F_1$  females (leaf-spray technique and the flowable cyhexatin formulation).

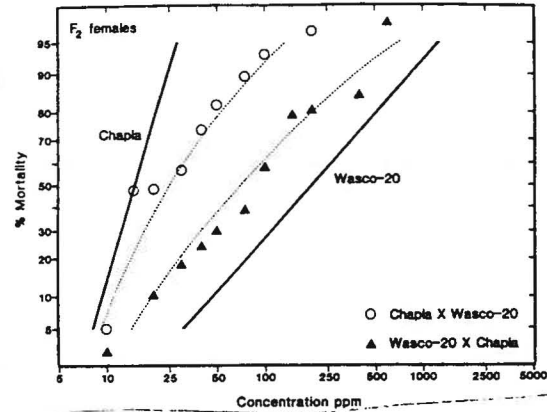


Fig. 4. Concentration/mortality curves for reciprocal  $F_2$  females derived from crossing Wasco-20 females with Chapla males and Chapla females with Wasco-20 males. Dashed lines represent responses expected if cyhexatin resistance in *T. pacificus* were determined by a single major semirecessive gene.

pletely dominant or recessive (Tsukamoto 1963). The lines for the reciprocal  $F_1$  females are not the same but are parallel (Savin et al. 1977). The reasons for the differences between the concentration/mortality lines of the reciprocal  $F_1$  females are unknown, but they could be due to cytoplasmic inheritance, maternal effects, or experimental error. The estimate of dominance (D) for the  $F_1$  females from the Wasco-18 female  $\times$  Chapla male cross was  $-0.308$ . For the reciprocal cross, D was  $-0.087$ . Both values indicate that cyhexatin resistance is incompletely recessive (Georghiou 1969).

Reciprocal  $F_1$  males yielded concentration/mortality lines that were similar to the males from the susceptible Chapla or resistant Wasco-18 colonies, respectively (Table 1A; Fig. 3). The  $LC_{50}$  for the Wasco-18 colony males was 121 ppm; for the males derived from the cross between Wasco-18 females  $\times$  Chapla males, the  $LC_{50}$  was 80 ppm (Table 1A). These lines are equal (Savin et al. 1977). The equality of the lines confirms the arrhenotokous

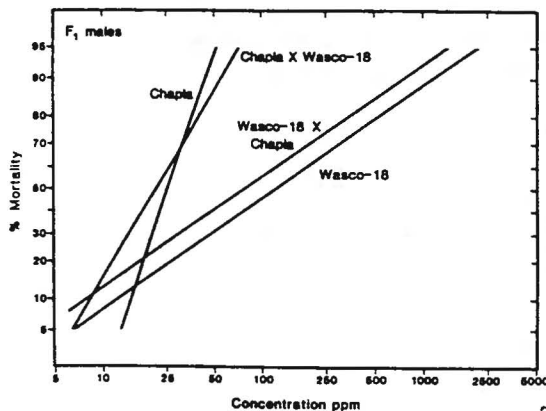


Fig. 3. Concentration/mortality lines of reciprocal  $F_1$  males compared with males from the susceptible (Chapla) and resistant (Wasco-18) lines of *T. pacificus*.

mode of inheritance for *T. pacificus*, in which haploid sons inherit their resistance, and other, genes from their mothers only. This also confirms that the  $F_2$  female progeny are genetically equivalent to backcross progeny. The larger confidence limits for males (Table 1A), compared with the lines of the females, are probably due to the smaller sample sizes (50 males per concentration tested), and the fact that males tended to run off the leaf disks more than females did, even if females were present.

The concentration/mortality lines for the reciprocal  $F_2$  females were significantly different, as expected (Fig. 4; Table 1B). The  $LC_{50}$  for the Wasco-20 colony was 201 ppm, the  $LC_{50}$  for the susceptible Chapla colony was 15 ppm, the  $F_2$  females derived from the  $RS \times R$  cross had an  $LC_{50}$  of 87 ppm,

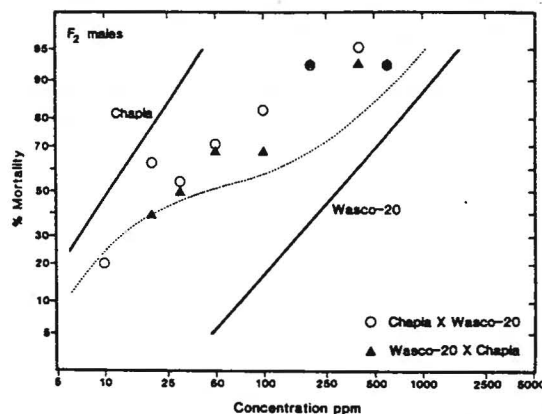


Fig. 5. Concentration/mortality lines for reciprocal  $F_2$  males derived from the crosses described in Fig. 4. The dashed line represents the responses expected if cyhexatin resistance were determined by a single semirecessive gene.

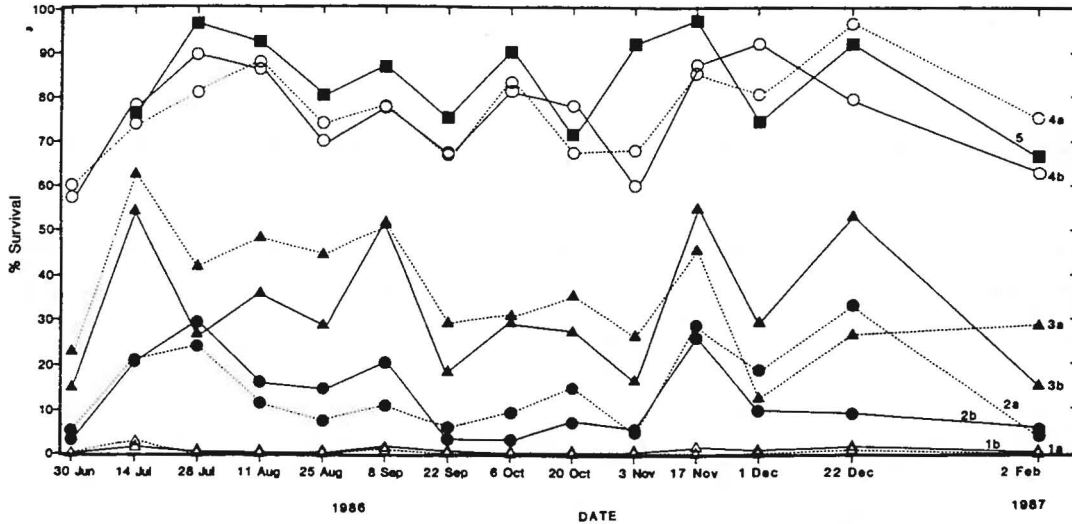


Fig. 6. Stability of cyhexatin resistance in colonies of *T. pacificus* derived from known genotypes that were untreated with cyhexatin. Lines 1a, 1b are of the susceptible Chapla colony; lines 2a, 2b are of a colony derived from F<sub>1</sub> females from the cross (Chapla female × Wasco-18 male); lines 3a, 3b are from the reciprocal F<sub>1</sub> cross (Wasco-18 female × Chapla male); 4a, 4b are of the resistant (Wasco-18) colony without cyhexatin selection, while the Wasco-18 colony in line 5 continued to receive cyhexatin selections an additional five times. Survival of 200 adult females was assessed using 45 ppm flowable cyhexatin with a leaf-spray technique.

and the F<sub>2</sub> females derived from the SR × S cross had an LC<sub>50</sub> of 23 ppm (Table 1B).

The dotted lines in Fig. 4 give the values expected for F<sub>2</sub> females, if we assume that cyhexatin resistance is determined by a single major semirecessive gene. Chi-square analysis of the goodness-of-fit of the observed mortality to that expected, assuming the probit model, was not significantly different ( $\chi^2 = 8.29$ ; df = 11;  $P < 0.7$ ) for the F<sub>2</sub> females (RS × R) from the cross Wasco-20 female × Chapla male. F<sub>2</sub> females (SR × S) from the reciprocal cross (Chapla female × Wasco-20 male) give a significant departure from expected ( $\chi^2 = 36.9$ ; df = 9;  $P < 0.0005$ ). When the points were examined individually, four of the nine points

were significantly different (mortality at 15, 20, 50, and 75 ppm was higher than expected).

The concentration/mortality lines for F<sub>2</sub> males are shown in Fig. 5. Again, the dashed lines give the expected mortality if a single semirecessive gene confers cyhexatin resistance. The LC<sub>50</sub> for the Wasco-20 males was 289 ppm and that of the F<sub>2</sub> males derived from the Wasco-20 female × Chapla male cross was 35 ppm (Table 1B). The F<sub>2</sub> males derived from the Chapla female × Wasco-20 cross had an LC<sub>50</sub> of 20 ppm; the Chapla colony had an LC<sub>50</sub> of 11 ppm (Table 1B).

Chi-square analysis of the goodness-of-fit of the observed mortality to mortality expected, assuming the probit model for F<sub>2</sub> males derived from the Wasco-20 female × Chapla male cross, indicated no significant departure from the model ( $\chi^2 = 13.0$ ; 8 df;  $P > 0.10$ ). In contrast,  $\chi^2$  analysis of the reciprocal F<sub>2</sub> males derived from the Chapla female × Wasco-20 male cross indicated significant departure from the model ( $\chi^2 = 22.6$ ; df = 8;  $P < 0.005$ ). Effects of five concentrations of eight were significantly different from values expected, assuming the probit model. Possible reasons for this poor fit include lack of purity of the S or R colonies (or both), experimental error, and the influence of additional modifying genes. Of these possibilities, we conclude colony purity is least likely to influence the results, and that one or more modifying genes are likely to influence cyhexatin (and fenbutatin-oxide) resistance(s). Without suitable genetic markers, however, discrimination between inheritance determined by a major semirecessive gene with modifier(s) and other more complex in-

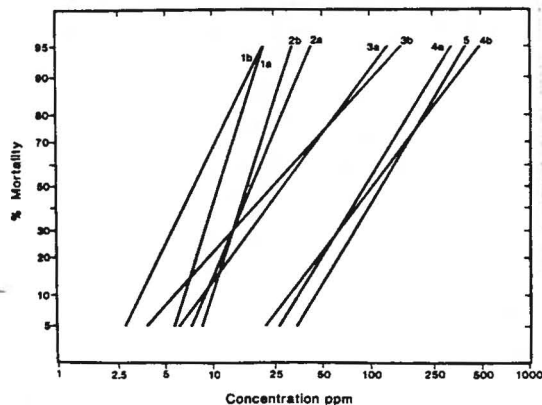


Fig. 7. Concentration/mortality lines of the colonies described in Fig. 6 done 6 mo after starting the stability experiment (leaf-spray technique).

Table 2. Stability of cyhexatin-resistance after 6 mo without treatment with cyhexatin or fenbutatin-oxide determined by comparing concentration/mortality data for a cyhexatin-resistant *T. pacificus* colony (Wasco-18), a susceptible colony (Chapla), and progeny derived from reciprocal crosses, using a leaf-spray technique

| Colony  | No. ♀♀ tested | LC <sub>50</sub> ppm | 95% CL | LC <sub>90</sub> ppm | 95% CL  | Slope ± SE  |
|---|---------------|----------------------|--------|----------------------|---------|-------------|
| 1a. Chapla (susceptible)                      | 613           | 11.1                 | 9-13   | 18.6                 | 17-22   | 5.72 ± 0.79 |
| 1b. Chapla (susceptible)                      | 630           | 7.7                  | 6-10   | 17.1                 | 15-21   | 3.70 ± 0.39 |
| 2a. Chapla ♀ × Wasco-18 ♂                     | 614           | 17.6                 | 13-21  | 42.9                 | 35-62   | 3.31 ± 0.43 |
| 2b. Chapla ♀ × Wasco-18 ♂                     | 628           | 16.9                 | 12-20  | 32.5                 | 27-44   | 4.51 ± 0.52 |
| 3a. Wasco-18 ♀ × Chapla ♂                     | 623           | 28.2                 | 19-36  | 92.7                 | 69-151  | 2.48 ± 0.25 |
| 3b. Wasco-18 ♀ × Chapla ♂                     | 616           | 24.5                 | 17-31  | 103.8                | 79-161  | 2.05 ± 0.23 |
| 4a. Wasco-18 (resistant)                      | 625           | 93.8                 | 72-116 | 250.7                | 194-373 | 3.00 ± 0.31 |
| 4b. Wasco-18 (resistant)                      | 622           | 103.7                | 54-144 | 353.4                | 255-680 | 2.41 ± 0.36 |
| 5. Wasco-22 (selected resistant) <sup>a</sup> | 609           | 117.5                | 75-152 | 305.7                | 229-564 | 3.09 ± 0.44 |

<sup>a</sup> This colony was selected four times with cyhexatin during the 6-mo interval and serves as a selected R control.

heritance patterns will be difficult (Tsukamoto 1963).

The data were also examined to determine whether they fit a polygenic model, in which multiple alleles contribute equally and additively to the resistance. In this model, concentration/mortality lines for the F<sub>1</sub> females should have been approximately equidistant between the R and S lines. This did not occur, nor were the lines for the reciprocal F<sub>2</sub> females equidistant between the F<sub>1</sub> lines and the R and S lines, respectively. Thus, the simplest model providing the best fit to the data is the semirecessive gene model.

**Stability of Cyhexatin Resistance.** Fig. 6 shows that survival of females tested every 2-6 wk from June 1986 through February 1987 varied from sample date to sample date. However, the slope of regression lines estimated for each replicate did not differ significantly from zero in any case. Lines 1a, 2a, 2b, and 5 do not have a slope significantly different from zero ( $P > 0.25$ ); lines 1b ( $P = 0.22$ ), 3a ( $P = 0.72$ ), 3b ( $P = 0.116$ ), 4a ( $P = 0.219$ ), and 4b ( $P = 0.074$ ) also did not have a slope significantly different from zero.

Fig. 7 shows complete concentration/mortality lines obtained for all the colonies in October, ca. 6 mo after the start of the trial. The two replicates of each colony clearly yielded similar concentration/mortality lines. Although the lines and slopes for the susceptible Chapla colonies (1a, 1b) are significantly different from each other ( $P < 0.05$ ), the absolute values are similar (Table 2). The differences suggest that genetic drift, or a small amount of immigration of resistant mites into one cage, may have occurred. The unselected resistant Wasco-18 colonies (4a, 4b) were not significantly different from the Wasco-22 colony (line 5), which was selected an additional four times (Fig. 7, lines 4a, 4b, and 5; Table 2). This suggests that no contamination with susceptible mites occurred. The cyhexatin resistance was stable in lines 4a and 4b over the 9 mo of the test.

Concentration/mortality lines for the two colony replicates derived from crossing Chapla females × Wasco-18 males also yielded different lines with different slopes. Again, while these lines (2a, 2b,

Fig. 7) are significantly different ( $P < 0.05$ ), absolute values are similar (Table 2). Such differences could be due to experimental error, genetic drift, or differential fitness of the RR, RS, SR, and SS genotypes. As expected, the two colony replicates derived from crossing Wasco-18 females × Chapla males had higher LC<sub>50</sub>'s than the colonies derived from the reciprocal crosses, and lines 3a and 3b are the same and are parallel (Table 2).

These data support the hypothesis that cyhexatin resistance in this colony of *T. pacificus* is stable under these conditions; failure of colonies 4a, 4b, 3a, 3b, and 2a, 2b to develop a negative slope (Fig. 6) suggests that there was no selection against the cyhexatin resistance allele(s) in the absence of cyhexatin selection. If substantial reductions in fitness were associated with the cyhexatin resistance allele(s), the slopes should have declined over the 9 mo of the project, during which a *minimum* of 27 generations (3 generations per month × 9 mo) could develop.

## Discussion

This colony of *T. pacificus* has a high level of cyhexatin and fenbutatin-oxide resistances, and the congruence of the concentration/mortality lines for the two acaricides (Fig. 1) supports the hypothesis that there is cross resistance, particularly since both acaricides are similar chemically and could therefore have elicited an identical selection response. Until the mechanism(s) of resistance are investigated, however, we cannot make a stronger conclusion regarding the number of genes involved in cyhexatin or fenbutatin-oxide resistances (or both). The data on mode of inheritance of resistance to cyhexatin (and fenbutatin-oxide) are incompletely recessive ( $D = -0.308$  and  $-0.087$ ). The concentration/mortality lines of the reciprocal F<sub>2</sub> females and males are consistent with the model involving a single semirecessive gene. This does not eliminate the possibility that modifier genes are also involved (Tsukamoto 1963). Stability of the cyhexatin resistance in the replicated cage populations initiated with known genotypes (RR, RS, SR, or SS females) suggests that little contamination occurred between

the cages, and that the cyhexatin resistance is stable under these test conditions. The stability of the resistance suggests that the fitness of the cyhexatin resistance allele(s) is (are) similar to the wild type allele in this population of *T. pacificus*. The RS and SR cages were most likely to have shown a decline in resistance allele frequency, and this did not occur. These data suggest that, once populations of *T. pacificus* become homozygous for cyhexatin resistance, reversion to susceptibility is unlikely to occur rapidly unless large scale immigration of susceptible mites were to occur.

#### Acknowledgment

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# Selection for Abamectin Resistance in *Tetranychus urticae* and *T. pacificus* (Acari: Tetranychidae)

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**ABSTRACT** Three colonies of *Tetranychus urticae* Koch and two colonies of *Tetranychus pacificus* McGregor were selected in the laboratory and greenhouse with abamectin (avermectin B<sub>1a</sub>). Two selection methods were used. One challenged ability of gravid females to survive 48 h on dried residues using concentrations that resulted in 40–60% mortality. The other technique selected against all life stages on bean plants. Concentration/response bioassays were used to determine whether a selection response was achieved. The three *T. urticae* colonies were selected 4, 6, or 15 times. LC<sub>50</sub>'s for two of these colonies before selection ranged from 0.06 to 0.15 ppm on different dates. After selection, the *T. urticae* colonies' LC<sub>50</sub>'s were 0.12 and 0.24 ppm (values not significantly different from those of their respective base colonies). Likewise, for the two *T. pacificus* colonies (selected 2 and 15 times), no response to selection occurred. LC<sub>50</sub>'s of the unselected *T. pacificus* base colonies ranged from 0.04 to 0.12 ppm on different dates. Selected lines had LC<sub>50</sub>'s of 0.06 and 0.08 ppm, values not significantly different from LC<sub>50</sub>'s of their base colonies. These negative results do not preclude the possibility that resistance to abamectin will develop in other populations of *T. urticae* or *T. pacificus*. Because at least one of the *T. urticae* and *T. pacificus* colonies tested was resistant to propargite, cyhexatin, and fenbutatin-oxide, we suggest that cross-resistances are unlikely.

**KEY WORDS** *Tetranychus urticae*, *Tetranychus pacificus*, abamectin, resistance, selection, avermectin B<sub>1a</sub>.

SPIDER MITES are major agricultural pests around the world. One reason for their economic importance is their propensity to develop resistance to pesticides used to control them (Dittrich 1975, Cranham & Helle 1985). Spider mites have become resistant to DDT, organophosphorus insecticides, sulfur, carbamates, and others. Often, new acaricides have been used for only 1 or 2 years before spider mites become resistant. Recently, spider mite populations have developed resistances to propargite (Chapman & Penman 1984, Keena & Granett 1985; unpublished data), cyhexatin (Edge & James 1982, 1983, Croft et al. 1984, Hoyt et al. 1985, Carbonaro et al. 1986; unpublished data), and fenbutatin-oxide (unpublished data). These three acaricides have been especially useful in integrated mite management programs in deciduous orchards and vineyards because they are selective for insect and phytoseiid predators of spider mites. As resistances to them become more prevalent, other acaricides will be substituted. Hopefully, these new acaricides will be selective for phytoseiid predators.

Abamectin (avermectin B<sub>1a</sub>) is an effective acaricide and shows potential as a selective acaricide for western predatory mite, *Metaseiulus occidentalis* (Nesbitt) (also treated as *Typhlodromus* or *Galendromus*). However, selectivity may only be achieved if methods to use it at lower-than-proposed label rates are developed (Grafton-Cardwell

& Hoy 1983, Hoy & Cave 1985). Acquisition of the field data requisite to integrating abamectin into integrated mite management programs will require time and funds and will not be initiated if cross-resistances to abamectin are known to exist in spider mite populations that are resistant to propargite, cyhexatin, or fenbutatin-oxide.

Here we describe the results of selecting with abamectin three colonies of *Tetranychus urticae* Koch and two of *Tetranychus pacificus* McGregor having variable levels of propargite, cyhexatin, and fenbutatin-oxide resistances.

## Materials and Methods

### Colony Sources and Rearing Methods

**Berkeley Greenhouse Colony.** A *T. urticae* colony resident in the greenhouses at Oxford Tract, University of California, Berkeley, was isolated during April 1984. About 100 females initiated the colony, which is susceptible to propargite, cyhexatin, and fenbutatin-oxide (unpublished data).

**Chico Almond Colony.** This *T. urticae* colony was derived from seven colonies collected in April 1984 from almond orchards in the Chico-Durham area in Butte County, Calif. The mites had experienced strong selection with propargite and cyhexatin, as well as carbaryl, pyrethroids, and organophosphorus insecticides during the past 5 years. Twenty-five females from each of the seven

colonies were pooled and, after several generations had elapsed, selection was begun. Five of the original seven colonies were tested with cyhexatin and propargite. All were susceptible to propargite; three were moderately resistant to cyhexatin (unpublished data).

**Watsonville Strawberry Colony.** A colony of *T. urticae* was collected on 15 March 1985 from a second-year field near Watsonville, Calif., in Santa Cruz County. About 150 females were used to initiate the colony. Its susceptibility to acaricides was not assessed, but Croft et al. (1984) found *T. urticae* from that area to be resistant to cyhexatin. Cyhexatin resistance is widespread in strawberries in that area (C. Pickel, personal communication).

**Bakersfield Almond Colony.** This *T. pacificus* colony was derived from three colonies collected in Kern County near Bakersfield, Calif., during July 1984. A pooled colony was initiated with ca. 50 females from each of the three colonies, and selection was begun two or three generations later. The original colonies were resistant to cyhexatin, fenbutatin-oxide, and propargite (unpublished data).

**Modesto Almond Colony.** This *T. pacificus* colony was collected on 17 April 1985 in Stanislaus County, Calif. At least 50 females were used to initiate the colony. It was tested with propargite and cyhexatin and exhibited moderate resistance levels to both acaricides (unpublished data).

**Colony Rearing Method.** Spider mite colonies were reared on pinto bean plants, *Phaseolus vulgaris* L., in cages in the greenhouse. Plants were grown in pots (20 cm diameter) or in flats (25 by 34 by 8 cm) in a mixture of vermiculite and University of California potting mixture. Newly emerged plants were inoculated with active stages of mites from infested foliage of the old cultures and held in wooden cages (53 by 42 by 81 cm) screened with a fine-meshed white cotton organza. The cages were placed on water moats to reduce mite movement from cage to cage. Cultures were kept at ca. 20–40°C in the greenhouse under a photoperiod of 18:6 (L:D).

### Selection and Assay Methods

**Leaf Dip Selection Method.** Whole pinto bean leaves with cut edges were dipped into abamectin solutions or water, placed underside up on moist cotton in plastic trays, and allowed to dry. Fresh solutions were made for each selection using distilled water and a wetting agent (0.1% Triton AG-98). Fifty active gravid females were placed on the undersurface of each leaf, and an average of 735 females (400–1,000) was selected each time. About 100 females (50–100) were placed on control leaves, and ca. 200 females (100–200) of the base colony were tested each time for comparison after the first selection. After 48 h at 25–28°C under continuous light, mites were scored as dead or alive. Dead mites were those obviously dead plus

those that were immobilized. Immobilized mites do not deposit eggs and do not recover. Survivors could walk when touched with a brush; this included mites that dragged their hind legs. Leaves were then placed on bean plants in cages in the greenhouse and the mites were reared for several generations before the next selection. The Berkeley greenhouse and Chico *T. urticae* colonies and the Bakersfield *T. pacificus* colony were selected with 0.04 ppm abamectin using this technique.

**Bean Flat Selection Method.** Selection was conducted by spraying infested or uninfested flats of pinto beans until runoff. The plants were allowed to dry. If uninfested with mites, they were infested by cutting the old plants, placing the foliage on the new flat, and allowing the spider mites to walk on to the new, freshly treated foliage. Flats were then held in cages in the greenhouse as described in the colony rearing methods. This technique exposed all stages of spider mites to abamectin for an extended time period. Concentrations used for *T. urticae* selection ranged from 0.008 to 0.25 ppm; for *T. pacificus*, concentrations ranged from 0.004 to 0.50 ppm. Treatments were applied every 1–8 weeks, depending on the size of the population. Survival of the selected population was estimated by counting the number of dead and living adult females on four leaves after 48 h. This information was used to determine doses for subsequent selections. Concentrations were increased whenever survival increased to more than ca. 80%. Concentration/mortality lines were obtained after two to seven selections to compare the base and selected colonies.

The Chico *T. urticae* and Bakersfield *T. pacificus* colonies that had been selected using the leaf dip method for three selections were subsequently selected with this method. The Watsonville strawberry and Modesto almond colonies were selected exclusively by this method.

**Concentration/Mortality Lines.** Concentration/mortality lines were obtained using a dipped bean leaf disk method. Five doses of abamectin and a water control were made fresh each test date. The solvent was distilled water and a wetting agent (0.1% Triton AG-98). Leaf disks (1.75 cm diameter) were dipped into solutions, placed on moist cotton in plastic trays, and dried. Five females were placed on each disk. At least eight replicates per concentration (range, 8–16) were tested. Females were held at 25–28°C under continuous light for 48 h. Survivors were scored as described in the selection methods. Data were analyzed using the POLO program (Russell et al. 1977). Concentration/mortality lines of the selected and base colonies were compared by the likelihood ratio test (Savin et al. 1977).

### Results

Three colonies, Berkeley greenhouse *T. urticae*, Chico almond *T. urticae*, and Bakersfield almond

**Table 1. Concentration responses to abamectin of the Chico base and selected colonies of *T. urticae* from almonds by leaf dip analysis**

| Test colony             | No. tested | LC <sub>50</sub> (ppm) | 95% CL       | LC <sub>90</sub> (ppm) | 95% CL       | Slope ± SE  |
|-------------------------|------------|------------------------|--------------|------------------------|--------------|-------------|
| A) Chico Base           | 540        | 0.06                   | 0.05-0.06    | 0.09                   | 0.08-0.11    | 6.32 ± 1.00 |
| Chico-III               | 540        | 0.06                   | 0.05-0.06    | 0.09                   | 0.08-0.12    | 6.75 ± 1.07 |
| B) Chico Base           | 450        | 0.12                   | 0.10-0.21    | 0.30                   | 0.18-2.16    | 3.17 ± 0.86 |
| Chico-VIII <sup>a</sup> | 450        | 0.11                   | 0.08-0.16    | 0.41                   | 0.25-1.05    | 2.19 ± 0.27 |
| C) Chico Base           | 110        | 0.14                   | <sup>b</sup> | 0.28                   | <sup>b</sup> | 4.51 ± 2.00 |
| Chico-XV                | 110        | 0.12                   | 0.10-0.15    | 0.20                   | 0.15-0.46    | 6.03 ± 1.48 |

Test A was conducted after females were selected three times using the leaf dip technique. Tests B and C were conducted after the colony was selected on whole plants in the greenhouse an additional 5 and 12 times, respectively.

<sup>a</sup> Lines and intercepts not the same ( $P < 0.05$ , likelihood-ratio test [Savin et al. 1977]).

<sup>b</sup> Confidence values were not calculated because the value of  $g$  was  $>0.50$  (Russell et al. 1977).

*T. pacificus*, were selected by the leaf dip method. No selection response was observed after four, three, and three selections, respectively, based on a comparison of the survival of the selected and base colonies after treatment with 0.04 ppm abamectin. Concentration/response tests with the Chico and Bakersfield colonies confirmed this (Tables 1, A and 2, A). Because of a virus disease (M.A.H., unpublished data), no concentration/response test was conducted on the Berkeley greenhouse base and selected colonies and this selection was discontinued. Survival rates were 46 and 44%, respectively, after four selections.

Selection of the Chico *T. urticae* and Bakersfield *T. pacificus* colonies was continued using the bean flat method. All stages of the Chico *T. urticae* colony were selected 12 more times with concentrations of abamectin increasing from 0.008 to 0.25 ppm. Concentration/response lines were obtained after the 8th and 15th selections (Table 1, B and C). A significant difference in lines and intercepts was found after the eighth selection, but the LC<sub>50</sub> of the selected colony was lower (0.11 ppm) than that of the base colony (0.12 ppm). The LC<sub>90</sub>, in contrast, was higher (0.41 ppm) for the selected colony than that of the base colony (0.30 ppm). Because no significant differences were found in these colonies after the 15th selection, we conclude

that the differences were due to normal variation (Table 1, B and C). The Bakersfield *T. pacificus* colony was selected 12 more times on bean flats using concentrations ranging from 0.004 to 0.50 ppm. Concentration/response tests were done after the 8th, 12th, and 15th selections (Table 2, B, C, and D). Significant differences were found between the Bakersfield-XII (12th selection) and base colony (Table 2, C), but this shift did not persist when concentration/response lines were compared later (Table 2, D).

The Watsonville *T. urticae* colony was selected six times on bean flats with concentrations ranging from 0.01 to 0.5 ppm. The concentration/response lines of the base and sixth-selection (Watsonville-VI) colonies were compared; no significant differences were found. LC<sub>50</sub>'s (90% CL) for the base and Watsonville-VI colonies were 0.15 (0.13-0.21) and 0.24 (0.18-0.74) ppm, respectively. LC<sub>90</sub>'s were 0.24 and 0.51 ppm, respectively. Slopes (±SE) were 6.07 ± 2.25 and 5.72 ± 2.74. The virus disease in these colonies prevented further selections.

The Modesto *T. pacificus* colony was selected twice on bean flats with 0.2 ppm abamectin. Concentration/response lines of the base and selected colonies gave LC<sub>50</sub>'s (95% CL) of 0.05 (0.04-0.06) and 0.06 (0.03-0.07) ppm, respectively. LC<sub>90</sub>'s were 0.10 (0.09-0.13) and 0.12 (0.11-0.14) ppm. Slopes

**Table 2. Concentration responses to abamectin of the Bakersfield base and selected colonies of *T. pacificus* by leaf dip analysis**

| Test colony                   | No. tested | LC <sub>50</sub> (ppm) | 95% CL                 | LC <sub>90</sub> (ppm) | 95% CL                  | Slope ± SE  |
|-------------------------------|------------|------------------------|------------------------|------------------------|-------------------------|-------------|
| A) Bakersfield                | 240        | 0.12                   | 0.08-0.49 <sup>a</sup> | 0.41                   | 0.18-25.80 <sup>a</sup> | 2.36 ± 0.92 |
| Bakersfield-III               | 240        | 0.09                   | <sup>b</sup>           | 0.14                   | <sup>b</sup>            | 6.00 ± 2.35 |
| B) Bakersfield                | 340        | 0.04                   | 0.02-0.06              | 0.10                   | 0.08-0.15               | 3.43 ± 0.60 |
| Bakersfield-VIII              | 340        | 0.05                   | 0.03-0.06              | 0.09                   | 0.08-0.11               | 5.23 ± 1.04 |
| C) Bakersfield                | 360        | 0.05                   | 0.04-0.06              | 0.12                   | 0.10-0.17               | 4.12 ± 0.73 |
| Bakersfield-XIII <sup>c</sup> | 360        | 0.08                   | 0.07-0.09              | 0.12                   | 0.10-0.15               | 8.16 ± 1.86 |
| D) Bakersfield                | 420        | 0.07                   | 0.06-0.08              | 0.13                   | 0.12-0.16               | 4.80 ± 0.74 |
| Bakersfield-XV                | 420        | 0.08                   | 0.06-0.10              | 0.15                   | 0.13-0.20               | 5.28 ± 0.97 |

Test A was conducted after females were selected three times using the leaf dip technique. Tests B, C, and D were conducted after the colony was selected on whole plants in the greenhouse an additional 5, 9, and 12 times, respectively.

<sup>a</sup> 90% CL (95% CL could not be calculated because the value of  $g$  was  $>0.50$  [Russell et al. 1977]).

<sup>b</sup> Confidence values were not calculated because the value of  $g$  was  $>0.50$  (Russell et al. 1977).

<sup>c</sup> Lines and intercepts are not the same and lines are not parallel (Savin et al. 1977).



( $\pm$ SE) were  $4.35 \pm 0.59$  and  $4.31 \pm 0.51$ . The concentration/response lines were not significantly different.

### Discussion

Resistance to abamectin was not obtained in any of the five colonies of *T. urticae* and *T. pacificus* selected. Both the Bakersfield *T. pacificus* and Chico *T. urticae* colonies were pooled colonies from three and seven sites, respectively, and, therefore, may have greater genetic variability than if they were collected from a single source. The colonies were also of diverse geographic origin, ranging from Butte County in the northern part of the Central Valley of California to Kern County in the south. The colonies had experienced diverse pesticide applications. The Watsonville *T. urticae*, Bakersfield *T. pacificus*, and Chico *T. urticae* colonies each had detectable levels of resistances to propargite, or cyhexatin and fenbutatin-oxide before selection with abamectin. Thus, our selection tested the hypothesis that cross-resistances to abamectin do not occur in propargite-, or cyhexatin- and fenbutatin-oxide-resistant populations. Such cross-resistances were not expected because of the possibly unique mode of action of abamectin (Fritz et al. 1979), but predictions of potential cross-resistances are always risky. Thus, these data suggest that field trials to develop optimal methods for the selective use of abamectin are justified.

Such negative data do not preclude the possibility that resistance to abamectin will develop in populations of *T. urticae* and *T. pacificus*, however. Both species have a history of developing resistances to nearly every acaricide introduced. The longevity of cyhexatin and propargite use (ca. 20 years in deciduous orchards and vineyards) seems to be unusual.

It will be interesting to review the resistance development history for abamectin in the future to determine whether this evaluation was a useful predictor of resistance development or not. Recently, Roush & Wright (1986) tested pesticide-resistant strains of house fly, *Musca domestica* L., to determine if cross-resistances to abamectin exist. Those authors also failed to find cross-resistances. However, as Roush & Miller (1986) noted, monitoring for resistance in arthropods is difficult. Detecting (with a high degree of confidence) the existence of rare resistant individuals in a population requires large sample sizes. Our colonies were selected for abamectin resistance and, hence, rare resistance alleles, if present, would have increased in frequency. However, our selection project required >2 years and is, therefore, not an inexpensive method. Because negative selection results are rarely recorded in the literature, we have little information on which to judge whether laboratory selections are useful predictors of resistance development or not. At the least, our data establish

baseline information on field-collected colonies of *T. urticae* and *T. pacificus* for future comparisons.

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# Benefit/Cost Analysis of an Integrated Mite Management Program for Almonds

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**ABSTRACT** An integrated mite management program for almonds is being implemented in California. Implementation includes using a predatory mite, *Metaseiulus occidentalis* (Nesbitt), genetically selected in the laboratory; monitoring predator/spider mite populations; and using reduced rates and numbers of applications of acaricides selective to this predator. Economic analysis suggests that growers who adopt the program will save \$60/ha (\$24/acre) to \$110/ha (\$44/acre). Programmatic benefit/cost analysis suggests that annual return on the research investment will range from 280 to 370%. This high rate of return on the original research investment may be due, in part, to the fact that more than half of the resources were allocated to field testing and implementation research.

**KEY WORDS** almonds, predatory mites, economic analysis, biological control, research investment

SPIDER MITES are economic pests in the majority of the 155,000 ha (395,000 acres) of almonds grown in California. Growers apply zero to three acaricides per season if they use a conventional acaricide program, and, conservatively, average about 1.5 acaricide applications per season (W. Barnett, personal communication; unpublished data). These applications cost about \$187.50/ha (\$75/acre) for the 1.5 applications per season if growers use standard label rates of propargite, cyhexatin, or fenbutatin-oxide (W. Barnett, D. Cahn, D. Castro, C. Kitiyama, and B. Wilke, personal communications; unpublished data, Bowen 1985).

During the past 7 yr, an integrated mite management (IMM) program was developed for almonds. This program is based on integrating chemical and biological control of spider mites through the use of insecticides for the navel orangeworm, *amcyolais transitella* (Walker), that are less toxic to the predatory mite, *Metaseiulus* (*Typhlodromus* or *Galendromus*) *occidentalis* (Nesbitt); use of lower-than-label rates of acaricides that are also less toxic for *M. occidentalis*; and the release of pesticide-resistant *M. occidentalis* (Acarinae: Phytoseiidae) in orchards where native organophosphorus-resistant *M. occidentalis* are lacking or are too rare to achieve effective control (Hoy 1982, 1984, 1985a, Hoy et al. 1982a, 1984, 1985). IMM requires that orchards be monitored using either a presence-absence system as described by Wilson et al. (1984) and Zalom et al. (1984), or a brush-and-count method as described in Hoy (1984, 1985a). Monitoring is necessary to ensure that an adequate predator/spider mite ratio exists to prevent economic damage. Only the effect of *Tetrany-*

*chus* species has been evaluated on almonds, using *Tetranychus urticae* Koch and *T. pacificus* (McGregor) (Barnes & Andrews 1978, Andrews & LaPre 1979, Welter et al. 1984). The effects of the European red mite, *Panonychus ulmi* Koch, feeding on almonds are unknown.

The IMM program can be used in almond orchards with native *M. occidentalis* because native predator populations usually have sufficient levels of resistance to organophosphorus (OP) insecticides so that azinphosmethyl, phosmet, and diazinon can be used to control the key insect pest, the navel orangeworm. Carbaryl and permethrin cannot be used with native *M. occidentalis* without causing disruption (Hoy et al. 1984, Hoy 1985a). If growers wish to use carbaryl for control of navel orangeworms, they can release the laboratory-selected strain of *M. occidentalis* that is resistant to carbaryl, sulfur, and OP insecticides (Hoy 1982, 1984, 1985a, Hoy et al. 1982a, 1984). This strain can be mass reared using two methods (Hoy et al. 1982b), and became commercially available from several firms in California during 1983. This strain will become established, persist for at least 5 yr in the orchard, and provide substantial to complete control of spider mites. A genetically improved strain, it makes carbaryl an option for control of the navel orangeworm; releases are also desirable if native *M. occidentalis* are rare in the orchard.

Integrated pest management (IPM) programs are rarely as simple as conventional chemical control programs. Before growers adopt the new technology, they must decide whether the new method is cost-effective, as well as efficacious. Efficacy of the IMM program has been demonstrated. The purpose of this paper is to compare the cost of a conventional mite control program using label rates of propargite (Omite), cyhexatin (Plictran), or fenbutatin-oxide (Vendex) with the costs of using lower-than-label rates of these materials in combina-

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**Table 1. Research expenditures for development of IMM program for almonds in California**

| Date                               | Expenditures | Value in 1985<br>with interest<br>compounded<br>at 12% |
|------------------------------------|--------------|--|
|                                    | Phase 1      |  |
| 1976-77                            | \$ 16,636    | \$ 41,190  |
| 1977-78                            | 31,596       | 69,849   |
| 1978-79                            | 36,073       | 71,202   |
| 1979-80                            | 63,719       | 112,295  |
| Total phase 1                      | \$148,024    | \$294,536  |
|                                    | Phase 2      |  |
| 1980-81                            | \$133,393    | \$209,896  |
| 1981-82                            | 77,859       | 109,366  |
| 1982-83                            | 76,140       | 95,510   |
| 1983-84                            | 102,245      | 114,514  |
| Total phase 2                      | \$389,637    | \$529,306  |
| Total cost phase 1<br>plus phase 2 | \$537,661    | \$823,842  |

tion with biological control by native strains of *M. occidentalis*. In addition, we compare the costs of conventional control with the costs of releasing the laboratory-selected carbaryl/OP/sulfur-resistant strain of *M. occidentalis* combined with lower-than-label acaricide rates. Both IMM tactics require monitoring of the predator/prey ratio to achieve effective control, and these costs are included in the comparisons. Finally, we compare the costs of the research conducted during the period July 1978-July 1984 to develop the IMM program with the benefits expected to accrue under differing levels of adoption by almond growers. This comparison provides a measure of the return on investment in this agricultural research.

#### Materials and Methods

**Sources of Information.** The data used to develop the cost savings budgets were obtained from interviews and a survey. Seventy-one almond growers completed a survey while attending either the December 1984 Almond Research Conference sponsored by the California Almond Board or one of three University of California extension meetings. These surveys provided information on the numbers of acaricide applications per season during the past 5 yr, average cost of applications over the past 5 yr, and names and rates of acaricides used. In addition, William Barnett, University of California Pest Management Specialist, and Darryl Castro, Dan Cahn, Cliff Kitiyama, and Barry Wilke (independent pest management consultants) provided similar information as well as estimates of the area requiring *M. occidentalis* releases each year, and the expected acceptance rates by growers of the IMM guidelines.

Data for the programmatic costs were developed by compiling the salary and benefits of the principal investigator (M.A.H.), half salary and benefits

for a laboratory technician for 5 yr, plus extramural funds to support the project, including supplies, travel expenses, technical assistance, and equipment. The cost data are presented for two phases, phase 1 representing the research phase and phase 2 representing the development and implementation phase, and are found in Table 1.

Interest on the expenditures was compounded annually to reflect the value of these resources to society had they been used by the private sector rather than as public research expenditures. Other costs that are a part of this program are costs of the University of California IPM program and costs of state IPM specialists who were involved in the phase 2 research to test and implement the IMM program. These costs cannot be documented, but are believed to be  $\leq 10\%$  of the documented expenditures. Additional investments by these agencies will be required, however, to complete the adoption by growers and to adapt the program as conditions change, but are not included in this analysis.

**Assumptions of Analyses of Cost Effectiveness.** Analysis of cost effectiveness was used to compare the IMM program with conventional acaricide control. We assumed the IMM program would result in almond meat yields at least equal to those achieved with conventional control. Given this assumption, the program benefits accrue in the form of cost savings. If the IMM program resulted in cost savings, those savings were treated as benefits to the new technology. Cost saving benefits projected for the future were discounted at an annual interest rate of 12% for 5 yr to provide a present value of the cost savings in 1985. (Present value is a sum that one would be willing to exchange today in return for a series of annual payments, and which includes interest on the unpaid principal.)

Two forms of evaluations were completed. First, programmatic cost savings were computed for the entire industry and a net present value of cost savings over a 5-yr period was computed by subtracting the value of the research investment necessary for developing the program. This analysis indicates the degree of social payoff from the research investment. Second, the cost savings per hectare to growers were computed and discounted over a 5-yr period to provide individual growers with an estimate of the profitability of IMM.

Analyses were conducted for three programmatic alternatives. Assumptions for the first alternative are as follows. First, 80% of 158,000 ha (126,400 ha or 316,000 acres) has spider mite problems requiring intervention; and second, 25% of this problem area (31,600 ha or 79,000 acres) will use the IMM program in the 1st yr. Third, growers managing 20% of the area (6,320 ha or 15,800 acres) using the IMM program will need to make releases of *M. occidentalis* because native predators are absent or rare. Fourth, the balance of the problem area (94,800 ha or 237,000 acres) continues to use conventional acaricide programs during

Table 2. Projected annual programmatic cost savings from adopting IMM programs in California almonds

| Item  | Unit     | 25% adoption<br>yr 1 | 50% adoption<br>yr 2 | 75% adoption<br>yr 3 |
|---|----------|----------------------|----------------------|----------------------|
| 1 Total almond area   | ha acres | 158,000/395,000      | 158,000/395,000      | 158,000/395,000      |
| 2 Area requiring spider mite treatment                                | ha acres | 126,400/316,000      | 126,400/316,000      | 126,400/316,000      |
| 3 Total conventional treatment cost                                   | \$       | 23,700,000           | 24,885,000           | 26,129,250           |
| 4 Projected area under IMM  | ha acres | 31,600/79,000        | 63,200/158,000       | 94,800/237,000       |
| 5 Projected area under conventional treatment                         | ha acres | 94,800/237,000       | 63,200/158,000       | 31,600/79,000        |
| 6 Cost for projected conventional treatment area                      | \$       | 17,775,000           | 12,442,500           | 6,532,312            |
| 7 Cost for IMM area, acaricides including application plus monitoring | \$       | 2,449,000            | 5,142,900            | 8,100,067            |
| 8 Cost for predator releases  | \$       | 316,000              | 663,600              | 1,045,170            |
| 9 Total cost for IMM adoption (line 6 + line 7 + line 8)              | \$       | 20,540,000           | 18,249,000           | 15,677,550           |
| 10 Cost reduction due to IMM (line 3 - line 9) <sup>a</sup>           | \$       | 3,160,000            | 6,636,000            | 10,451,700           |

<sup>a</sup> The cost reduction is total conventional treatment cost minus the sum of IMM costs of acaricides, predator releases, and the cost for conventional treatment on the area with mite problems.

the 1st yr. Fifth, releases of *M. occidentalis* cost \$50/ha (\$20/acre); mite monitoring, which is needed by all growers using the IMM program, costs \$25/ha (\$10/acre); conventional acaricide costs are estimated to average \$157.50/ha (\$75/acre) if 1.5 treatments are applied.

The second alternative assumes the same conditions as the first except that by the 2nd yr, 50% of the area (63,200 ha or 158,000 acres) with spider mite problems is being managed using the IMM guidelines and 20% of the area (12,640 ha or 31,600 acres) in the program requires releases of *M. occidentalis*.

Finally, the third alternative is identical to alternatives 1 and 2 except that we assume that by the 3rd yr, 75% of the area with spider mite problems (94,800 ha or 237,000 acres) is being managed under the IMM guidelines. We further assume that 20% of the area (18,960 ha or 47,400 acres) will require releases of *M. occidentalis* each year.

A cost-savings budget for a grower presents two plans. The first plan includes the cost savings from adopting only the lower-than-label rate of acaricide use in conjunction with monitoring spider mites and native *M. occidentalis* populations. This cost is estimated to be \$52.50/ha (\$21.00/acre) based on use of 10% of the normal amount of propargite, or other acaricide, plus about \$25/ha (\$10/acre) for monitoring (Hoy et al. 1984a,b, Hoy 1985). The second plan includes the cost savings if growers adopt the lower-than-label rate of acaricide and monitor spider mites, but resistant *M. occidentalis* releases are needed at the beginning because native *M. occidentalis* are lacking or very rare, or the grower wishes to use carbaryl for control of the navel orangeworm. We assume releases of *M. occidentalis* cost \$50/ha (\$20/acre).

### Results

Table 2 contains the results of the analysis that pertain to the entire almond-producing industry in California.

**Industry Results.** All three adoption rates (25, 50, and 75%) provide a high degree of cost effec-

tiveness relative to the research investment (Table 2). The net present values were positive for each adoption rate. Net present values for 25, 50, and 75% adoption rates for the IMM program that involves use of lower-than-label rates and releases of *M. occidentalis* in 20% of the area in the program each year were \$11,626,684, \$21,255,816, and \$28,239,860, respectively. These dollar values represent the values in 1985 of a 5-yr stream of annual cost savings net of the initial research investment costs, which were compounded at a 12% interest rate from the date of receipt. The benefit/cost ratios for these three adoption alternatives range from 14:1 to 34:1.

Without question, the IMM program is economically justified by the cost savings it has the potential to generate in only 5 yr. If 25% adoption is achieved, the cost reduction per total area requiring treatment is \$25.00/ha. If 50 or 75% of the area becomes part of the IMM program, average cost reduction is \$50.00 or \$82.50/ha for the total problem area, respectively (Table 1). The rate of return on the investment, i.e., a rate of interest that makes the present value of the 5 yr of cost savings equal to the initial research investment, ranges from 280 to 370%.

**Individual Grower Results.** For a grower, the decision to adopt the IMM program depends, at least in part, on the expected cost savings. Two plans for the grower are shown in Table 3. Plan 1 shows the cost savings per unit area for a grower who adopts the program by using lower-than-label rates of acaricides and monitoring, but does not need releases of *M. occidentalis*. The savings are expected to be \$110.00/ha with a 5-yr present value of \$396.55/ha.

If a grower adopts the IMM program and needs releases of *M. occidentalis*, plan 2 in Table 3 is applicable. The cost savings under this plan are \$60.00/ha for the 1st yr (due to the cost of predators) and \$110.00/ha in succeeding years. The 5-yr present value of the cost savings for plan 2 is \$351.90/ha.

The magnitude of the cost savings under either plan is a function of the cost of the conventional



Table 3. Grower cost savings analysis for IMM in almonds

| Plan 1 No releases of <i>M. occidentalis</i> necessary                                |           |           |           | \$/ha     | \$/acre                              |
|---|-----------|-----------|-----------|-----------|--------------------------------------|
| Cost of conventional treatment (includes material plus application per unit area)     |           |           |           | \$187.50  | \$75.00                              |
| Minus low acaricide rate treatment (includes material plus application per unit area) |           |           |           | 52.50     | 21.00                                |
| Minus cost of mite monitoring per unit area   |           |           |           | 25.00     | 10.00                                |
| Cost reduction per unit area  |           |           |           | \$110.00  | \$44.00                              |
| Value of cost savings per ha (or acre)  |           |           |           |           |                                      |
| Yr 1  | Yr 2      | Yr 3      | Yr 4      | Yr 5      | Present value <sup>a</sup><br>at 12% |
| \$110.00  | \$110.00  | \$110.00  | \$110.00  | \$110.00  | \$396.55                             |
| (\$44.00)   | (\$44.00) | (\$44.00) | (\$44.00) | (\$44.00) | (\$158.62)                           |
| Plan 2 Release of <i>M. occidentalis</i> necessary                                    |           |           |           | \$/ha     | \$/acre                              |
| Cost of conventional treatment (includes material plus application per unit area)     |           |           |           | \$187.50  | \$75.00                              |
| Minus low acaricide rate treatment (includes material plus application per unit area) |           |           |           | 52.50     | 21.00                                |
| Minus cost of mite monitoring per unit area   |           |           |           | 25.00     | 10.00                                |
| Minus cost of 1st yr predator releases per unit area                                  |           |           |           | 50.00     | 20.00                                |
| 1st yr cost reduction per unit area   |           |           |           | \$60.00   | \$24.00                              |
| Second and following year cost reduction per unit area                                |           |           |           | \$110.00  | \$44.00                              |
| Value of savings ha (or acre)   |           |           |           |           |                                      |
| Yr 1  | Yr 2      | Yr 3      | Yr 4      | Yr 5      | Present value <sup>a</sup><br>at 12% |
| \$60.00   | \$110.00  | \$110.00  | \$110.00  | \$110.00  | \$351.90                             |
| (\$24.00)   | (\$44.00) | (\$44.00) | (\$44.00) | (\$44.00) | (\$140.76)                           |

<sup>a</sup> Present value = [savings 1 (1.12)] - [savings 2 (1.12)<sup>2</sup>] - [savings 3 (1.12)<sup>3</sup>] + [savings 4 (1.12)<sup>4</sup>] + [savings 5 (1.12)<sup>5</sup>

spider mite control program. Obviously, the higher the costs of the conventional control program, the greater the potential for cost savings. Some growers will save more than \$110.00 or \$60.00/ha per year, and some will save less. The average is expected to be about \$110 for plan 1 and \$60 for the 1st yr of plan 2. These cost savings are roughly equivalent to the cost of pruning and brush disposal, respectively, or ca. 5-10% of cash production costs per hectare.<sup>3</sup>

### Discussion

The study reported here is unusual because it evaluates the programmatic benefits of a pest management practice and compares those benefits with the research investment cost. Other studies of the economic feasibility of biological control of insects such as that conducted by Reichelderfer (1979) examine the cost effectiveness of the technique, but have no data on the research investment required to develop the control method. The IMM program is also unusual in that one component involves the use of a laboratory-selected predator; genetic manipulation of biological control agents has long been discussed but rarely effected (Hoy 1985b).

The IMM program has the potential to generate considerable cost savings. We anticipate that these potential cost savings will stimulate growers to adopt

the approach relatively quickly (Headley & Hoy 1985). A major benefit of this technology is that it does not increase yields. Therefore, adoption of the program should not reduce almond meat prices, at least in the short run. Rather, it should increase the efficiency of production, resulting in increased net incomes to the adopting growers.

Furthermore, because the IMM program results in cost savings with no yield increases, all of the benefits go to the growers. In contrast, a good part of the benefits from yield-increasing technology goes to consumers in the form of lower prices. Because the farming community in general, and the almond producers in particular, are under severe financial constraints, there should be a strong incentive to adopt the IMM program.

Commercial implementation of the IMM program began in 1984. By May of 1985, ca. 4,800 ha of almonds in California had received releases of the resistant *M. occidentalis* and about 36,000 ha were being managed under IMM guidelines, but did not require predator releases (B. Wilke, C. Kitiyama, D. Castro, D. Cahn, W. Barnett, and R. Curtis, personal communications).

The total identified costs of research to develop the IMM program were \$537,661. Phase 1, which included the laboratory selection of the pesticide-resistant predators and the laboratory and small-plot testing, cost \$148,024. Phase 2, which involved large-scale field testing and commercial adaptation of the program, cost \$389,637. Thus, the second phase cost more than 2.6-fold that of the first. When these costs were compounded annually from the

<sup>3</sup> Cost estimates based on unpublished work by Karen Klonsky, Dep. of Agric. Econ., Univ. of California (Davis).

date of receipt at a 12% rate of interest, the total research and development costs, as of 1 January 1985, amounted to \$823,842.

The benefits to almond growers of adopting the IMM program are savings in mite control costs through fewer acaricide applications, and, where applications are applied, through the reduced rate of acaricides applied per hectare. The programmatic cost savings are a function of the area of almond orchards in need of an alternative to conventional chemical control of spider mites and the rate of adoption by growers.

This economic analysis encompasses the entire range of activity from selecting a strain of insecticide-resistant predators to the field testing. An operational program at the grower level is assured with numerous University of California personnel contributing to the project.

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# Toxicity of pesticides to western predatory mite

Marjorie A. Hoy □ Janet Conley

**B**ecause of its effectiveness in controlling spider mites, the western predatory mite plays an important role in integrated mite management in California almond orchards. When monitoring indicates that the predator, *Metaseiulus occidentalis* (Nesbitt), needs help in controlling the pest mites, however, it is necessary to apply acaricides that will suppress the spider mites but not the predators. Use of selective insecticides and fungicides to control insect pests and diseases is also crucial, since some materials can disrupt predator effectiveness through direct or indirect toxicity.

Evaluating the survival of *M. occidentalis* in the field after treatment with pesticides in replicated spray plots is expensive and time consuming. Such field trials do not always provide useful data on pesticide selectivity, since predator populations can be reduced through starvation and emigration from the orchard or increased through immigration into the orchard on air currents. Laboratory tests provide repeatable and replicated information at a substantially lower cost.

We have used a leaf spray technique to assess mortality of *M. occidentalis* after treatment with pesticides. We sprayed

both predators and spider mites with a pesticide on a leaf substrate, and then noted their survival on the residues after 48 hours. The laboratory trials cannot be translated into field efficacy with full reliability, however, because coverage in the field is rarely as complete, and such trials provide no information on the effect of weather on residues.

## Test methods

Leaf spray tests involved placing five young gravid females per pinto bean leaf (*Phaseolus vulgaris* L.) disc about ¼ inch in diameter with two-spotted (*Tetranychus urticae* Koch) and Pacific (*T. pacificus* McGregor) spider mites as prey. Fifty females from each colony were tested at each dose. The leaf discs, resting on moist cotton in plastic trays, were sprayed thoroughly with formulated pesticides for about five seconds with a fluorocarbon spray system (Crown Spra-Tool). After keeping the treated discs at 78° to 84°F for 48 hours, we recorded the number of

TABLE 1. Toxicity of pesticides listed in UC Leaflet 21343 to western predatory mite, *Metaseiulus occidentalis* (M.o.), as determined in laboratory tests

| Material (formulation) and rate tested as field rate* | Toxicity to predatory mite            | Comments  | Material (formulation) and rate tested as field rate                | Toxicity to predatory mite | Comments  |
|---|---------------------------------------|---|---|----------------------------|---|
| Azinphos-methyl (Guthion 50 WP) 4 lb 50 WP/400 gal    | Low to moderate                       | Most native <i>M.o.</i> populations are resistant, but variability exists. COS-resistant strain is resistant.   | Lorsban (see chlorpyrifos)  |                            |   |
| Benomyl (Benlate 50 W) 0.5 lb AI/100 gal              | High or low, depending on colony used | Reduces egg production of native <i>M.o.</i> COS strain is resistant.   | Maneb (Manzate, Dithane M-22) 8 lb 80 WP/400 gal                    | Low                        | Both well fed and starved females tolerated sprays well.  |
| Captan (Orthocide 50 W) 8 lb 50 WP/400 gal            | Low                                   | Both well fed and starved females tolerated sprays well.  | Methidathion (Supracide 2 E) 6 qt 2 E/400 gal                       | High                       | Toxicity rating could be lower in winter since predators are in diapause and hidden in crevices. (100% mortality at half the field rate on leaves.) |
| Carbaryl (Sevin Sprayable) 4 lb 50 WP/100 gal         | High or low                           | Native <i>M.o.</i> are susceptible, COS strain is resistant.  | Oils. Supreme or superior-type narrow-range oils at 4 to 8 gal/acre | Low                        | Rating based on tests with summer oils and literature data.   |
| Chlorpyrifos (Lorsban 4 E) 2 qt 4 E/400 gal           | Moderate                              | Toxicity rating could be lower in orchards in winter, since predators are in diapause and hidden in crevices. (60% mortality occurred at field rate on leaf discs [50 W].)                                    | Omite (see propargite)  |                            |   |
| Cyhexatin (Plictran 50 W) 2 lb 50 WP/400 gal          | Low with WP formulation               | Flowable formulation was also tested and was more toxic than WP formulation to <i>M.o.</i> at equivalent rates. (Flowable formulation is not yet registered in almonds. Field rate of flowable is not known.) | Orthocide (see captan)  |                            |   |
| Diazinon (Diazinon 50 W) 1 lb 50 WP/100 gal           | Low                                   | Most native <i>M.o.</i> are resistant. COS strain is also resistant.  | Parathion   | Low                        | Based on literature data.   |
| Dithane (see maneb)                                   |                                       |   | Phosmet (Imidan 50 WP) 1 lb 50 WP/100 gal                           | Low                        | Most native <i>M.o.</i> populations are resistant to Imidan, as is the COS strain.  |
| Fenbutatin-oxide (Vendex 4 L) 2 pt 4 L/400 gal        | Low                                   | Only 4 L formulation was tested at field rate (2 pt/acre).  | Plictran (see cyhexatin)  |                            |   |
| Guthion (see azinphos-methyl)                         |                                       |   | Propargite (Omite) 2.5 lb 30 WP/100 gal                             | Moderate to high           | Only WP formulation tested: moderate at 5-10 lb/100 gal; high at 10-20 lb/100 gal. Strains of <i>M.o.</i> could vary in tolerance.                  |
| Imidan (see phosmet)                                  |                                       |   | Sevin (see carbaryl)  |                            |   |
|   |                                       |   | Supracide (see methidathion)  |                            |   |
|   |                                       |   | Thiophanate methyl (Topsin M) 2 lb/400 gal                          | Low                        | No mortality at 5 times field rate.   |
|   |                                       |   | Vendex (see fenbutatin-oxide)                                       |                            |   |
|   |                                       |   | Ziram (Ziram 76 W) 12 lb 76 W/400 gal                               | Low                        | Both well-fed and starved females tolerated sprays well.  |

NOTE: Toxicity ratings to western predatory mite (*M.o.*) are based on the laboratory leaf spray technique. Actual field toxicities could be different, and individual orchard populations of the predator could vary in their responses.

\*Materials: WP = wettable powder; W = wettable; AI = active ingredient; E = emulsifiable; L = liquid; ED = emulsifiable concentrate; SC = suspension concentrate.



predators alive, dead, run off, and absent. Mites able to walk when touched lightly with a fine camel's hair brush were recorded as alive, all others as dead.

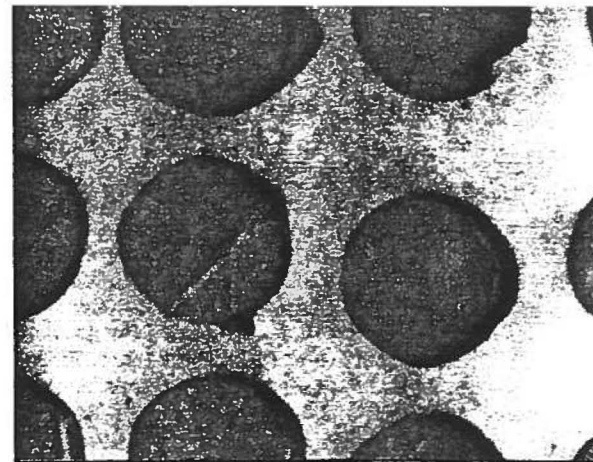
Pesticide rates tested included a control (water only) and one-fourth, one-half, one, and five times the field rate. Pesticides were rated as having a high toxicity if over 50 percent of the predators were killed at one-fourth and one-half the field rate. Pesticides were considered low in toxicity if predators were unaffected by rates of one or five times the field rate. A moderate toxicity rating was given if about 50 percent of predators were killed at the field rate. We also noted whether the material was toxic to the spider mite prey.

Native *M. occidentalis* populations vary in their dose responses to organophosphorus (OP) insecticides. We believe these differences reflect past treatment histories in specific orchards or vineyards. Such variability has been found in California pears, grapes, and almonds. We therefore tested two colonies of *M. occidentalis* with the pesticides listed in Leaflet 21343, *A Guide to Controlling Almond Pests, Diseases, and Micronutrient*

*Deficiencies* (UC Division of Agriculture and Natural Resources, 1983). The two colonies were a wild strain collected from an almond orchard in Stanislaus County with a moderate level of resistance to OP insecticides, and the carbaryl-OP-sulfur-resistant (COS) strain, which is being mass-reared commercially and released in almond orchards.

### Toxicity ratings

Because all ratings are from laboratory data, they may need to be amended if field data indicate the pesticides are more or less toxic to the predatory mite. Based on our experiences, and discussion with several experienced farm advisors and pest control advisors, we believe it is likely that pesticides with low toxicity ratings in the laboratory will have a low impact in the field. Likewise, pesticides with a high rating will probably cause high mortality in the field. The most difficult ratings to interpret are those in the moderate category. Such pesticides could have a high, moderate, or low rating, since a number of factors influence their field impact: thoroughness of spray coverage, duration of residues, effect of pesticides on



In toxicity tests, mite predators were placed on leaf discs along with spider mites as prey. They were sprayed with various pesticides and later checked to record the number of predators alive, dead, or missing.

spider mite prey, and pesticide formulation.

If some ratings are controversial, we suggest that field trials be conducted to resolve them. Because populations of western predatory mite from different orchards vary in their responses, particularly to organophosphorus insecticides such as Guthion (azinphosmethyl), Diazinon, and Imidan (phosmet), ratings should be

TABLE 2. Toxicity of pesticides not listed in Leaflet 21343 (1983) and those not currently registered, in laboratory tests on western predatory mite

| Material (formulation and rate tested as field rate*)      | Type† | Toxicity to predatory mite | Comments  | Material (formulation and rate tested as field rate*) | Type† | Toxicity to predatory mite | Comments   |
|--|-------|----------------------------|---|---|-------|----------------------------|--|
| Abamectin (see avermectin)                                 |       |                            |   | Iprodione (not registered) 3 g 50 WP/100 liter        | F     | Low                        | COS strain is very tolerant.   |
| Ambush (see permethrin)                                    |       |                            |   | Kelthane (see dicofol)                                |       |                            |  |
| Apollo (see clofentezine)                                  |       |                            |   | Malathion 8 lb 25 WP/100 gal                          | I     | Low to moderate            | Native and COS strains may tolerate low field rate (0.5 lb AI/100 gal). Less than 50% survival at 2 lb AI/100 gal.                                 |
| Avermectin B <sub>1a</sub> (not registered) 3 ppm          | A,I   | Moderate to high           | This experimental material is less toxic to <i>M.o.</i> than to spider mites, but at this field rate would be likely to kill most <i>M.o.</i> | Naled   | I     | Moderate                   | Survival of 16% at field rate and 44% or 80% at half the field rate, for native and COS strains respectively.                                      |
| <i>Bacillus thuringiensis</i>                              | I     | Low                        | Not toxic to <i>M.o.</i> if lacking the beta-exotoxin.  | Permethrin 2 g AI/100 liters                          | I     | High                       | Toxic to all native <i>M.o.</i> tested.  |
| Clofentezine (not registered) 1 oz 50 SC/100 gal           | A     | Low                        | No negative effects found.  | Phosalone 6 pt 3 EC/100 gal                           | I     | High                       | COS strain is slightly more tolerant than native strains.  |
| Danitrol (see fenpropathrin)                               |       |                            |   | Pounce (see permethrin)                               |       |                            |  |
| Dibeta (see thuringiensin)                                 |       |                            |   | Pydrin (see fenvalerate)                              |       |                            |  |
| Dibrom (see naled)   |       |                            |   | Rovral (see iprodione)                                |       |                            |  |
| Dicofol (not registered)                                   | A     | High                       | Toxic to predator, based on literature data.  | Savay (see hexythiazox)                               |       |                            |  |
| Endosulfan 4 lb 50 WP/400 gal                              | I     | Low                        | Native and COS-resistant strains are tolerant.  | Sulfur 4 lb 80 WP/100 gal                             | F,A   | Low to high                | Toxic to native <i>M.o.</i> in almonds, <i>M.o.</i> from many vineyards and COS strain tolerate sulfur.  |
| Fenpropathrin (not registered) 6 gal/100 liters            | I,A   | High                       | Toxic to COS strain.  | Thiodan (see endosulfan)                              |       |                            |  |
| Fenvalerate (not registered for almonds) 0.2 lb AI/400 gal | I     | High                       | Toxic to all native <i>M.o.</i> tested.   | Thuringiensin (not registered) 20 g AI/100 gal        | A,I   | Moderate to high           | This experimental material is less toxic to <i>M.o.</i> than to spider mites, but at proposed field rates would be likely to kill most <i>M.o.</i> |
| Funginex (see triforine)                                   |       |                            |   | Triforine (not registered) 16 oz 1.6 EC/100 gal       | F     | Low                        | Native and COS strains are tolerant.   |
| Hexythiazox (not registered) 5 oz AI/400 gal               | A     | Low                        | No negative effects found in the laboratory.  | Zolone (see phosalone)                                |       |                            |  |

NOTE: See table 1 NOTE.

\* See table 1 asterisk (\*) footnote.

† Type: A = acaricide; I = insecticide; F = fungicide.

used as general guidelines only. Actual toxicities in specific orchards may be different.

In addition to testing the pesticides listed in Leaflet 21343, we tested several that were not listed, including some that are not currently registered for use in almonds or are not registered in California at this time. We included those to determine which are most promising for future incorporation into an integrated mite management program for almonds.

## Results

Pesticides in table 1 are from Leaflet 21343. Table 2 lists pesticides that are not registered or are not recommended in the leaflet.

Acaricides that were low in toxicity to *Metaseiulus occidentalis* included Plictran, Vendex, Omite, Apollo, and Savey. The Plictran, Vendex, and Omite application rates used are important, because the higher recommended rates were toxic to this predator. The integrated mite management program has thus encouraged the use of low rates of these materials to preserve both the predator and the prey. Abamectin, Dibeta, and Kelthane were toxic to the predator. Abamectin and Dibeta, which are currently unregistered, might be used in a selective manner if rates were very low, but such use would have to be determined by field trials.

Insecticides that were generally low in toxicity to the predator included Guthion, Diazinon, Parathion, Imidan, *Bacillus thuringiensis*, and Thiodan. Sevin had low toxicity to the COS strain, but was toxic to native populations. At higher rates, Guthion and Malathion showed moderate toxicity to native strains of the predator. Lorsban, Dibrom, and Dibeta were moderately toxic to all colonies tested. Supracide, Pounce, Danitol, Pydrin, Ambush, and Zolone appeared to be highly toxic to the predator.

Among the fungicides, Captan, Maneb, Ziram, Funginex, and Rovral appeared to have low toxicity to the western predatory mite. Benlate and sulfur had low toxicity to the COS stain, but were generally toxic to the native populations from almond orchards.

Our laboratory assays suggest that growers and pest control advisors wishing to preserve *M. occidentalis* populations can choose among several options in controlling diseases, insects, and spider mites.

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