Project No. 87-D2 - Insect and Mite Research
Miticide Resistance Management

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Objectives: (1) Characterize the genetics of Omite resistance in Tetranychus pacificus (Pacific spider mite) and T. urticae (Two spotted spider mite) collected from almonds. (2) Assess the influence of predator activity, irrigation practices, pesticide history and miticide resistance on field efficacy of miticide treatments in almonds.

Interpretive Summary: Purified Omite-resistant (R) and susceptible (S) T. pacificus colonies were reciprocally crossed and backcrossed and concentration-response lines were estimated. There was a 24-fold difference in susceptibility between the R and S colonies used in this study. The reciprocal crosses responded the same and were as susceptible as the S colony, indicating that the resistance is inherited recessively and that there is no apparent cytoplasmic inheritance involved. The concentration-response curve for the backcross to the R colony plateaued at approximately the 50 percent mortality level, demonstrating that the resistance is inherited as a single factor (one gene or a group of closely linked genes). This mode of inheritance for Omite resistance in T. pacificus is the most advantageous for resistance management since interbreeding of Omite-resistant and -susceptible individuals will lower the frequency of individuals exhibiting the resistance trait.

Purified Omite-resistant and susceptible T. urticae colonies, with a 59-fold difference in susceptibility, are ready for use in crosses and backcrosses. The mode of inheritance of Omite-resistance in T. urticae is scheduled to be determined during January and February 1988.

The influences of biological, cultural and chemical factors on the efficacy of miticide treatments were assessed in the field. Resistance in the spider mite populations did not alone cause field failure. Five sites tested had high frequencies of resistant mites, of these only one had a field failure.

The field studies showed that rapid assays of field collected spider mites are satisfactory estimators of field susceptibility (field efficacy for the most part) but not of the frequency of resistant spider mites present in the orchard. Omite and Plictran rapid bioassays of spider mites taken directly from the orchards before treatment, indicated that at most sites the mites were susceptible to both miticides. Laboratory colonies were started using some of the spider mites collected from each field site. Laboratory bioassays (both cell bioassays and rapid bioassays) of colonies reared in the laboratory indicated varying levels of susceptibility. These results emphasize that other factors, in addition to resistance, must be involved in determining field efficacy and that field rapid assays can only estimate field susceptibility at one point in time, immediately before treatment.

Experimental Procedure:

A. Selection Procedure and Inheritance Studies

The Omite-resistant T. pacificus and T. urticae colonies were purified by use of mass selection (called cage-spray in 1986 report). The original colonies were colonies started in cages The most resistant colonies collected in the field surveys were used to start colonies in cages which are labeled as the original colonies in this study. Replacement plants put into each of the caged colonies were treated with Omite. Each round of selection consisted of adding treated plants to the cage until the entire supply of plants in the cage were treated. Untreated plants were then rotated in and the mites allowed to reach testable levels. Bioassays were than run to estimate concentration-responses lines. Both colonies received 3 rounds of selection, in the first 2 rounds the plants were treated with 1,000 ppm and in the third with 3,162 ppm Omite. In both cases, after the third round of selection, little change in bioassay response was detected and the concentration-response lines indicated the colonies were highly homogeneous.

Reciprocal crosses and backcrosses were accomplished by isolating individual virgin females in the teliochrysalis stage, from the appropriate colonies, on stickem ringed cotton cotyledons. The cotton seedlings were held in test tubes containing a nutrient water solution. Flats of test tubes for each purified colony, cross or backcross were isolated in styrofoam box enclosures (Keena and Granett 1987) and held at 83 $\pm$ 3 F in constant light. Two adult males from the appropriate colony were added the following day to each ringed cotyledon. The females were allowed to mate and lay eggs for 3 days before they were removed. When the resulting female progeny had become adults, concentration-response lines were estimated using the residual cell method (Keena and Granett 1987).

B. Factors Influencing Miticide Efficacy

The factors that were assessed were broadly broken down into 3 categories: chemical, biological, and cultural.

Chemical	Biological	Cultural
Resistance levels	Predator numbers	Irrigation practices
Spray coverage	Spider Mite numbers	Orchard floor management
Spray rate	Predator/Prey ratio	
Spray volume		
Formulation used		
Pesticide use history		

1. Selection of Test Sites

Almond farm advisors from Butte, Glenn, San Joaquin, Stanislaus, Merced, Madera, Fresno, Tulare, and Kern counties were asked to supply names, addresses, and phone numbers of cooperators they felt would be willing to take part in this study. Cooperators were then contacted and asked to notify us, 24-48 hours in advance, when a miticide treatment was to be applied to one or more of their orchards. The sites, therefore, were sites where the grower or pest control advisor had determined that a miticide treatment was necessary to control the spider mites present in their orchard and were not chosen based on any other criteria

. 2. Chemical

Field resistance levels were estimated for both Omite and Plictran using the rapid bioassay method (1986 annual report) using spider mites collected directly from the orchard before treatment. Laboratory resistance levels were determined for both Omite and Plictran by using both the rapid bioassay and residual cell bioassay methods. Between 100-250 short sections of branches (2-5 in. long) with obvious adult female spider mites on the leaves were collected from a minimum of 50 trees from throughout the orchard for use in the field rapid assays and in order to start single species laboratory colonies to use in the laboratory bioassays. Three concentrations were used in the rapid bioassays for Omite (316, 1,000 and 3,162 ppm) and 3 for Plictran (5.62, 10.0 and 17.8 ppm). In the residual cell method 7 concentrations were used for Omite (0, 31.6, 100, 316, 1,000, 3,162 and 10,000 ppm) and 6 were used for Plictran (0, 10.0, 31.6, 100, 316, 1,000 ppm). Four replications were run for each miticide and concentration in the laboratory and 1-4 replications of the rapid bioassays were run using field collected spider mites.

Spray coverage was assessed by placing water sensitive paper (both facing up and down) at 5 foot intervals on PVC poles placed next to three trees in each orchard before treatment. The dry water sensitive papers were ranked on a scale of 1-10 (separate scales were used for high and low volume sprays) with 1 being virtually no coverage and 10 being complete coverage. orchard means were calculated and spray coverage was labeled as excellent (8-10), good (4-8), fair (2-4) or poor (1-2).

The rate, volume, formulation used, and pesticide use histories for each site were obtained from the cooperators.

3. Biological

Predator numbers were assessed before and 6-10 days after treatments by counting the feeding stages present on 15 leaves collected from 10 trees spread through out the orchard (the same trees were used for both estimates). Five leaves were collected from the lower-interior, 5 from the lower-exterior, and 5 from the upper exterior (approximately 10-15 feet on taller trees).

Spider mite numbers were estimated by using a stereomicroscope to count all stages present on the leaves described above for predator numbers. The number of infested leaves per tree was also determined.

The predator to prey ratio was determined by using the spider mite per leaf and predator (only six-spotted thrips and predatory mites) per leaf values estimated by dividing the total spider mite or predator numbers estimated above by the number of leaves collected.

4. Cultural

Information on irrigation timing and methods were obtained from the cooperators. Surrounding crops and orchard floor management were recorded when collections were made.

C. Survey Methods

The survey questions attached as appendix 1 were distributed to all of the cooperators in the field study, as well as, a few from previous year's studies. The survey questions attached as appendix 2 were distributed only to cooperators which had supplied an orchard for the field study.

<u>Results and Discussion</u>:

A. Genetics

Figures 1 and 2 show the results of selection for Omite-resistant <u>T. pacificus</u> and <u>T. urticae</u>. The original field collected colony's concentration-response line and the response lines for after the 1,000 ppm selections (A)and after the final 3,162 ppm selection (B) are shown. Notice that in both cases the original line had a shallow slope and after selection the lines had a much steeper slope. This change indicates that both of the original colonies contained individuals that responded with susceptibility. The selection process removed susceptible individuals, thus purifying the colony. It should be noticed that resistant individuals were present before selection and selection just altered the frequency of the resistance.

Figure 3 shows the results of the reciprocal crosses (RS) and the backcross to the resistant line (RSxR) for <u>T. pacificus</u>. There is a 24-fold difference in susceptibility between the SS and RR colonies, which have similar slopes indicating similar purity. The two RS lines have different slopes but are not significantly different from each other, indicating no apparent cytoplasmic inheritance. The S male x R female cross is significantly different from the SS line over part of its range, but the RS cross is not significantly different from the SS line. Therefore, we conclude that the resistance is inherited recessively. The fine dotted line in figure 3, running between the RS and RR lines is the expected concentration-response line that would result from an RSxR cross if the resistance were inherited as a single factor. The boxes are the means and the bars are the 95% confidence intervals for the actual data for the RSxR cross. Although the actual data does not exactly follow the expected it does plateau at approximately 50% mortality and the 95% confidence intervals do overlap the expected. Therefore, we conclude that the resistance is inherited as a single factor with possibly some small influence from a minor modifying gene or just large variation in response.

Single factor, recessively inherited resistance is the most advantageous mode of inheritance for resistance management, since interbreeding of Omite-resistant (RR) and - susceptible individuals (RS or SS) will lower the frequency of individuals exhibiting the resistance.

The mode of inheritance of Omite-resistance in T. urticae remains to be determined this coming year. The resistant and susceptible colonies that will be used have a 60-fold difference in susceptibility.

B. Factors Influencing Miticide Efficacy

Table 1 summarizes most of the important factors which influence efficacy and figure 4 gives an indication of which orchards were at treatment levels before (a) and after (b) treatment, based on the presence-absence sequential sampling plan. If we look at figure 4 we see that, based on the presence absence sequential sampling plan, that only sites 1, 3, and 4 had reached treatment infestations (with predators present) and sites 2 and 7 were approaching treatment levels. Sites 5, 6, 8, 9, and 10 based on this should not have been treated when they were, unless it was early in the season and they wished to balance their predator to prey ratio. If we now look at the spider mite per leaf values in table 1 and use 2.25 spider mites per leaf as a treatment threshold (this is based on the presence absence sequential sampling plan treatment threshold) we see that sites 1, 2, 3, 4, 6, 7, 8, and 10 had almost reached or surpassed the treatment threshold. Based on spider mite per leaf values only sites 5 and 9 should not have been treated when they were. If we now consider the predator to prey ratios we see that sites 3, 5, 9, and 10 had excellent ratios and the spider mites may have been controlled with out any treatment by the predators alone. Thus there appears to be some question as to when it is appropriate to treat for spider mites.

1. Chemical

If we compare the field and laboratory resistance levels we see that at sites where resistant spider mites were detected in the laboratory the spider mites in the field responded as if they were susceptible, except for sites 2 and 6 where intermediate resistance was detected both in the field and in the laboratory. While a comparison of field resistance levels with the level of control achieved (percentage reduction in spider mites/leaf) shows that sites which tested susceptible in the field achieved good or excellent control and sites that showed intermediate resistance levels achieved fair to poor control. In general, the rapid bioassays run using field collected spider mites were good indicators of efficacy but not of resistance level as measured in the laboratory. Thus, indicating that resistance levels alone do not determine efficacy.

Two observations were made concerning the effect of coverage on efficacy. First, the bottom water sensitive papers got better coverage on average than did the top papers. This is important since the majority of the T. pacificus were found on the upper surface of the almond leaves and would therefore, have been contacted by less of the chemical. Second, it appears that coverage is less important at sites where the spider mites are susceptible to the miticide being used than where resistance was detected.

Below-label rates were used at seven of the 10 sites (half of which some level of resistance was detected) and at all but one of those sites good or excellent control was achieved. Here, again, we see that resistance alone does not determine the effectiveness of the spray.

We observed that Omite seemed to be more effective than Vendex where resistance was a factor and, of the 2 Omite formulations, Omite 6E performed better than Omite 30W.

From the pesticide use histories it appears that when a particular miticide has been used on an orchard in the past, some level of resistance to that miticide can be detected in the spider mites from that orchard. A particular miticide need never have been used on an orchard in order for resistance to be present, since spider mites can move from field to field.

2. Biological

The number of predators present in the orchard appeared to have more impact on the efficacy of the miticide where resistance was detected or where the number of spider mites/leaf was high. The predators may have had an impact on the field resistance level by making the spider mites more susceptible to the miticide. The predators may weaken the spider mites by puncturing and feeding on them with out killing them (studies must be run to verify this conjecture). When spider mite/leaf numbers were high the treatment did not remove all of the spider mites and adjusted the predator to prey ratio in most cases so that the remaining spider mites were controlled by the predators. Site 9 is an exception, at this site the trees had been pruned in such a way as to make each major branch arising from the trunk an island, thus impeding the natural movement of the predators and resulting in poor long term control in trees that were quite tall.

A comparison of the predator to prey ratio with the control level shows that, if the ratio was approximately 1:20 or less the predators seemed to be an important factor in controlling the spider mites.

3. Cultural

Water stress was a primary cause for the build up of spider mites in a small section of the orchard used for site 5; a drip line was plugged or compromised in some way for a number of weeks allowing a row of trees to become stressed. Spider mites were only found on trees with in the water stressed row or on trees within 5 rows of it. One variety in another orchard was found to have much higher numbers of spider mites, because it was susceptible to problems associated with over watering so it received less water than the other varieties in the block.

Spider mites found at site 10 were susceptible before the first treatment and the first treatment controlled the spider mites very well. Spider mites blowing in from an alfalfa field up-wind from the orchard at site 10 were the major causes for this site having to be resprayed later in the season. The alfalfa may also, have been partially responsible for the particular combination spider mite species present at this site.

The maintenance of a cover crop that can support spider mites may contribute to the spider mite load in the trees. I observed the mowing of more than one orchard, and found that a large quantity of dust and potentially spider mites was thrown high into the trees. If an orchard were to be mowed shortly after a miticide treatment the treatment might appear to be ineffective due to the reintroduction of mites into the trees.

4. Significance

The significance of these observations and results is that many factors influence spider mite populations and ultimately treatment timing and efficacy, therefore consideration of a few alone is insufficient for predictive purposes. Persons responsible for spider mite control in almonds must consider all of these factors if the integrated pest management program is to be successful.

C. Summary of Survey

The last 2 columns of table 1 show what the cooperator estimated concerning the long term effectiveness of the treatment and the number of predators present in their orchard prior to treatment (see appendix 2 for the questions used). In general, there was good correlation between our estimation of control level at 6-10 days after treatment and the cooperators estimate of control beyond 2 weeks, with exception of site 9 which has already been discussed. Their estimation of predator level was found to be different in some cases from ours, which is probably the result of a quick observation on their part or of an over estimation on our part.

The results of the more general survey (appendix 1) are as follows.

1. Omite was the preferred miticide 12/16 (half preferred Omite 6E and half Omite 30W)
Most said they would use between 2-4 lbs (or pts.) of Omite.

2. Gallons used per acre 4 preferred 350-800
6 " 150-250
9 " 25-100

3. Most said they were using lower rates and fewer applications, primarily because of better navel orange worm and spider mite control using the IPM program

4. Most said they determine when to spray for spider mites in almonds by a visual assessment of both spider mite and predator levels.

5. When asked what impact the loss of Plictran would have in the future they responded by writing one of the following:

(8) Plictran was good for right before harvest, so its loss would cause more insurance sprays to be applied.

(7) Plictran was an alternative product that could be used in rotation with Omite for resistance management, so there may be more problems with resistance.
(2) Plictran was not a viable option, so its loss will have no impact.
6. When asked if they had ever had an acaricide fail to adequately control spider mites in almonds 13 responded yes and 1 responded no. When asked why they responded as follows:

Poor coverage	Hot conditions
Too low a rate	Air application
Water stress	Too few predators
Heavy infestation	Resistance
Low volume	

Publications:

Keena, M. A. and J. Granett. 1985. Variability in toxicity of propargite to spider mites (Acari: Tetranychidae) from California almonds. J. Econ. Entomol. 78: 1212-1216.
Keena, M. A. and J. Granett. 1987. Cyhexatin and propargite resistance in populations of spider mites (Acari: Tetranychidae) from California almonds. J. Econ. Entomol. 80: 560-564.

Table 1

SUMMARY OF FIELD EFFICACY STUDIES

Site	Species County	Rate Formulation	Gal./Acre	Resistance Level Field	 Laboratory	Control Level	Pretreatment Predator/Prey Ratio	 Spider Mites/Leaf	Coverage	Cooperator Estimation Long Term Control	 Predator Level
1	P Kern	3 pts. Omite 6E	100 250	(Omite) S	 R	Good 86%	1:29 Good	13.6 High	5.5 Good	Good	Low
2	P Kings	1 lb. Vendex 50W	150	(Plictran) I	 I	Poor -30%	1:83 Fair	7.9 High	5.7 Good	Poor	Low
3	P Kern	2 pts. Omite 6E	350	(Omite) S	 R	Excellent 100%	1:14 Excellent	8.8 High	8.0 Excellent	Good	Medium
4	P/T Kern	3 lbs. Omite 30W	421	(Omite) S	 R/S	Good 78%	1:33 Good	16.3 High	8.7 Excellent	Good	Medium
5	P/U San Joaquin	1.5 lbs. Vendex 50W	50	(Plictran) ---	 S/S	Excellent 100%	1:9 Excellent	0.9 Low	5.1 Good	Ex.	Low
6	P Madera	3 lbs. Omite 30W	25	(Omite) I	 I	Fair 70%	1:143 Poor	1.9 Medium	3.0 Fair	Poor	Low
7	P/U Madera	.25/.5 lb. Plic./Ven.50W	107	(Plictran) S	 S/S	Good 75%	1:72 Fair	25.5 High	5.3 Good	Ex.	Medium
8	P/U Madera	.5/.5 lb. Plic./Ven.50W	30	(Plictran) S	 I/S	Excellent 100%	1:50 Fair	2.0 Medium	3.5 Fair	Ex.	Medium
9	P/T Fresno	3 lbs. Omite 30W	100	(Omite) ---	 I/S	Good 79%	1:4 Excellent	0.7 Low	4.2 Good	Poor	High
10	U/T Tulare	4 lbs. Omite 30W	100	(Omite) S	 S/S	Excellent 100%	1:10 Excellent	1.5 Medium	3.9 Fair	Ex.	High

P = *T. pacificus*
U = *T. urticae*
T = *T. turkestani*

R = Resistant
I = Intermediate
S = Susceptible

--- = insufficient spider mites to do rapid bioassays
Control level = percent decrease in mean spider mites/leaf

Figure 1

SELECTION OF OMITE RESISTANCE IN THE PACIFIC MITE

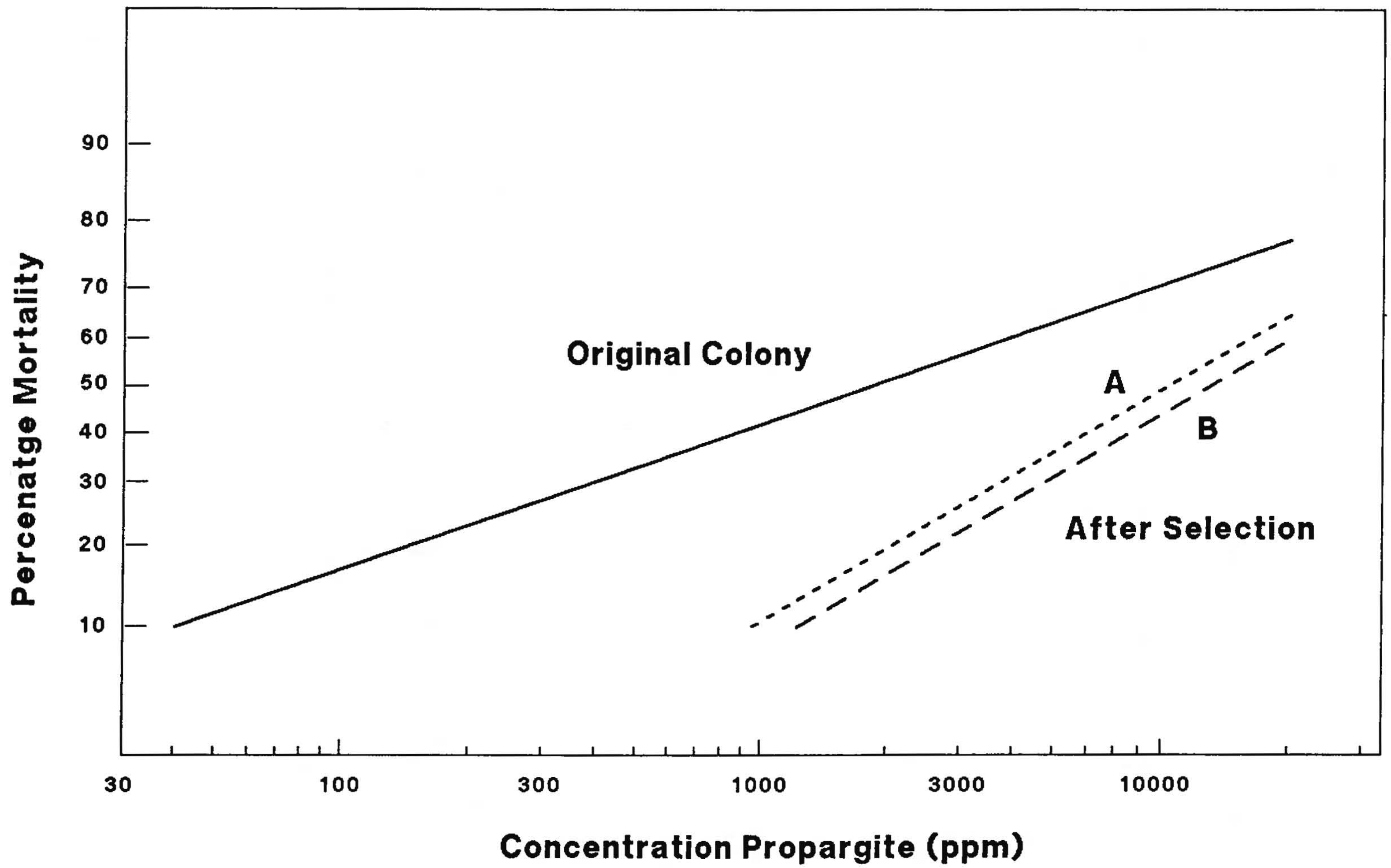


Figure 2

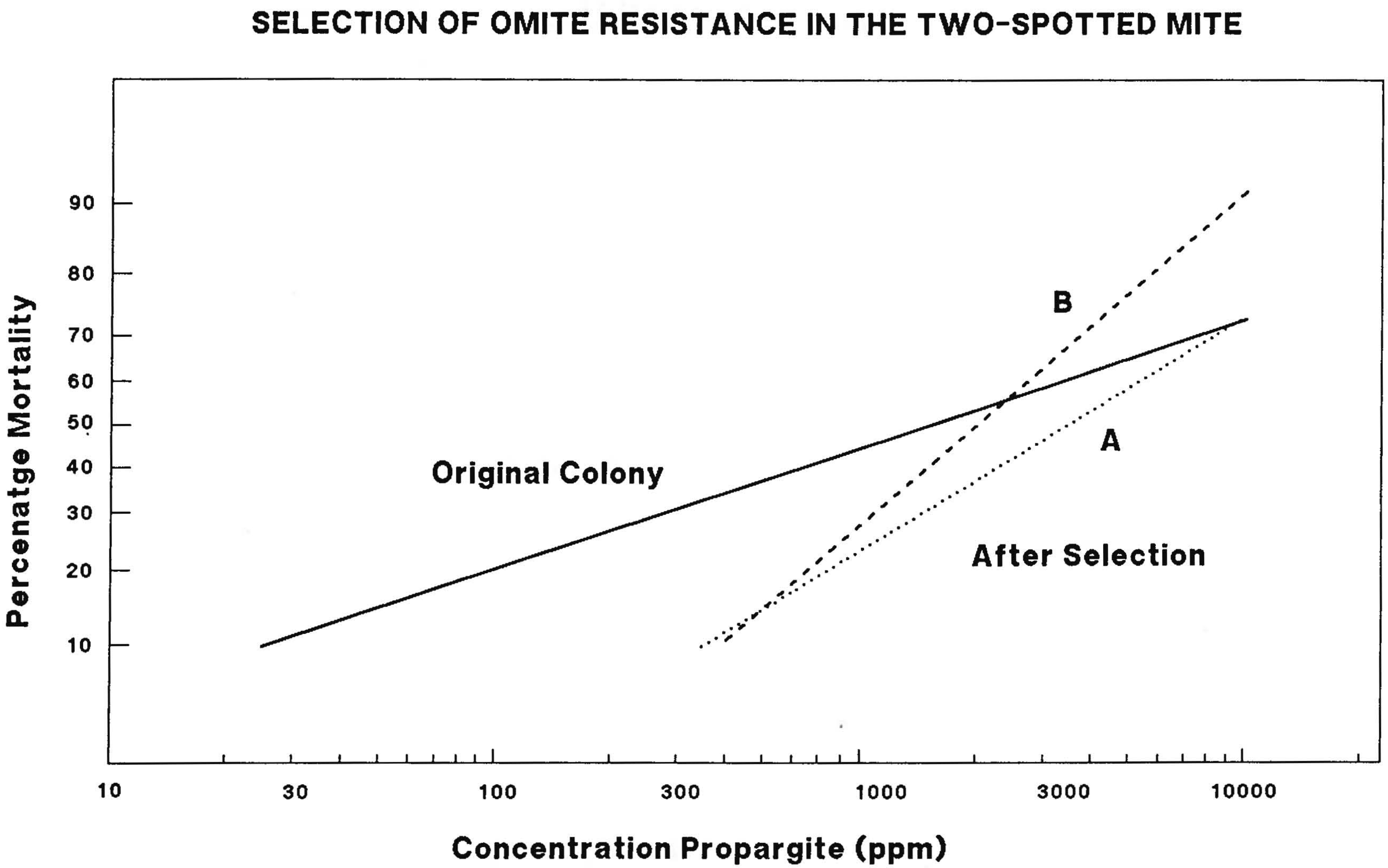
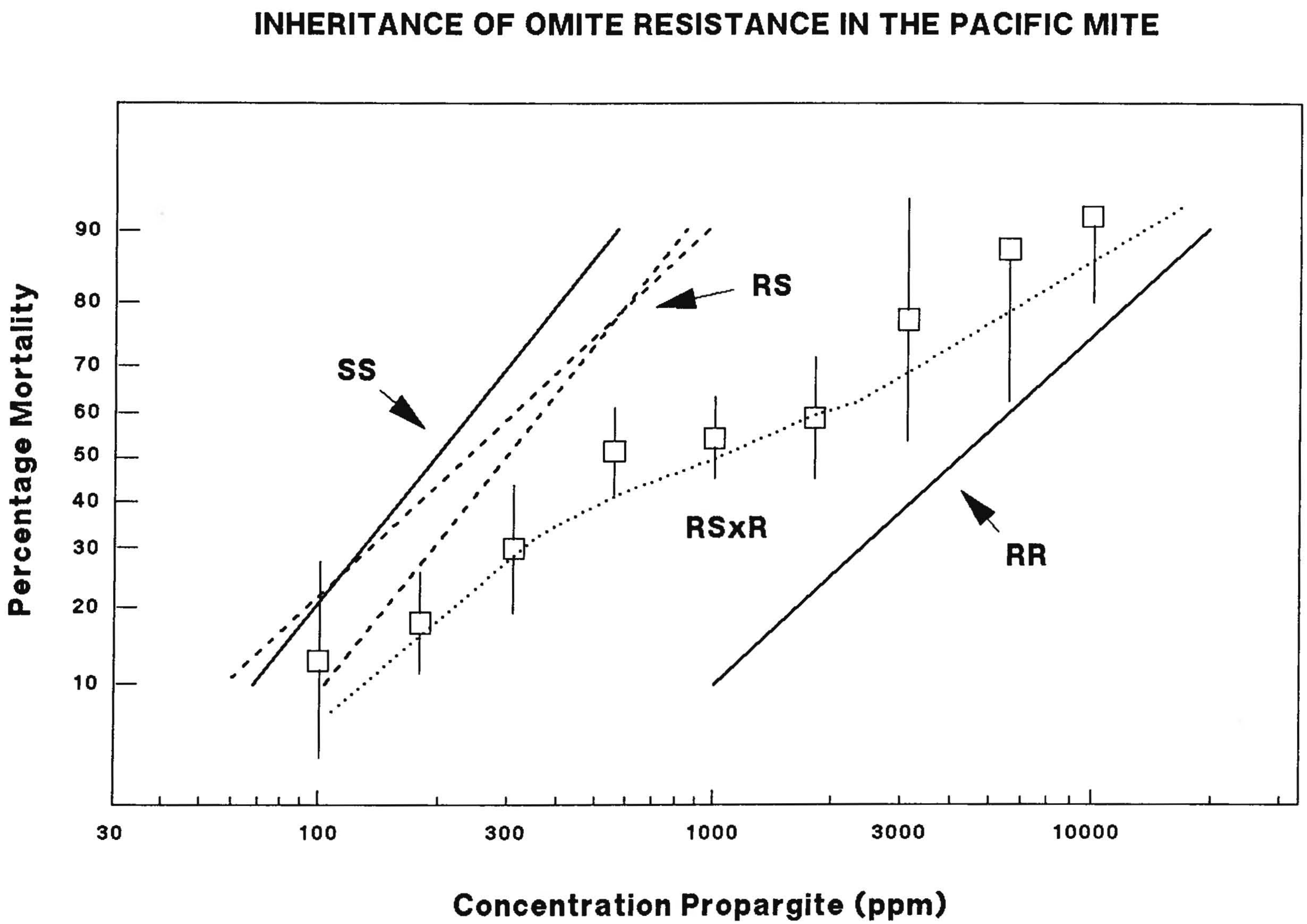
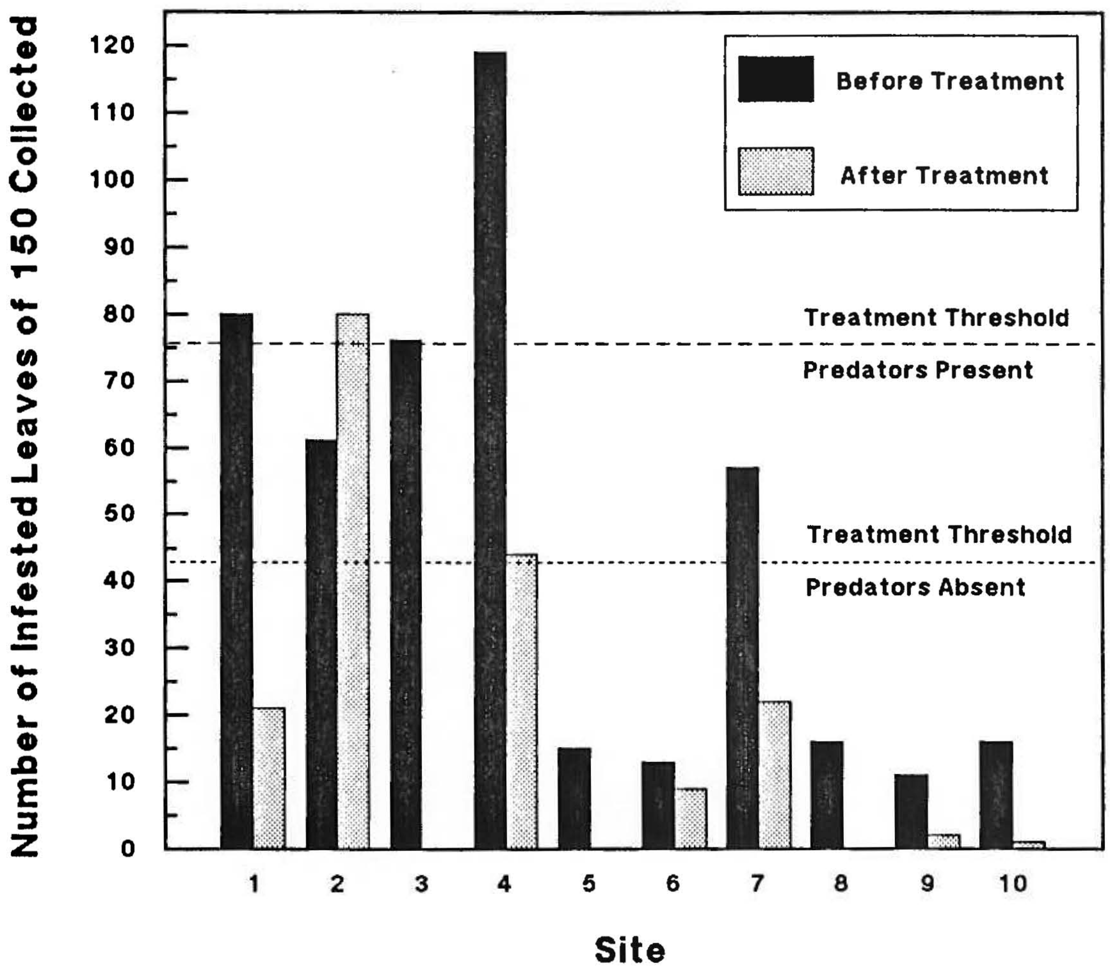

SELECTION OF OMITE RESISTANCE IN THE TWO-SPOTTED MITE
Percenatge Mortality
90
80
70
60
50
40
30
20
10
B
A
Original Colony
After Selection
10
30
100
300
1000
3000
10000
Concentration Propargite (ppm)

Figure 3



INHERITANCE OF OMITE RESISTANCE IN THE PACIFIC MITE
Percentage Mortality
90
80
70
60
50
40
30
20
10
SS
RS
RSxR
RR
30
100
300
1000
3000
10000
Concentration Propargite (ppm)

Figure 4



Number of Infested Leaves of 150 Collected
120
110
100
90
80
70
60
50
40
30
20
10
0
Before Treatment
After Treatment
Treatment Threshold
Predators Present
Treatment Threshold
Predators Absent
1
2
3
4
5
6
7
8
9
10
Site

COOPERATOR SURVEY

Name:____________________________________

Orchard Location:____

____

Date of treatment in question:____________________

1. Do you feel that the acaricide treatment applied to the sampled orchard was effective in controlling the spider mites?

Over the short term (up to 2 weeks)	Over the long term (over 2 weeks)	
☐	☐	Very Effective
☐	☐	Effective
☐	☐	Adequate, but not perfect
☐	☐	Inadequate

2. Did you feel that the number of predators present in the orchard was....

☐ High ☐ Low

☐ Moderate ☐ Nonexistent

ALMOND SPIDER MITE RESISTANCE MANAGEMENT SURVEY

1. Which acaricide is (or was in the case of Plictran) your first choice for spider mite control in almonds?

- ☐ Omite 6E
- ☐ Vendex 50WP
- ☐ Omite 30W
- ☐ Vendex 4L
- ☐ Plictran 50W

On average what rate of the acaricide you checked would you apply?

2. In general, how many gallons an acre do you use in applying acaricides to almonds? (you may mark more than one)

- ☐ 350-800, High-volume spray
- ☐ 150-250, Mid-volume spray
- ☐ 25-100, Low-volume spray
- ☐ 5-20, Ultra-low-volume spray

3. In general, would you say you are using lower rates and/or fewer applications of acaricides than you did in the past?

Lower rates	Fewer applications
☐ Yes	☐ Yes
☐ No	☐ No

Why?

4. How do you determine when it is time to spray for spider mites in Almonds?

- ☐ Presence-absence sequential sampling
- ☐ Brush and count or actual spider mite counts
- ☐ Visual assessment of both spider mite and predator levels
- ☐ Amount of webbing and/or stippling on the leaves
- ☐ Quick check for the presence of spider mites
- ☐ Other ____

____

5. What do you think the impact of the loss of Plictran for use in almonds will have in the future?

6. Have you ever had an acaricide treatment fail to adequately control spider mites in Almonds?

☐ Yes ☐ No

If so, under what conditions?

ORIGINAL

Project No. 86-D1 - Navel Orangeworm, Mite and Insect Research
Miticide Resistance Management

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Objectives: The overall goal of this work is to assess and manage spider mite resistance to selective miticides used on almonds. To accomplish this goal, several years of work will be required. The following are the objectives for 1986: (1) Better determine the field relevance of the laboratory bioassay results. (2) Characterize the genetics of cyhexatin (Plictran) and propargite (Omite) resistance in *T. pacificus* and *T. urticae* collected from almonds. (3) Develop a rapid, field practitioner accessible bioassay for Plictran resistance and verify the one for Omite on almonds. (4) Determine the overwintering stability of the Plictran and Omite resistant spider mites. (5) Determine the role of Omite and Plictran repellency on spider mite control. (6) Conduct preliminary studies on the potential of resistance of Vendex.

Interpretive Summary: Emphasis this year was placed on field verification of our laboratory bioassay results. Two field trials were completed with treatments of Omite, Plictran, water, and Vendex or Morestan. In both orchards, *T. pacificus* predominated and colonies of these spider mites responded with low levels of resistance to both Omite and Plictran in the laboratory. All miticides effectively controlled spider mites in the first field trial which had high levels of predator activity. In the second field trial the miticides were ineffective beyond 10 days and predator activity was low. This indicates that factors other than resistance can have a substantial impact on control of spider mites. Measurements of the number of mites per leaf demonstrated that clumping of spider mites decreased after miticide treatments. The presence-absence sampling method did not correlate well with these results.

Propargite resistance was found to be stable over winter or to increase in *T. pacificus* while in *T. urticae* it was less stable, decreasing at 33% of the orchards sampled. Plictran resistance in *T. pacificus* was unstable, levels of resistance increased, decreased or remained the same. No substantial resistance to Plictran was detected in *T. urticae*. Based on the stability of Omite resistance, populations of *T. pacificus* are potentially the most difficult to control.

Homogeneous Omite-resistant *T. pacificus* and *T. urticae* colonies are being selected in the laboratory for use in inheritance studies. Early observations indicate the inheritance of the resistance is not due to a simple single gene effect and may be different in the two species. Polygenic inheritance is favorable for resistance management.

Rapid field practitioner accessible bioassays have been developed to detect Plictran and Omite resistance. Both assays must still be verified in the field.

The role of Omite and Plictran repellency on spider mite control was not assessed, but similar work has already been carried out (Warwick and Wrensch J. Econ. Entomol. 79: 1472-1476 (1986)).

A method for detecting Vendex resistance is being developed and will be tested on *T. pacificus* colonies which are Plictran-susceptible and Plictran-resistant. Work with *T. urticae* conducted in Australia has demonstrated cross resistance between Plictran and Vendex (Edge and James J. Econ. Entomol. 79: 1477-1483 (1986)).

Experimental Procedure

A. Field Trials

Three to 4 rows of commercially grown Nonpareil almond trees (a minimum of 5 rows from any edge of the orchard) were used in each field trial (except at the Beltran orchard which was done in cooperation with Mobay Chemical Company and had additional experimental treatments). Each treatment tree was bounded by guard trees on all sides to prevent problems with drift of chemicals between treatments. Treatments were randomly assigned to each of the test trees. A handgun sprayer was used to apply miticides and trees were sprayed to run off. The following are the lbs.or pints AI per acre for each treatment:

Low label	High Omite (30 WP)	4.5 lbs./acre AI
	Low Omite (30 WP)	.6 lbs./acre AI
Low label	High Plictran (50 WP)	1.0 lb /acre AI
	Low Plictran (50 WP)	.25 lbs./acre AI
	Vendex (4 L)	.84 pints/acre AI
	Pounce (3.2 EC)	.15 pints/acre AI

An equivalent of 500 gal./acre of water was used.

Spider mite populations were estimated using both presence-absence sequential sampling and actual spider mite counts per leaf. Estimates of spider mite population size were made every 2 weeks until treatments were applied and then 6 days and 18-20 days after treatments. Predator counts were also made in conjunction with the spider mite counts.

B. Bioassay Methods

We used the residual cell bioassay technique for both Plictran and Omite, which was developed in our laboratory (Keena and Granett 1985) to estimated log concentration/probit mortality lines and to detect resistance using critical concentrations. Cotton cotyledons were dipped in solutions of Plictran or Omite, air dried and placed in cells. The treated leaves were then infested with 20-30 adult female spider mites and held at 29 $\pm$ 1° C. Mortality was assessed at 72 hours. Four to 6 replications were made for each concentration tested on spider mites from each colony. A Vendex bioassay using the residual cell method is in the developmental stage (optimum temperature and duration must be determined)

The rapid bioassay methods for Plictran and Omite consist of coating the inside of a tightly-fitting petri dish with different concentrations of formulated Omite (30 WP) of Plictran (50 WP) in 95% ethanol. Twenty adult female spider mites are placed in each dish and held at 80-85° F for 24 hours. Mites are considered to be alive if they are able to move vigorously.

C. Species Identification

Species were identified both visually and using the slide squash-technique of Kono and Papp (Handbook of Agricultural Pests 1977). A minimum of 10 males from each colony were used.

D. Selection Procedures Used For Inheritance Studies

The following three methods have been used for both Plictran and Omite:

1) Cell Method. Colonies where high frequencies of resistant spider mites were present were treated with the LC_{90} miticide concentration in cell bioassays. Survivors were saved and used to start a new colony. Once the colony was established concentration mortality lines were run.

2) Cage-spray. Replacement plants put into colonies with high frequencies of resistant spider mites were treated with increasing concentrations of miticide. After each spray the population were allowed to build up, and concentration mortality tests were run.

3) Isolated Female Lines. Individual females were isolated from resistant colonies and once population numbers (of the single females progeny) had increased concentration mortality lines were run on each single female family line. This method is currently in use and results are not yet complete.

Results and Discussion

Since this is the first formal annual report we felt it was important to summarize previous research results so that this years work would be easier to understand and so that the part it plays in the whole resistance management program can be clearly seen.

DESCRIPTION OF PLICTRAN RESISTANCE

The log-concentration/probit-mortality (lc/pm) lines for the most homogeneously Plictran-susceptible colonies collected for each of the three species are given in Fig. 1. The lc/pm lines for *T. turkestani* , *T. urticae,* and *T. pacificus* are statistically the same, indicating similar responses.

No significant Plictran-resistance was detected in the remainder of the *T. turkestani* or *T. urticae* colonies collected. However, in 14% of the *T. urticae* colonies, 3 to 5% of the individuals tested were able to survive a concentration of 31.6 ppm Plictran, indicating the presence of resistant individuals at very low frequencies.

Varying frequencies and intensities of Plictran resistance were detected in the *T. pacificus* colonies collected. Figure 2 shows the lc/pm lines for the most Plictran susceptible and resistant colonies collected and a colony with an intermediate response. At the LC_{90} level there is a significant separation between the lc/pm lines, while at the LC_{10} level the 95% confidence intervals for the lc/pm lines of the three colonies overlap. This indicates that susceptible individuals were present in each of these populations.

The results for Plictran suggest that *T. turkestani* and *T. urticae* are still controllable, while Plictran resistance in *T. pacificus* may reduce the efficacy of this acaricide in some orchards. However, the presence of susceptible individuals in even the most Plictran-resistant *T. pacificus* colony collected allows for intermating to eventually lower the frequency of resistant individuals in the population.

DESCRIPTION OF OMITE RESISTANCE

The lc/pm lines for the most homogeneously Omite-susceptible colonies collected for each of the three species are shown in Fig. 3. The most Omite-susceptible *T. pacificus* colony collected responded with an LC_{50} 8-fold greater than that of the most susceptible *T. urticae* colony, while the most susceptible *T. turkestani* colony had an LC_{50} 2.5-fold greater. The significant, 8-fold difference exhibited by *T. pacificus* indicates that this species may have a natural tolerance for Omite, thus requiring higher treatment rates to achieve effective control.

No significant Omite-resistance was detected in any of the *T. turkestani* colonies collected, but varying frequencies and intensities of Omite-resistance were found in the *T. urticae* and *T. pacificus* colonies. The lc/pm lines for field collected *T. urticae* and *T. pacificus* colonies representative of extreme and intermediate Omite susceptibilities are shown in Figs. 4 and 5. Several *T. urticae* and *T. pacificus* colonies responded with intermediate or high intensities and frequencies of Omite-resistance. However, at the LC_{10} level the 95% confidence intervals for the intermediate and resistant colonies overlap those of the most susceptible colony, indicating the presence of susceptible individuals in all of the colonies.

The results for Omite suggest that *T. urticae* and *T. pacificus* are more difficult to control than *T. turkestani*, and that *T. pacificus* may be more difficult to control than *T. urticae*. The presence of susceptible individuals in even the most resistant colonies, however, is favorable for resistance management.

SURVEY RESULTS

PLICTRAN In 1985, 20 almond orchards were sampled for spider mites in April and August and were tested with Plictran to estimate lc/pm lines. From this work 2 levels of Plictran susceptibility were defined:

1. Plictran-susceptible
   $>$ 80% mortality at 31.6 ppm
2. Intermediate Plictran-resistance
   $\leq$ 80% mortality at 31.6 ppm

All of the *T. urticae* colonies collected in 1985 were classified as susceptible. Table 1 gives the percent of sites where *T. pacificus* was collected that fit the susceptible or intermediate categories established and the counties from which they were collected. We have not found a colony that we feel contains a high enough frequency of resistant types (ca. $\leq$ 80% mortality at 316 ppm) to be truly designated resistant, although our intermediate response may in itself cause some loss of efficacy in the field. These results emphasis that *T. pacificus* may be more difficult to control with Plictran than *T. urticae* and that, based on our collections, Plictran-resistance is developing in Kern, Tulare, and San Joaquin Counties.

OMITE In 1985, 20 almond orchards were sampled for spider mites in both April and August and lc/pm lines for Omite were estimated for each single species colony. From this work 3 levels of susceptibility were defined:

1. Omite-susceptible
   T. turkestani and *T. urticae*
   $>$ 80% mortality at 316 ppm
   T. pacificus
   $>$ 80% mortality at 1,000 ppm
2. Intermediate Omite-resistance
   T. urticae
   $>$ 80% mortality at 3,162 ppm and
   $\leq$ 80% mortality at 316 ppm
   T. pacificus
   $>$ 80% mortality at 3,162 ppm and
   $\leq$ 80% mortality at 1,000 ppm

3. High Omite-resistance
T. urticae and *T. pacificus*
$\leq$ 80% mortality at 3,162 ppm

The percentage of sites with spider mites which responded to Omite in each of these categories are given in Table 2. These results reemphasis that *T. pacificus* may be difficult to control that *T. urticae.* This also shows that Omite resistance is more likely to cause loss of efficacy in Tulare or Kern Counties than in the other counties where collections were made.

DYNAMICS OF RESISTANCE

In developing strategies for management, the following factors, which are known to influence resistance development and stability, must be studied.

SEASONAL SHIFTS In addition to the 1985 collections from 20 sites, 10 of the same orchards were sampled in April 1986 (5 resistant and 5 susceptible in 1985). for each species The percentage of sites which responded with statistically significant shifts in the lc/pm lines (non overlap of the 95% confidence limits at one or more of the concentrations tested) were determined. Figures 6-9 show the shifts observed between April 1985, August 1985, and April 1986.

PLICTRAN No significant shifts in Plictran response were observed for *T. turkestani* and *T. urticae,* since no significant resistance was detected. For *T. pacificus,* during the 1985 season, only shifts toward an increase in susceptibility were observed and between the 1985 and 1986 seasons only shifts toward increased resistance were observed. Increases in resistance could be the result of sprays selectively removing susceptibles from the population, migration of resistant individuals into the orchard, resistant individuals having a higher relative fitness then the susceptible individuals in the population or any combination of these factors. Conversely, decreases in resistance could be the result of intermating of resistant and susceptible types in the absence of sprays, migration of susceptible individuals into the orchard, susceptible individuals having a higher relative fitness then the resistant individuals, or some combination of these factors operating together. These results for *T. pacificus* and Plictran indicate that Plictran resistance in *T. pacificus* is unstable during the season and between seasons. The increases in resistance between seasons may make *T. pacificus* more difficult to control in April than in August.

OMITE For Omite no shifts in response were observed for *T. turkestani.* For *T. urticae* increases in resistance were observed both within the 1985 season and between the 1985 and 1986 seasons, while decreases in resistance were only observed between seasons. This indicates that Omite-resistance in *T. urticae* is stable or increasing during the season but is relatively unstable between seasons.

For *T. pacificus,* only increases in Omite-resistance were observed, indicating that Omite-resistance in *T. pacificus* is stable or tends to increase. These results suggest that management of Omite-resistance in *T. pacificus* may be more difficult to manage than in *T. urticae.*

SPECIES SHIFTS Shifts in the species composition collected from a given orchard were observed at 8 of the sites sampled. These shifts may cause shifts in the average resistance present in an orchard, since *T. pacificus* colonies collected were, in general, more resistant to Omite and Plictran.

MULTIPLE AND CROSS RESISTANCE Six colonies of *T. pacificus* responded with intermediate Plictran-resistance and Omite-resistance, while at other sites, *T. pacificus* responded with an intermediate or high resistance to only one of the two acaricides. This indicates that *T. pacificus* responds with multiple resistance, not cross resistance, to Plictran and Omite. Between Plictran and fenbutatin-oxide however, cross resistance is suspected because they are both tin compounds.

MODE OF INHERITANCE Preliminary studies of the mode of inheritance for both Plictran- and Omite-resistance indicate that more than one gene may be involved, and there may be differences in the mode of inheritance between the species. Using methods 1 and 2 listed in the procedures section, the purification of resistant colonies was extremely slow (very little shift in the lc/pm lines after each round of selection), so method 3 has been employed to attempt to speed up the process. If the mode of inheritance is polygenic, predictions about the rate of development of the resistance will be difficult and it may explain why resistance to these two acaricides has developed slowly (> 10 years of efficacy).

FIELD EFFICACY TRIALS

Extrapolation from laboratory detected resistance to the field cannot be made without field trials to determine whether a specific frequency and intensity of resistance, estimated by laboratory bioassays will result in a loss of efficacy in the field. We established 4 such field trials in 1986, in orchards where *T. pacificus* predominated. In two of the orchards — Beltran, Stanislaus County and Wood, Madera County — the *T. pacificus* were susceptible to both Omite and Plictran, and in the other two orchards — Kern Farming, Kern County and Apex, Kern County — the *T. pacificus* were resistant to both Omite and Plictran (intermediate resistance category).

At the Beltran orchard there was a light infestation of spider mites and high predator activity (Table 3). As shown in Fig. 10 treatments were applied before spider mite numbers per leaf had reached the control action threshold and all acaricide treatment gave good control. When predators were eliminated with a treatment of permethrin (Pounce^R) the number of spider mites per leaf rapidly increased indicating that predator activity in this orchard was a major control factor.

At the Wood orchard, spider mites reached the control action threshold earlier than the Beltran orchard and predator activity was low (Table 3). As shown in Fig. 11 treatments were applied after the number of spider mites per leaf had reached the control action threshold. At 6 days post treatment, good control was achieved with fenbutatin-oxide, while the other treatments gave only marginal to poor control (Fig. 11). At 18 days post treatment, all treatment trees were defoliating. Of all the treatments, fenbutatin-oxide gave the best control. These results suggest that in the absence of sufficient predator activity susceptible *T. pacificus* are difficult to control.

At the Kern Farming orchard, there was a moderate spider mite infestation and medium to low predator activity (Table 3). This field plot accidentally received a commercial Omite application, but pre- and post treatment spider mite counts were obtained. At 5 days before the commercial application was applied, a mean of 1.19 spider mites per leaf were found and at 8 days post treatment a mean of 1.25 spider mites per leaf were found.

Mite densities were still below the control action threshold, yet spider mite densities were increasing.

The Apex orchard, had a low spider mite infestation and high predator activity (Table 3). The spider mite per leaf counts in this orchard never reached the control action threshold, so no treatments were applied. This indicates that an orchard can contain resistant *T. pacificus*, and be effectively controlled by predators.

These field trials indicate that many factors influence the spider mite control achieved in a given orchard using these acaricides, especially the intensity of predator activity.

Conclusions

Laboratory bioassays have shown that, *T. pacificus* is more difficult to control than *T. turkestani* and *T. urticae* with Plictran or Omite, based on frequency, intensity, and stability of resistance. However, susceptible individuals were present in all of the colonies collected. This allows growers to stop using the chemical in orchards where resistance is a problem, and potentially use the chemical successfully at some point in the future. This is possible since in the absence of sprays, more susceptible individuals will be present to intermate with resistant individuals, thus more effectively lowering the frequency of resistance. As the field trials have indicated, many factors other than pesticide resistance, such as predator activity, cultural practices, and potentially pesticide use histories influence field efficacy of Plictran and Omite.

For a resistance management program to be of value to the grower, it must provide implementable strategies and methods for manageing Plictran and Omite resistance problems. Verifying the rapid bioassays we have developed to detect Plictran and Omite resistance and assessing the effects of the different factors on field efficacy during the next growing season is the logical next step in the development of a resistance management program for almonds.

The benefits resulting from resistance management are both immediate and long term. Management of resistance using the rapid bioassays, will decrease expenses for almond growers and increase predictability and so effectiveness of spider mite control, using Plictran and Omite. If field failure is due to resistance, then the grower can avoid the wasted expense of repeated, ineffective applications at high rates. Instead the grower can use an alternative miticide that is effective and/or release predators to enhance predator activity. If the rapid bioassay demonstrates that the spider mites in the orchard are not resistant, then the grower can work on improving application techniques, cultural practices or enhancing predator activity. This method will also show PCA's, chemical company representatives and/or growers the true incidence and distribution of the resistance problem.

Over the long-term, using Plictran and Omite only in orchards where the frequencies of resistant spider mites are low will help lengthen the effective life of these miticides. In addition, by making growers and PCA's more aware of the influence of other factors (cultural and biological controls) on spider mite control, the utilization of these factors will subsequently increase and the use of miticides will decrease, even in areas where resistance to these miticides is currently a problem.

Publications

Keena, M. A., and J. Granett. 1985. Variability in toxicity of propargite to spider mites (Acari: Tetranychidae) from California almonds. J. Econ. Entomol. 78: 1212-1216.

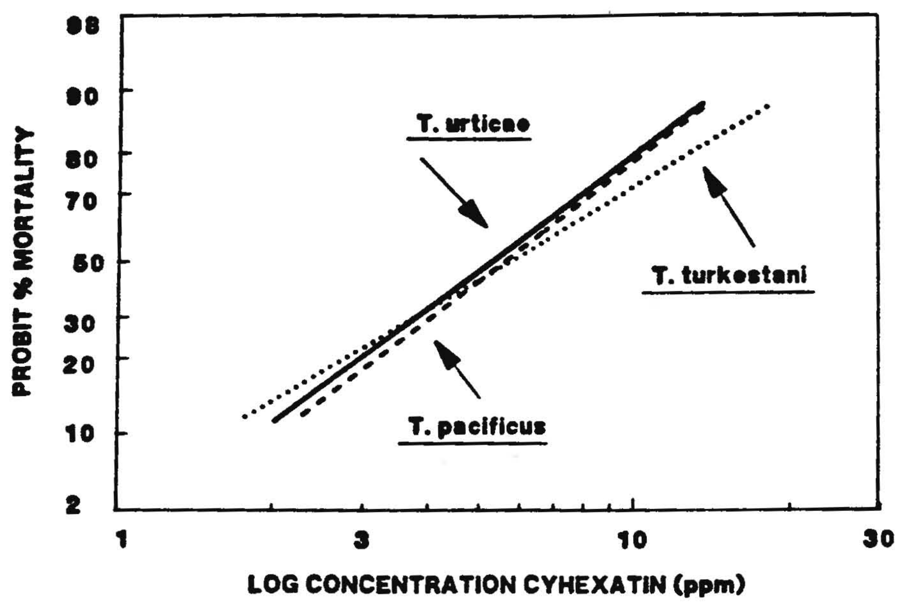
Keena, M. A., and J. Granett. 1987. Cyhexatin and propargite resistance in populations of spider mites (Acari: Tetranychidae) from California almonds. J. Econ. Entomol. (In Press).

Keena, M. A., and J. Granett. 1987. Monitoring for propargite and cyhexatin resistance in spider mites (Acari: Tetranychidae) from California almonds. (In Preparation).

(1)

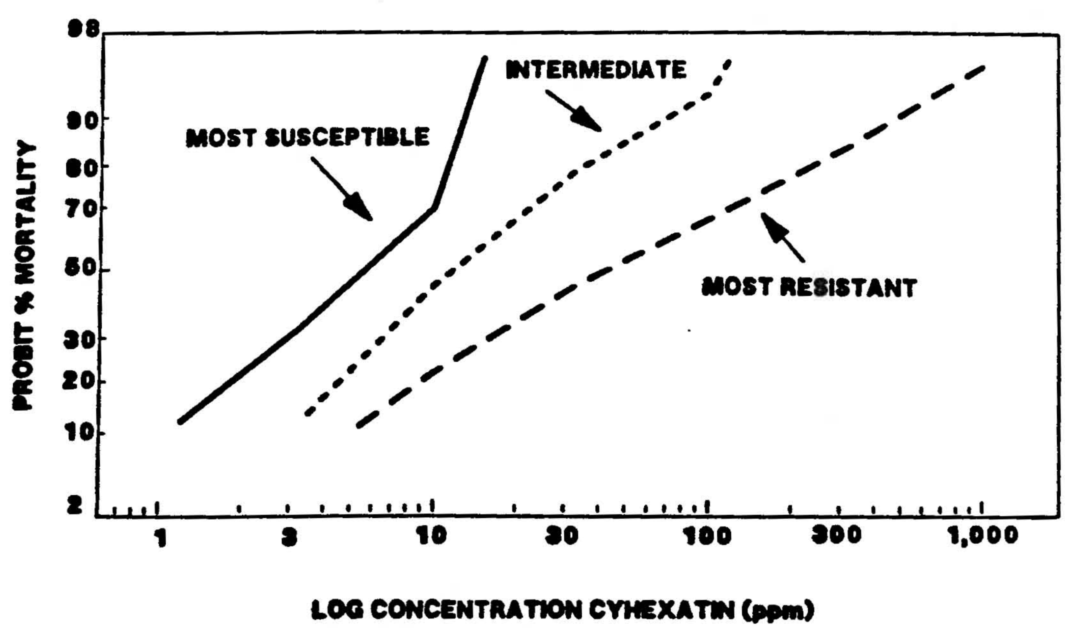
RESPONSES OF THE MOST SUSCEPTIBLE COLONY

FOR EACH SPECIES



(2)

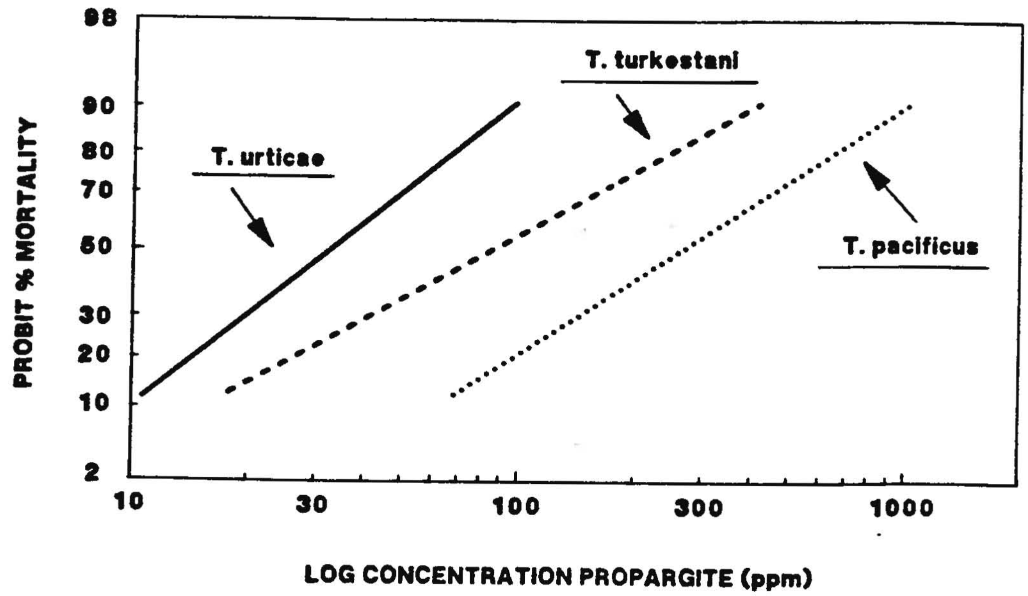
CYHEXATIN REPSONSES OF REPRESENTATIVE T. PACIFICUS COLONIES



(3)

RESPONSES OF THE MOST PROPARGITE SUSCEPTIBLE COLONY

FOR EACH SPECIES



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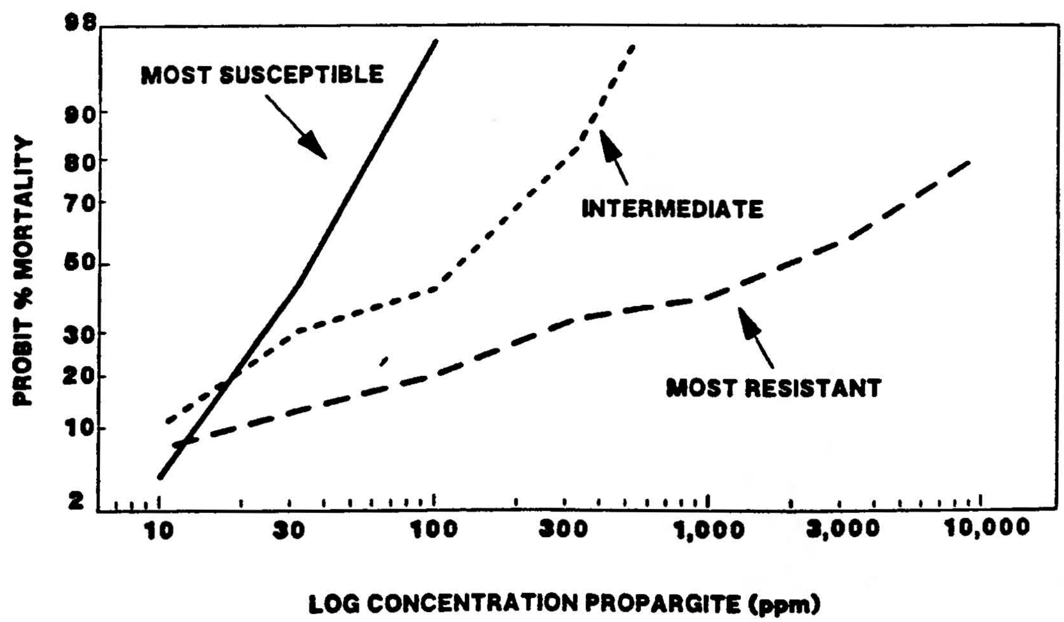
PROPARGITE RESPONSES OF REPRESENTATIVE T. URTICAE COLONIES



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PROPARGITE RESPONSES OF REPRESENTATIVE T. PACIFICUS COLONIES

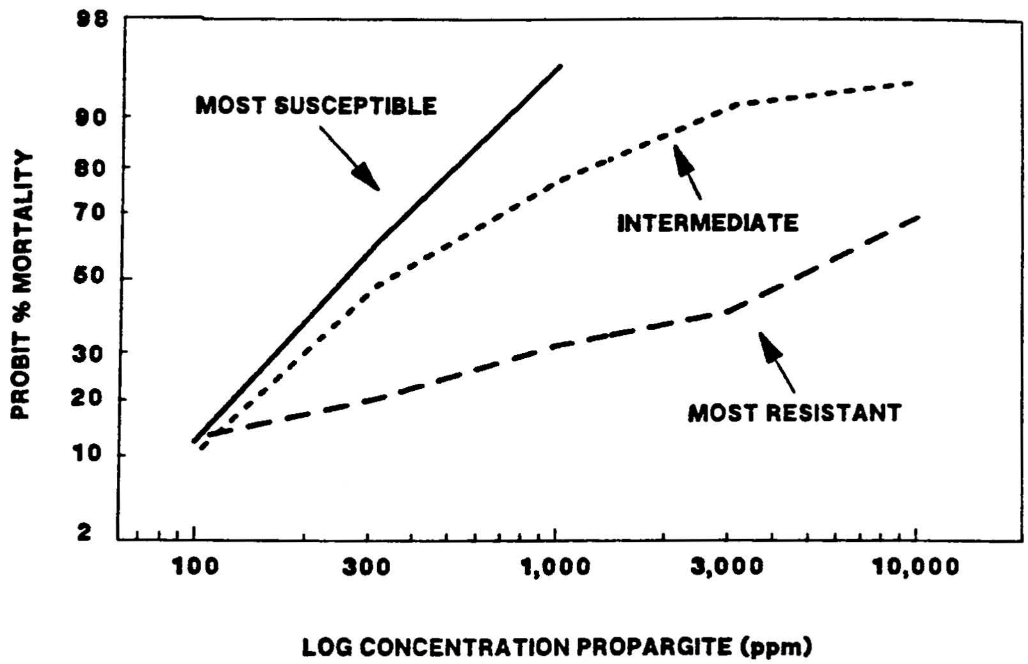


Table (1)

1985 REGIONAL MONITORING SUMMARY FOR CYHEXATIN

SPECIES		SUSCEPTIBLE	INTERMEDIATE RESISTANCE
T. URTICAE			
	- APRIL	100%	0%
	- AUGUST	100%	0%
	- COUNTIES	BUTTE, GLENN, SAN JOAQUIN, STANISLAUS, MADERA, MERCED, FRESNO, TULARE, KERN	
T.PACIFICUS			
	- APRIL	63%	37%
	- AUGUST	45%	55%
	- COUNTIES	BUTTE, GLENN, SAN JOAQUIN STANISLAUS, MADERA, FRESNO	SAN JOAQUIN, MERCED, TULARE, KERN

Table (2)

1985 REGIONAL MONITORING SUMMARY FOR PROPARGITE

SPECIES		SUSCEPTIBLE	INTERMEDIATE RESISTANCE	HIGH RESISTANCE
T. URTICAE				
	- APRIL	80%	20%	0%
	- AUGUST	67%	22%	11%
	- COUNTIES	BUTTE, GLENN, SAN JOAQUIN, MADERA,MERCED, FRESNO, TULARE	STANISLAUS, MADERA, KERN	TULARE
T.PACIFICUS				
	- APRIL	44%	12%	44%
	- AUGUST	50%	20%	30%
	- COUNTIES	GLENN, STANISLAUS, SAN JOAQUIN, MADERA, MERCED, FRESNO	BUTTE, FRESNO, KERN	TULARE, KERN

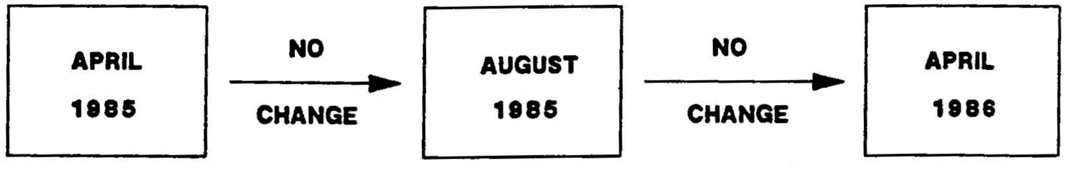
(6)

SHIFTS IN CYHEXATIN SUSCEPTIBILITY

T. PACIFICUS SITES

(7)

SHIFTS IN CYHEXATIN SUSCEPTIBILITY
T. URTICAE



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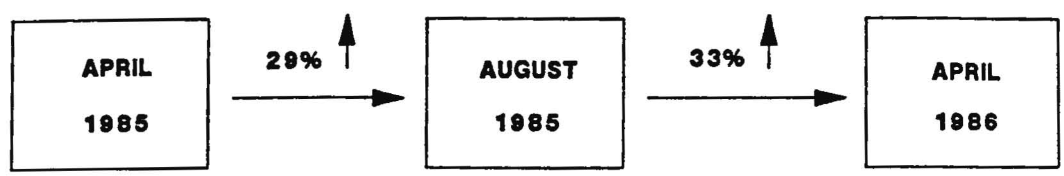
SHIFTS IN PROPARGITE SUSCEPTIBILITY

T. PACIFICUS SITES



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SHIFTS IN PROPARGITE SUSCEPTIBILITY

T. URTICAE SITES

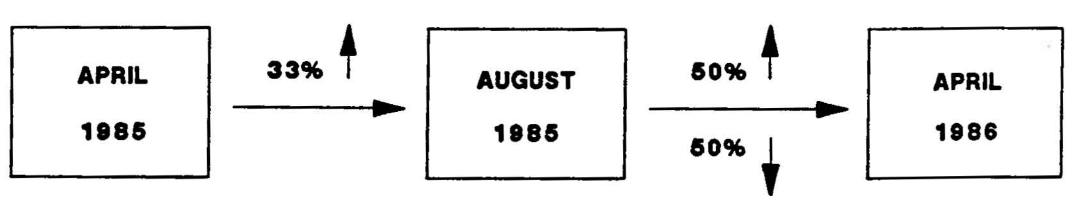


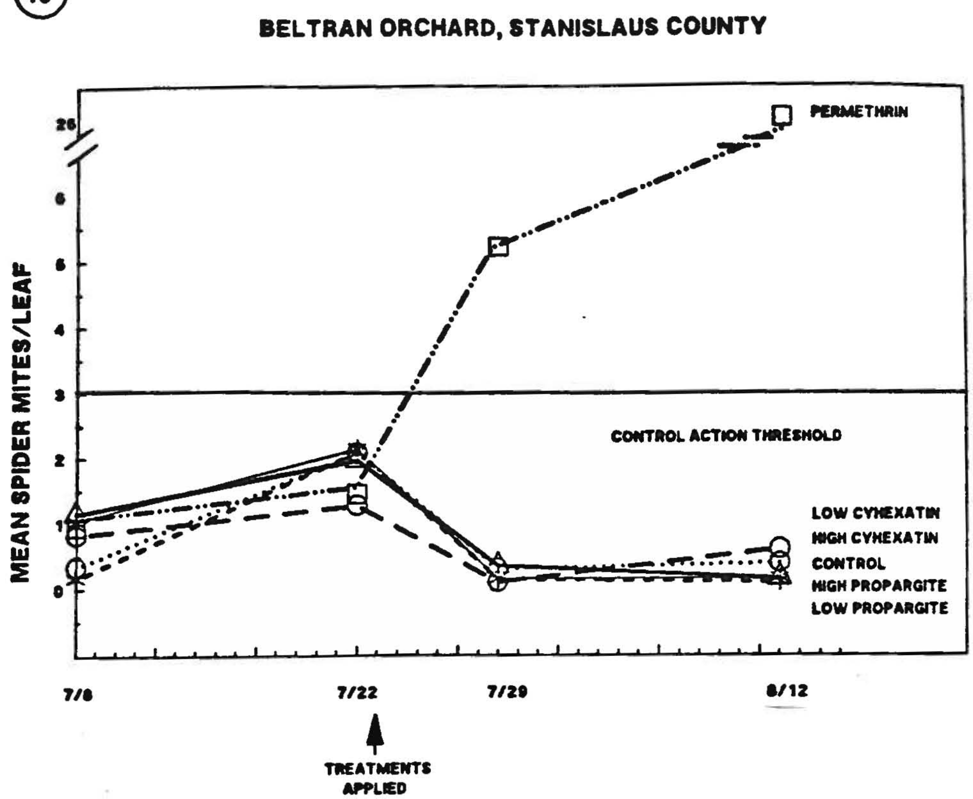
Table 3

FIELD TRIAL SUMMARY

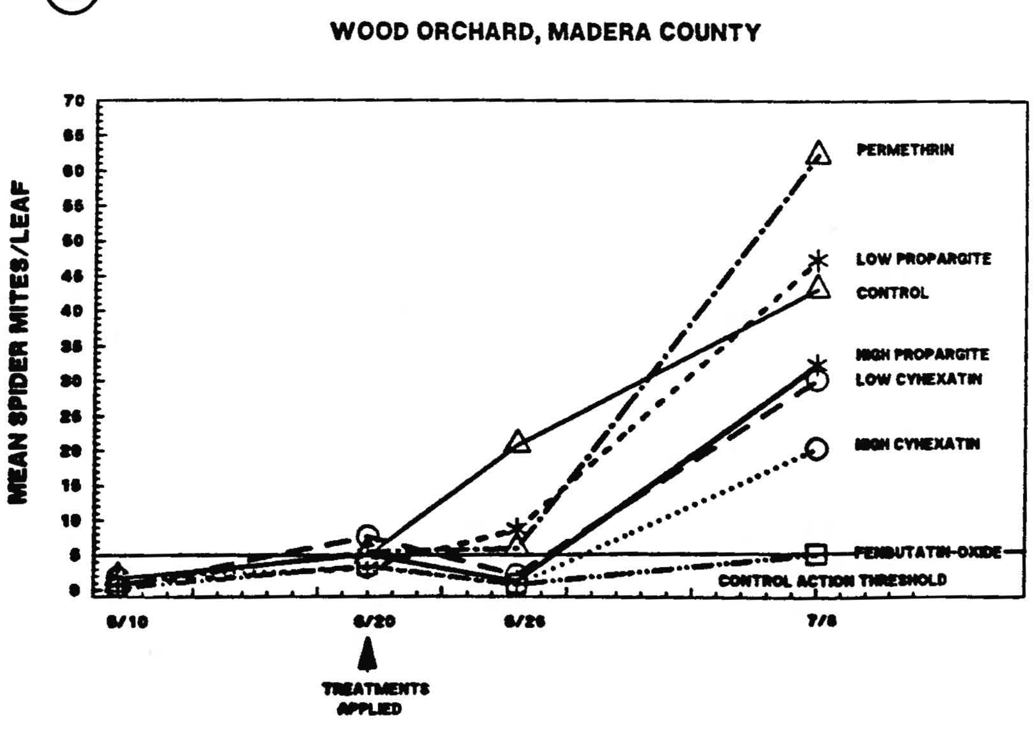
ORCHARD MEANS

ORCHARD	DATE	SPIDER MITES/LEAF	PREDATORS/LEAF	PREDATOR/PREY RATIO
BELTRAN	7/22	1.67	0.34	1/5
WOOD	6/20	4.75	0.06	1/79
KERN FARMING	6/26	1.19	0.04	1/30
APEX	7/22	0.58	0.13	1/5

(10)


BELTRAN ORCHARD, STANISLAUS COUNTY
MEAN SPIDER MITES/LEAF
PERMETHRIN
CONTROL ACTION THRESHOLD
LOW CYHEXATIN
HIGH CYHEXATIN
CONTROL
HIGH PROPARGITE
LOW PROPARGITE
7/8
7/22
7/29
8/12
TREATMENTS APPLIED

(11)


WOOD ORCHARD, MADERA COUNTY
MEAN SPIDER MITES/LEAF
PERMETHRIN
LOW PROPARGITE
CONTROL
HIGH PROPARGITE
LOW CYHEXATIN
HIGH CYHEXATIN
FENBUTATIN-OXIDE
CONTROL ACTION THRESHOLD
TREATMENTS APPLIED