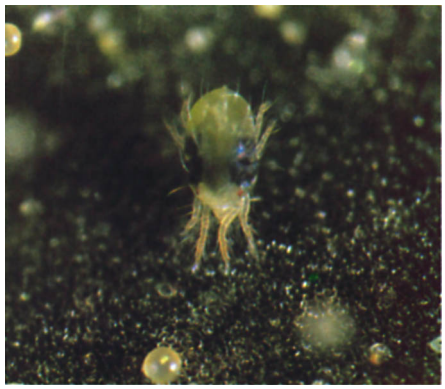
Quick tests for pesticide resistance in spider mites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The strawberry mite, two-spotted spider mite, and Pacific mite are the dominant spider mite pests of cotton in California's San Joaquin Valley. The damage they cause ranges from stippling of leaves to defoliation, influencing plant growth and production.

Three acaricides that allow beneficial insects and mites to survive are currently registered for control of spider mites in cotton. One of them, sulfur, is effective only against the strawberry mite, *Tetranychus turkestani* Ugarov and Nikolski. The other two, dicofol (Kelthane) and propargite (Comite), have been used successfully for 15 to 20 years to control all three species of spider mites. In the last few years, however, we have begun to detect populations of two-spotted spider mite, *T. urticae* Koch, and Pacific mite, *T. pacificus* McGregor, that are resistant to dicofol, propargite, or both.

Several characteristics of the spider mite/cotton system in the San Joaquin Valley make acaricide resistance more manageable than in many other situations. First, growers keep acaricide use down to one or two applications per season, resulting in a moderate selection pressure. Second, they avoid broad-spectrum pesticides that remove natural enemies. Third, agriculture, and thus pesticide practices, in the San Joaquin Valley are diverse; the use of many different acaricides lowers the selection pressure of any one. Fourth, because spider mites are very mobile and can drift from field to field on air currents, interbreeding of susceptible and resistant mites is enhanced. Finally, inheritance of resistance to dicofol and propargite is not dominant, and breeding with susceptible mites will rapidly reduce the frequency of resistance in a population.

The result is that high levels of dicofol and/or propargite resistance occur in relatively few cotton fields: 10 to 30 percent of those randomly sampled during 1982-85. If resistance becomes widespread because of mismanagement of these acaricides, however, and they are replaced with broader spectrum acaricides that kill natural enemies, the excellent integrated pest management program presently used in San Joaquin Valley cotton will be disrupted. It is therefore important to implement resistance management practices quickly for both dicofol and propargite.

In the past, acaricide resistance was detected by commercially treating a field and assessing spider mite densities 7 to 14 days after the application. If resistance was a problem, however, this was an expensive way to find out. Also, this method did not distinguish between lack of effectiveness due to resistance and that due to other factors, such as poor coverage, migration of spider mites from neighboring crops, low natural enemy populations, and plant water stress. Our laboratory has worked for the past five years on the development of a bioassay test that growers can use for an inexpensive, fast assessment of resistance in individual cotton fields before applying an acaricide.

A rapid bioassay that detects buildup of resistance can help growers avoid the expense and hazard of wasted pesticide treatments.

The benefits of a quick method for detecting resistance are both short-term and long-term. If resistance is a problem at a specific location, the grower will avoid the expense and hazard of a wasted acaricide application. If more than one acaricide is available, the grower can make a more accurate decision on which acaricide would be most effective. The rapid bioassay can also be used after an acaricide application to distinguish between loss of efficacy due to field failure and that resulting from other factors. Finally, temporarily avoiding the use of a problem acaricide gives susceptible mites a chance to survive and interbreed with resistant mites, resulting in a lower frequency of resistant individuals in the next generation.

Several areas of research had to be completed in developing the rapid bioassay. We first performed laboratory bioassays to characterize the levels of resistance in different populations of spider mites throughout the San Joaquin Valley. Then field trials determined which levels of resistance detected in the laboratory corresponded to resistance of populations in the field that cause the acaricide to lose effectiveness. Finally, the rapid bioassays were developed so that growers could determine, in 24 hours, which cotton fields had significant levels of resistant spider mites.

We report here on the development of the rapid bioassays for detecting dicofol and propargite resistance in spider mites in San Joaquin Valley cotton.

Rapid bioassays

The accurate evaluation of resistance depends on random sampling of mite-infested leaves. One to three species of spider mites may be found in a cotton field, and several levels of resistance may exist. Thus, 80 to 100 leaves are needed from

Damage caused by strawberry spider mite (at left), one of three mite pests of cotton, ranges from stippling of leaves, as shown here, to complete defoliation. The mites are hard to control without killing their natural enemies, and they can quickly develop resistance to some pesticides.

each field. Only one adult female mite is removed from each leaf. This type of sampling ensures that the spider mites are a cross-section of the various species and resistance levels present in the field. The mites can be stored in a refrigerator or ice chest (above 40°F) for up to 48 hours before testing.

For the dicofol rapid bioassay, small, tight-fitting plastic petri dishes are treated with 56.2 parts per million (ppm) active ingredient (AI) formulated dicofol in 95 percent ethanol (weight/volume). For the propargite rapid bioassay, the dishes are treated with 1000 ppm AI formulated propargite in ethanol. The control dishes are treated with ethanol alone. One milliliter of solution is placed in the test dish, the dish is closed, the liquid is swirled around the inside of the top, bottom, and sides, and the excess is poured off. The dishes are then air-dried and kept in the refrigerator (40° to 50°F) for at least one week and then used as needed. When stored in this way, the dishes remain effective for three months.

When the test is set up, the dish containing ethanol only, 56.2 ppm dicofol, or 1000 ppm propargite is placed on paper toweling on a block of frozen blue ice. The paper prevents condensation, and the ice cools the mites and prevents them from leaving the dish. Twenty adult females are removed from 20 sampled leaves and placed in a dish for each concentration tested. The test dishes are closed and left in indirect light or in darkness for 24 hours at 70° to 85°F for the dicofol-treated dish and 80° to 85°F for the propargite-treated dish.

After 24 hours, the mites are examined for activity. For dicofol, if more than 20 percent of the spider mites can walk the distance of one body length when prodded with a camel's hair artist's brush, then significant levels of resistance exist in that cotton field, and we recommend against use of dicofol. In the propargite test, the edge of the dish is tapped vigorously on a hard surface to knock the mites to one side. If more than 30 percent of the spider mites can move their legs vigorously, then significant levels of resistance exist in that cotton field, and we recommend against the use of propargite.

We want to avoid the error of discontinuing use of dicofol or propargite when resistance levels are low and the acaricides are still effective in controlling most of the spider mites. The dicofol and propargite rapid bioassays were therefore designed to detect only those populations of spider mites that have at least 20 and 30 percent resistant individuals, respectively.

If survival of the spider mites in the rapid bioassay dish is very close to 25 percent, other factors that influence acaricide efficacy must be carefully weighed, such as coverage, plant stress, and the number of natural enemies, before a decision to use the acaricide is made. For example, if the natural enemies are at high densities and resistance is borderline, control will probably be achieved.

We have found two sources of error in handling spider mites that alter their responses to dicofol or propargite. If we rear the spider mites in the laboratory, their resistance to propargite decreases. Rearing did not have as serious an effect on their level of resistance to dicofol. Also, if we store them in cold temperatures for more than 48 hours before testing, susceptibility to both acaricides increases. The rapid bioassay should thus be conducted within 48 hours of removal of spider mites from the field.

Validation of bioassays

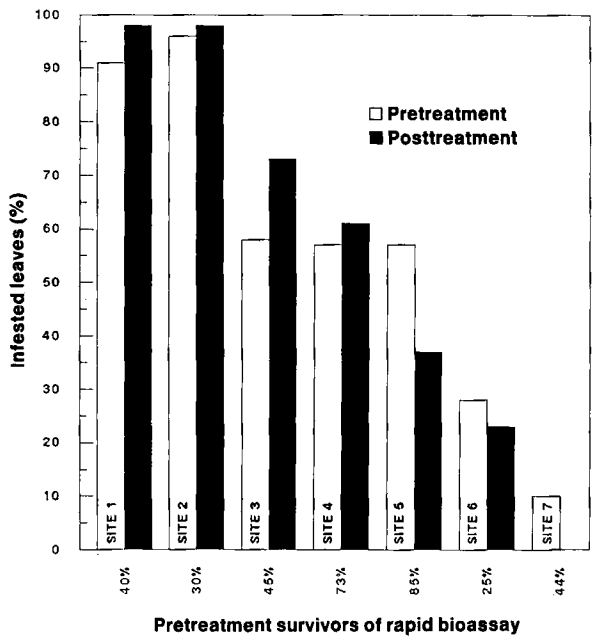
Our final step in validating the rapid bioassay was to use it before a commercial application of acaricide and see if it correctly predicted efficacy or lack of efficacy. We chose 16 cotton fields that were to receive a propargite application. We sampled each field twice using presence-absence sampling (percentage of 25 randomly collected leaves infested with mobile stages of spider mites) and conducted the propargite rapid bioassay immediately before the commercial application. One week to ten days after treatment, we returned to the fields and repeated the presence-absence sampling.

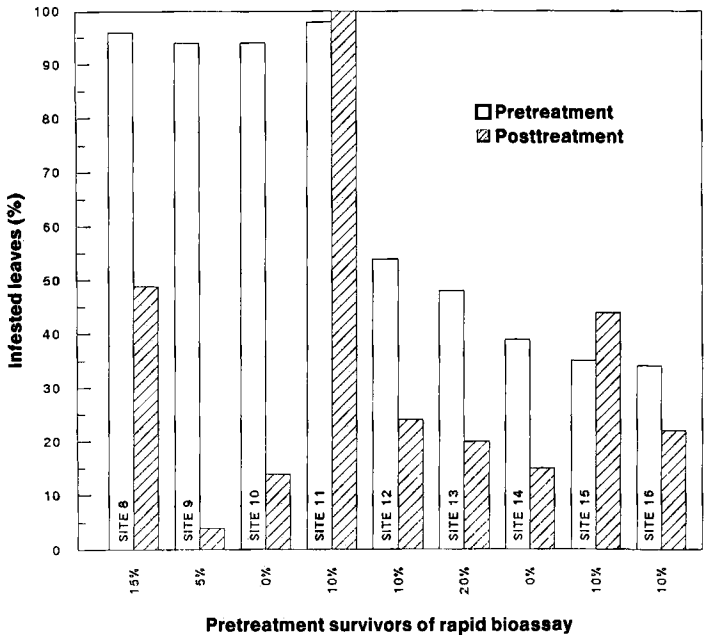
If the mites survived well in the propargite-treated dish, we predicted that they had high levels of resistance to the acaricide and that the percentage infestation would change very little after the application. Alternatively, we expected a large change in the percentage infestation of the cotton leaves if little or no resistance had been detected.

Spider mites from seven sites (fig. 1) had shown 25 percent or greater survival in the dish and thus had significant levels of resistance to propargite. Although 30 percent survival was the threshold we described earlier for highly resistant populations, 25 percent resistance was included in this group, because it is a borderline response and may also cause loss of field efficacy of the acaricide. In four out of seven of the sites, the percentage infestation actually increased. In site 7, a broad-spectrum insecticide applied with the propargite had an acaricidal action, and mite densities were greatly reduced.

Of the nine sites in which the spider mites showed 20 percent or less survival in the propargite-treated dish, six showed at least a 50 percent reduction in percentage infestation after treatment (fig. 2). Site 11 was next to a cornfield, and so, although the spider mites were susceptible, the propargite appeared to fail because more spider mites were blowing into the cotton from the corn. Site 15 received a propargite application for spider mites in combination with a pyrethroid application for Lepidoptera insect control. Since pyrethroids tend to cause mite populations to flare up, the propargite application did not lower the percentage of spider mite-infested leaves.

With a few exceptions, the propargite rapid assay was a very good predictor of





Rapid bioassay was a good predictor of resistance to propargite. In sites from which spider mites showed high resistance in laboratory bioassays, fig. 1 (left), pretreatment and posttreatment infestations were fairly close. Of sites from which spider mites showed susceptibility to propargite, fig. 2 (right), six had a significant reduction in infestation after treatment.

the effectiveness of commercially applied propargite. The spider mite populations that did not respond in the manner predicted by the rapid bioassay demonstrated that factors other than resistance, such as mite migration, natural enemies, and use of broad-spectrum pesticides, can also influence the apparent effectiveness of the selective acaricides.

Timing

The rapid bioassays are most useful if conducted immediately before an acaricide application is needed to determine which one will be effective. If the test predicts resistance to one acaricide, the other one should be used. If resistance to both acaricides is detected, another selective acaricide should be used if available. Broad-spectrum pesticides should be avoided if at all possible, because they destroy the natural enemies.

Because spider mite species and resistance levels can change during a season, one early-season measurement of resistance is not sufficient to predict efficacy of an acaricide throughout the season. If more than one acaricide application is needed, the rapid bioassay should be repeated.

The rapid bioassays can also be used 10 days or more after an acaricide application to see if noneffectiveness is due to resistance or some other factor. The rapid assays are also useful the following season to see if resistance levels have decreased to a level where the acaricide will be effective again.

Commercial use

During the 1986 field season, with the support of the California Department of Food and Agriculture, we cooperated with Dellavalle Laboratories in Fresno in a pilot program with the rapid bioassays. We used Dellavalle's facilities and courier service and trained a technician to process mite-infested leaf samples. Pest control advisors brought in these samples for resistance assessment, because they were concerned about a lack of effectiveness in particular fields. The results therefore do not represent the true distribution of acaricide resistance in San Joaquin Valley cotton.

TABLE 1. Number of "problem" cotton fields which produced susceptible or resistant spider mites when tested with the rapid bioassay during 1986

Samples tested with dicofol or propargite				
	Dicofol*		Propargite†	
County	Susc.	Resist.	Susc.	Resist.
Merced	3	1	0	0
Madera	3	0	1	0
Fresno	1	0	1	0
Tulare	3	4	3	8
Kings	5	10	9	5
Kern	7	7	5	5
Total	22	22	19	18

* Resistance is defined by >20% of the spider mites walking after 24 hours on 56.2 ppm dicofol.

† Resistance is defined by >30% of the spider mites moving vigorously after 24 hours on 1000 ppm propargite.

Of the 54 spider mite-infested leaf samples received from various counties in the San Joaquin Valley, approximately 50 percent revealed spider mite populations significantly resistant to dicofol, propargite, or both (table 1). For the remaining sites, the pest control advisor now knows to look for other factors that contribute to lack of effectiveness.

Cotton is the only crop in which the dicofol and propargite rapid bioassays have been validated. Propargite is used in other crops, however, such as almonds and seed alfalfa, and so we have begun work to validate that bioassay for those crops. The situation for perennial crops is more difficult, because factors other than resistance, such as natural enemies and water stress, exert greater influence on acaricide efficacy than they do in cotton.

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The rapid bioassay dishes and testing service are currently available from Insect Lore Products, P.O. Box 1535, Shafter, Ca 93263.