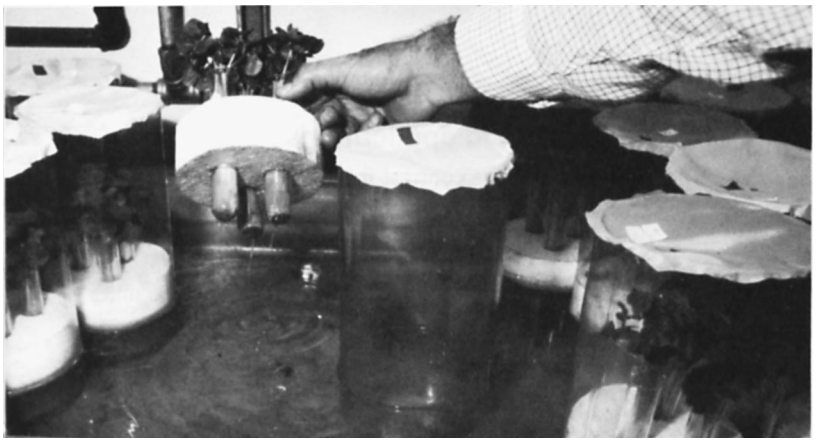
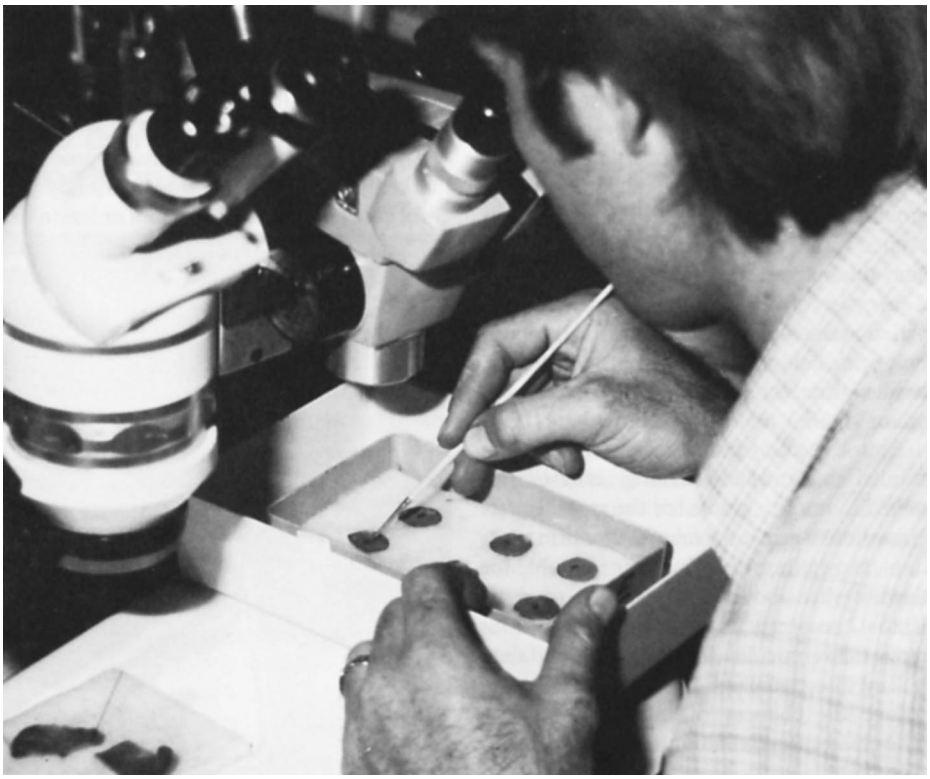


Hydroponically grown cotton plants and a water barrier (left) isolate small cultures of floating islands of spider mites. Below, author Timothy Dennehy prepares leaf-disc bioassay that will indicate miticide residual activity not shown by the conventional slide-dip method. In slide-dip assay (below left), mites are immersed in miticide test solution. Here, the mites are seen as small dots attached to the slide by double-stick tape.



A simplified bioassay system

Improved detection of dicofol-resistant spider mites in cotton

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In the past, resistant pest strains have been controlled by replacing noneffective chemicals with effective alternative chemicals. This tactic is becoming increasingly inappropriate because of escalating costs associated with developing replacement pesticides and incidences of cross and multiple resistance. As a result, some workers feel that pesticide resistance is the most important problem facing applied entomology.

Tactics used in integrated pest management (IPM), such as biological control, cultural methods, and pheromones, are key factors in suppressing pests without selecting for insecticide resistance. IPM programs that incorporate a chemical component, however, may be threatened by the development of resistance in pest populations.

No mechanism exists in California for monitoring the efficacy of pesticides used in the field. Growers who think they may have a resistance problem must resort to personal experience or that of other local growers and farm advisors in evaluating the inadequacies of their chemical control methods. Many questions basic to implementation of resistance-monitoring and management programs have not been answered.

In 1981, we began field and laboratory studies on documentation and management of spider mite resistance in San Joaquin Valley cotton. We investigated the variation in susceptibility to the widely used miticide dicofol by sampling spider mites from fields near Bakersfield, Corcoran, and Lemoore.

In this paper we describe a method that allowed us to perform resistance bioassays on numerous single-species spider mite cultures from cotton fields. We report on spider mite

populations found at three locations that appeared quite susceptible to dicofol in topical assays and yet quite tolerant of dicofol in residual assays.

Floating mite-islands

In studying miticide resistance, it is necessary to isolate and maintain genetically different spider mite populations. Mite cultures are difficult to isolate, because the mites are small and can be carried by air currents. We could not use existing methods of maintaining and isolating cultures for our studies, because space and labor were limited. The floating mite-island method allowed us to maintain numerous cultures in a relatively small laboratory area.

Floating mite-islands consisted of Styrofoam platforms containing hydroponically grown cotton seedlings. The platforms floated within a cylindrical, clear-sided, 0.01-inch-thick acetate-plastic cage. These cages were open at the submerged, basal end and closed at the top with a piece of finely woven polyester lining, the mesh of which was smaller than a newly hatched protonymph mite.

Cotton seedling bouquets in test tubes suspended within the floating mite-islands served as nursery plants for the spider mites. Greenhouse-grown, Acala SJ-2 cotton seedlings, grown in vermiculite, were suitable for this use when about 4 inches high. Groups of 10 to 12 seedlings were washed gently to remove the vermiculite from the roots and then bulked into bouquets. We took great care to reduce the chances of introducing spider mites inadvertently into mite-islands with replacement nursery plants or carrying them from one culture to another while working with the colonies.

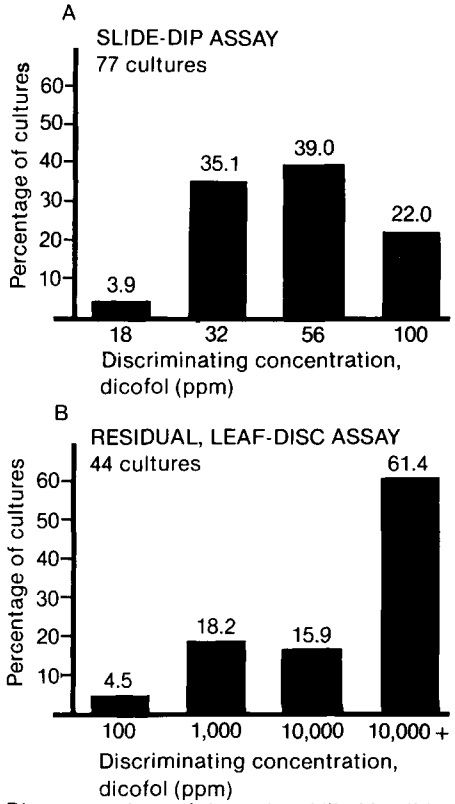
Slide-dip and residual assays

Because of the large number of cultures being assayed for susceptibility, we used a discriminating concentration method. A discriminating concentration was defined as the lowest concentration of dicofol tested that resulted in 100 percent mortality of samples of mites from a culture. We collected mite-infested leaves from fields in the three areas and brought them to the laboratory. Single-species cultures were started from individual females of *Tetranychus pacificus* or *T. urticae* by the mite-island culturing technique. Groups of 10 young, adult, female mites from each culture were treated with each test concentration of dicofol (formulated Kelthane 18.5 percent EC and 71 ppm Triton B1956 surfactant), and discriminating doses were recorded. Tests were replicated two to eight times.

We used two bioassay methods: the slide-dip and a residual-contact, leaf-disc method. In the slide-dip method, female mites were placed on their backs on double-stick tape attached to a microscope slide. The slide was then dipped for 5 seconds, totally immersing the mites in dicofol concentrations of 0, 10, 18, 32, 56, or 100 ppm. Slides were allowed to dry for 15 minutes, placed in slide boxes, and held at 27° C. Mortality of mites was assessed after 24 hours.

For the residual assay, leaf discs 20 mm in diameter were excised from cotton cotyledon leaves, dipped for 5 seconds in dicofol concentrations of 0, 10, 100, 1,000, or 10,000 ppm, and placed on moist fiber-cotton beds. After the leaf discs were air-dried, 10 female mites were transferred to each. Mortality was assessed after 48 hours at 27° C.

The slide-dip and residual assays produced markedly different data on susceptibility. Dicofol concentrations of 100 ppm or less in the slide-dip method killed mites from all cultures after 24 hours, whereas many of the cultures contained mites that survived 48 hours exposure to 10,000-ppm treatments with the residual contact method (see graphs). The survivorship rate with the residual assay at the 10,000-ppm concentration varied among cultures from 0 to 100 percent. Of 44 cultures tested by residual assay, 27 repeatedly had survivors of the 10,000-ppm rate.



Bioassays showed that mites killed in slide-dip tests with 100 ppm or less could survive 10,000-ppm residues (10,000+ category indicates cultures with mites able to survive 48-hour, 10,000-ppm residual assays).

Field applications

The contrast in results obtained from the two bioassay methods presents a problem. Which method can we rely on to indicate field susceptibility? If the topical, slide-dip assay is used, then our data would indicate that mites in fields from all locations sampled were approximately equal in susceptibility to dicofol. On the other hand, the residual bioassay indicates that susceptibility was extremely variable both within and between the locations sampled.

Cotton growers apply miticides with 20 to 30 gallons of water per acre. Conservative estimates are that about 20 percent of the mites present in a field are not directly contacted by the spray at the time of application. That 20 percent will either be killed by the residual chemical deposit or survive the treatment.

Our laboratory studies indicate that the reason for the poor results some growers have obtained with dicofol may be the presence of mites that, if not directly contacted by the miticide, can withstand contact with the residue. In essence, we hypothesize that dicofol has no or little residual activity in fields containing residue-tolerant mites. Field and laboratory studies are under way to test this hypothesis.

Conclusions

The floating mite-island method provided significant savings in time and space when culturing many, small, discrete populations of spider mites. Field-monitoring studies in 1981 with single-species colonies detected *T. urticae* and *T. pacificus* populations that were readily killed in slide-dip dicofol bioassays (discriminating concentration of 100 ppm or less) but that repeatedly survived 48-hour residual contact with 10,000 ppm dicofol treatments.

Dicofol sprays applied to fields containing residue-tolerant mites may effectively perform as a contact miticide. Resistant mites not directly contacted by the dicofol spray may survive the residual spray material so that the pest population rebounds rapidly. The possibility of resistance to residues in cotton fields underscores the importance of good spray coverage. Monitoring programs cannot rely solely on slide-dip assays for detection of field resistance.

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