Development and Evaluation of a Wax Immersion Technique Designed for Studies of Spider Mite (Acari: Tetranychidae) Population on Strawberries

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ABSTRACT A wax immersion technique was developed and evaluated for use in population ecology studies of *Tetranychus urticae* Koch on strawberries. This method prolonged the time period in which leaves were suitable for spider mite counts (2 weeks), thus increasing the number of leaf samples which could be collected and effectively counted at any given time. Since all *T. urticae* life stages were killed immediately following treatment in the laboratory or the field, problems associated with continued development or increased activity caused by handling were eliminated. Comparison of the wax immersion technique and an imprint method based on a protein-sensitive dye indicated that the wax immersion technique was more effective for calculating pretreatment counts of the developmental stages studied, and was therefore more suitable for estimating population density.

A STATISTICALLY adequate data base for development of sequential sampling, binomial sampling, or stable age distributions of spider mites on strawberries or other crops requires counts from substantial amounts of foliage. The small size, heavy webbing, and mobility of the twospotted spider mite, *Tetranychus urticae* Koch, make such extensive counts difficult.

Jeppson et al. (1975) discussed some of the problems associated with counting mites using a dissecting microscope. The primary problem was the stimulation of migratory behavior by handling leaves which led to counting individual mites more than once if the microscope field of vision was smaller than the total area to be examined. Refrigeration of destructively sampled leaves may eliminate some of the problem of continued development and oviposition by mites, but strawberry leaves deteriorate rapidly in cold storage; a combination of dehydration and discoloration due to oxidation and hydrolysis of starches to sugars makes counting difficult. Most studies using this technique have required that all foliage be examined within 24 h of removal from the plant (Kennedy et al. 1976, Oatman et al. 1977, Wyman et al. 1979, E. R. Oatman, personal communication). Freezing leaves sealed in plastic bags is possible in the field using dry-ice in coolers, but this technique is expensive, and cell damage from intra- and intercellular ice formation causes leaves to turn black and become watersoaked by the time they warm to room temperature. Also, counting becomes tedious and awkward. Thus, sample sizes are restricted by time and the availablity of technical help.

Unfortunately, the mite brushing machine, as described by Henderson and McBurnie (1943) and improved by Chant and Muir (1954) and Morgan et al. (1955), has not proven useful for estimating the density of all stages of spider mite populations on strawberries. The hirsute leaves and the webbing formed by the pests prevent clean separation of all developmental stages; broken hairs and associated trash make counting eggs and larvae virtually impossible. Therefore, we determined whether either a wax immersion technique or the imprinting method modified from Venables and Dennys (1941) and by Austin and Massee (1947) would be suitable for estimation of spider mite populations from the large samples of strawberry foliage necessary for studies of population dynamics.

Materials and Methods

Strawberry leaves with naturally occurring populations of *T. urticae* were collected from an untreated experimental planting of cv. 'Tufts' strawberries at the University of California South Coast Field Station in Orange County, Calif. Each leaf was chilled and then examined with a dissecting microscope; numbers of eggs, nymphs plus larvae plus adult males, and adult females were recorded. These leaves were then immediately subjected to either the imprinting or wax immersion procedures. Linear regression analyses were utilized to determine which technique provided the most accurate approximation of pretreatment counts. Numbers of leaves and ranges of each mite growth stage examined have been presented in Table 1.

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Table 1. Relationship between pretreatment counts of *T. urticae* on strawberry leaves and estimates based on an imprinting method using a protein-sensitive dye and a wax immersion technique[a]

Developmental stage	Filter paper imprinting				Wax immersion			
	n	Range	Slope	r^2	n	Range	Slope	r^2
Egg	25	5–1,474	0.13	0.675	52	0–197	0.81	0.953
Larvae + nymph + adult ♂	25	0–501	1.06	0.828	54	0–100	0.99	0.974
Adult ♀	25	0–36	2.15	0.320	54	0–33	0.91	0.953
Total count	25	6–1,577	2.36	0.787	52	0–231	0.90	0.958

[a] Imprint method utilized filter paper disks treated with acidified protein-sensitive dye, bromo phenol blue; original counts on untreated leaves were the independent variables, and counts from filter paper disks or wax-treated leaves were dependent counts.

Imprinting Technique. The imprinting procedure was evaluated by counting the number of small and large stains from crushed spider mites present on filter paper which had been firmly pressed against the abaxial surface of strawberry leaves. Unlike the red-pigmented spider mites for which this technique was developed, immature *T. urticae* produces a colorless stain on white filter paper. Therefore, the filter paper disks were treated with an acidified protein-sensitive dye, bromo phenol blue (available as a photographic supply) prior to use for imprinting. Approximately 50 mg of the powdered dye was mixed with 100 ml H_2O and then acidified to a pH of 3.0 with 25% HCl. The solution changes from a deep blue to yellow at a pH of 3.0, so repeated measurements for pH are not necessary. After drying, the filter paper disks were a pale yellow in color; green stains were produced when spider mites were crushed. Similar experiments utilizing basic solutions of bromo phenol blue or acidified bromo cresol green were also successful, but the acidified bromo phenol blue produced the sharpest contrast and was selected for this test.

Wax Immersion Technique. Spider mite infested leaves with known numbers of eggs, nymphs plus larvae plus adult males, and adult females were dipped in commercially available liquid floor wax (Thrifty Floor Care Formula) and placed in airtight plastic containers. Filter paper disks were placed below the leaves to absorb any excess wax. The leaf petioles were held with forceps to minimize the disturbance-induced problems discussed by Jeppson et al. (1975). Examination of wax remaining in dipping containers and on filter paper disks indicated that mites were neither washed off during the dipping process nor rubbed off when leaves were placed on the filter paper. Field tests where >500 infested leaves were collected using forceps and dipped in floor wax also indicated that spider mites were not dislodged during the dipping or storage procedures.

Results and Discussion

The wax immersion technique was more effective than the imprinting method for estimating densities of the various spider mite stages evaluated. The coefficients of determination (r^2) for pretreatment counts as independent variables vs. posttreatment counts as dependent variables were consistently higher with the wax immersion technique (Table 1). When the ranges of each *T. urticae* developmental stage examined for the imprinting method were reduced to values similar to the wax immersion technique by selecting leaves with lower posttreatment populations (n = 19–22 leaves), all r^2 values were reduced substantially; the highest r^2 value achieved was 0.570 for the egg stage. Thus, in the lower ranges of density at which strawberry producers would begin control measures, the wax immersion technique was superior.

Comparisons of the slopes also suggested that the wax immersion technique was best for estimating all stages. Although the wax immersion procedure underestimated densities of eggs, adult females, and total mite counts, these estimations were consistent and provided for an accurate prediction of larval density. This did not occur with the imprinting method, where variation was such that predictive ability (see low r^2 values, Table 1) was minimized, and estimates ranged from 87% below to 236% above pretreatment density. Additionally, intercepts (α values) for all regressions based on the wax immersion technique were not significantly different from 0 ($P < 0.01$ level, t test), while only the intercept for the adult female counts was not different from 0 for the imprinting method.

Difficulty in relating the size of stains (small or large) to *T. urticae* developmental stages made the imprinting method less suitable for estimating age distribution of the spider mite population. Multiple stains from dense groups of eggs, larvae, and nymphs tended to blend together and could have been misinterpreted as an adult female. Also, this method may not provide reliable estimates if more than one species of spider mite were present. However, the imprinting method would be suitable for making permanent records of spider mite distribution upon leaves, and would be more cost-effective than the wax immersion technique for use in nearest-neighborhood sampling plans or for a binomial sampling program, as proposed by Pielou (1960).

The wax treatment also prolonged the time during which the spider mites could be effectively counted, although the time necessary for assessment was the same on a per leaf basis. Water loss was minimized by sealing the leaf surfaces with wax; refrigerated leaves remained suitable for counting for ca. 2 weeks. Since spider mite development was stopped by the wax treatment, this technique would allow researchers to collect and subsequently count more foliage than was previously possible.

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