

A METHOD FOR HARVESTING AND SHIPPING LIVE CITRUS RUST MITES (ACARI: ERIOPHYIDAE)

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Field populations of citrus rust mite, *Phyllocoptruta oleivora* Ashmead in Florida have shown resistance to dicofol (Omoto et al. 1994) and shifts in susceptibility to abamectin (Bergh et al. 1999). Resistance monitoring of eriophyid mites has not been widely practiced, limiting the availability of reference strains and technical expertise. This study was prompted when a company expressed interest in testing the susceptibility of citrus rust mite populations from Texas to their acaricide. Under normal conditions, citrus rust mites do not live long off of the host (J. C. B., unpublished data), and importing them into Florida on citrus fruit or foliage requires quarantine. However, importing mites off of host plant tissue reduces the risk of introducing a new pest or plant pathogen and does not require their quarantine.

Our objective was to develop methods for removing large numbers of citrus rust mites from their host and shipping them live to other locations. Toward this end, we capitalized on two aspects of their biology. First, mites disperse by leaping from the plant and are borne away on air currents (Bergh and McCoy 1997). This behavior also occurs on excised pieces of citrus leaf as the tissue deteriorates (Omoto et al. 1994). Second, rust mites in Florida are regularly submerged in rain or dew and can withstand extended periods of immersion, remaining motionless until dried (J. C. B., personal observation).

Mites were harvested from 'Sunburst' mandarin leaves from seedlings maintained in a greenhouse at the Citrus Research and Education Center (CREC), Lake Alfred, FL. To examine the effect of temperature on the harvest of mites, heavily infested leaves were cut into 2 × 2 cm pieces, which were randomly assigned to 4 groups. Each piece of leaf was impaled on an insect pin and the pin was inserted into the side of a rubber stopper. Five stoppers with leaf pieces were placed in each of four translucent plastic boxes lined with wet paper towel. Each piece of leaf was positioned about 1.5 cm above the center of a filter paper disk (Whatman No. 50, 38 mm diam.) placed on the wet paper towel. The boxes were covered and placed in lighted environmental cabinets at 20, 25, 30, and 35°C. The filter paper disks were replaced at 2-h intervals from 09:00 to 17:00 hours, and the mites on each were counted using a dissecting microscope at 20×. The mean number of mites harvested on each of three days was compared among temperatures using PROC GLM of SAS (SAS Institute 1985) and the Tukey multiple range test at the 5% probability level.

To examine the effect of light on the harvest of mites, rubber stoppers with impaled leaf pieces were placed in uncovered boxes in chambers set at 30°C, with bright, overhead lights and continuous darkness. The filter paper disks were replaced at 2-h intervals from 08:00-16:00 hours and the mites were counted as described above. The *t*-test was used to compare the mean number of mites harvested in constant light and dark conditions on each of three days.

Five 2 × 2 cm pieces of peel were removed from areas on green 'Valencia' oranges heavily infested with mites, using a razor blade and flat-tipped forceps. The flavedo and albedo were separated from the edible portion of the fruit, taking care not to burst the oil glands. Each piece of peel was impaled on an insect pin and mites were harvested at 30°C, beginning at 11:30 hours on two days. The filter paper disks were replaced at 16:30 hours and at 08:30 and 16:30 hours the following day and the mites on each were counted.

The effect of cold storage on the survival of mites was measured, using mites harvested at 30°C from 5 leaf pieces between 09:00 and 13:00 hours. At 13:00 hours, each filter paper disk was placed in a 35 × 10 mm Petri dish, and a few drops of water were applied to the perimeter of each disk. A strip of Parafilm was used to seal the lid of each dish to the bottom half containing the paper disk. Twenty-five mites were also manually transferred to each of five filter paper disks, which were sealed in Petri dishes as described above. The dishes were held in a refrigerator at 6°C for 72 h, after which the disks were dried and the number of live and dead mites on each disk was recorded. The percentage of mites alive after cold storage was compared between those manually transferred to disks and harvested from leaf pieces, using the *t*-test with arcsine transformed percentages.

To determine if mites survive interstate shipment, 25-50 adult mites were transferred from fruit to six filter paper disks at the Citrus Center, Texas A&M University, Weslaco, TX. The disks were placed on two 50 mm diam circles of wet paper towel in 50 × 15 mm Petri dishes, and the dishes were sealed with Parafilm. The Petri dishes were placed in a covered Styrofoam box (30 × 20 × 18.5 cm) with Styrofoam packing and 3 ice packs. The Styrofoam box was placed in a cardboard box and surrounded by Styrofoam packing. Mites were also transferred to three other filter paper disks, which were sealed in Petri dishes with wet paper and held in a refrigerator (6°C) at the Citrus Center.

To prepare for the receipt of mites from Texas, five green 'Valencia' oranges were washed in distilled water, dried, and dipped in liquid paraffin wax, leaving an unwaxed area of about 1/3 of the surface. Approximately 24 h elapsed between when the mites were collected in Texas and delivered by courier service to the CREC in Florida. The filter paper disks from Texas were dried and the mites on each were transferred to the unwaxed area of a fruit. The fruit were placed in a covered plastic box lined with wet paper towel and held in an environmental cabinet set at 27°C and a photoperiod of 14:10 L:D. After 24 h, fruit were examined for surviving mites and eggs, and the development of mite populations was noted after 7 d.

Mites from one disk were transferred to a young (4 leaf stage) 'Sunburst' mandarin seedling, which was held in a Plexiglas cage in a naturally lighted greenhouse at 27°C. The seedling was checked after 7 d for mite population development. Upon delivery of the mites in Florida, the survival of mites that had been held in cold storage in Texas was assessed.

There was a significant effect of temperature on the number of mites harvested over 8 h (Table 1). Numerically, more mites were harvested from leaf pieces at 30°C than at other temperatures. Only 1 mite was harvested from leaf pieces held at 20°C. At 30 and 35°C, the majority of mites were harvested during the first 4 h (Table 1), whereas <50% of mites were harvested from leaf pieces held at 25°C during the same period.

At the end of the 8-h collection period there were many healthy mites feeding on the leaf pieces held at 20°C and the leaf tissue appeared fresh and succulent. At 25°C, there were many mites walking on the leaf pieces, which had slightly curled edges but did not appear overly desiccated. At 30°C, there were dead mites and a few live mites on the plant tissue, and the leaf pieces were dry and curled. At 35°C, no live mites and many dead mites were observed on leaf pieces, which were dry, curled, and crinkled.

TABLE 1. THE EFFECT OF TEMPERATURE ON THE HARVEST OF CITRUS RUST MITES FROM PIECES OF 'SUNBURST' MANDARIN LEAVES OVER EIGHT HOURS.

Date	20	25	30	35	F (df = 3,16)	P
30 March						
Mean \pm SD no. harvested from 0900-1700	0.0a	11.0 \pm 3.0a	43.0 \pm 14.3b	15.2 \pm 3.0ab	6.05	<0.01
Mean cumulative percentage \pm SD harvested after 4 h	NA	24.4 \pm 23.2	76.2 \pm 16.4	90.6 \pm 3.1		
31 March						
Mean \pm SD no. harvested from 0900-1700	0.2 \pm 0.2a	16.6 \pm 5.5a	53.4 \pm 12.9b	23.2 \pm 6.5ab	8.31	<0.01
Mean cumulative percentage \pm SD harvested after 4 h	NA	45.0 \pm 17.4	93.3 \pm 4.8	96.4 \pm 4.1		
2 April						
Mean \pm SD no. harvested from 0900-1700	0.0a	15.8 \pm 3.6a	52.8 \pm 14.4b	23.8 \pm 0.7ab	8.88	0.001
Mean cumulative percentage \pm SD harvested after 4 h	NA	29.5 \pm 17.0	82.3 \pm 6.4	90.1 \pm 12.0		

Means based on five leaf pieces. Mean number of mites harvested, within rows, followed by the same letter are not significantly different by ANOVA and the Tukey test at the 5% probability level.

The mean numbers of mites harvested did not differ between light and dark conditions (Table 2). The majority of mites were harvested from leaf pieces in the light and dark during the first 4 h.

At 30°C, very few mites were harvested from pieces of orange peel during the first five hours (11:30-16:30 hours); mean \pm SD = 4.6 \pm 7.5 and 4.6 \pm 4.9 mites on 13 and 17 August, respectively. The vast majority of mites were collected between 16:30 and 08:30 hours the following day; mean \pm SD = 81.6 \pm 55.3 and 70.2 \pm 27.1 mites on 14 and 18 August, respectively.

Following storage at 6°C for 72 h, there was no difference in the survival (mean \pm SD percentage) of mites that had been manually transferred to paper disks (89.6 \pm 7.8%, n = 25 mites per disk) or harvested from leaf pieces (92.6 \pm 3.1%, n = 29-61 mites per disk) ($t_{0.05, (21, 8)} = 0.743, P > 0.20$).

The interstate shipping of mites had no adverse effect on their survival; 83.5 \pm 5.4% SD and 72.3 \pm 3.3% SD of mites survived shipping to Florida from Texas and cold storage in Texas, respectively. Mites shipped to Florida oviposited on fruit during the first 24 h, and all life stages were present on fruit after 7 d. A building population of mites was also evident on the young seedling to which imported mites were transferred.

This method for harvesting and shipping live citrus rust mites off of host tissue should enable work with populations from different geographical locations at one laboratory, as is often done in resistance monitoring and other research on other arthropods (Campos et al. 1996).

We thank J. Villarreal and M. Jackson for collecting and preparing mites for shipment from Texas to Florida. Florida Agricultural Experiment Station Journal Series No. R-06595.

TABLE 2. THE EFFECT OF EXPOSURE TO LIGHT ON THE HARVEST OF CITRUS RUST MITES FROM PIECES OF 'SUNBURST' MANDARIN LEAVES OVER EIGHT HOURS.

Date	Light	Dark	t	P
8 April				
Mean ¹ \pm SD no. harvested from 0800-1600	61.2 \pm 60.3	48.4 \pm 17.0	0.457	>0.50
Mean cumulative percentage \pm SD harvested after 4 h	83.4 \pm 7.2	80.8 \pm 5.7		
9 April				
Mean \pm SD no. harvested from 0800-1600	25.6 \pm 10.2	36.0 \pm 11.9	-1.479	>0.10
Mean cumulative percentage \pm SD harvested after 4 h	79.1 \pm 18.7	72.3 \pm 19.3		
13 April				
Mean \pm SD no. harvested from 0800-1600	29.2 \pm 14.7	41.4 \pm 14.4	-1.418	>0.10
Mean cumulative percentage \pm SD harvested after 4 h	68.2 \pm 13.7	51.4 \pm 18.5		

¹ Means based on five leaf pieces.

