Physical Post-Harvest Techniques as Potential Quarantine Treatments Against Brevipalpus yothersi (Acarina: Tenuipalpidae)

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Physical post-harvest techniques as potential quarantine treatments against *Brevipalpus yothersi* (Acarina: Tenuipalpidae)

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Abstract

Brevipalpus mites (Acari: Tenuipalpidae) carrying citrus leprosis virus are considered serious quarantine pests. The objective of this research was to clarify the effectiveness of commonly used fruit cleaners, soaps, waxes, and mechanical brushing techniques (alone and in combination) on removal and/or mortality of mites (percentage of density reduction) from infested citrus fruits. Six bioassays were conducted with infested lemons, *Citrus limon* (L.) Burm.f. (Sapindales: Rutaceae), using non-virulent *Brevipalpus yothersi* Baker as a model species. In each bioassay, all stages (eggs, nymphs, and adults) of *B. yothersi* were recorded before and after treatment. Results indicated that none of the treatments provided 100% reduction of all stages of mites, as would be required for quarantine treatments. In general, mite reduction following single treatments (soap rinse, brushing, or waxing alone) was not significantly different from reduction obtained with a water drench control. However, several combination treatments were successful in achieving ~90% reduction of mites, particularly those that included application of a food-grade wax coating. Therefore, a combination of treatments, including a soap wash and mechanical brushing followed by a wax coating, may be the most effective method to achieve significant reduction of all stages of *Brevipalpus* mites from infested citrus.

Key Words: citrus leprosis virus; CiLV; mite reduction; infested citrus

Resumen

Especímenes de *Brevipalpus yothersi* (Acari: Tenuipalpidae) que sean vectores de la leprosis de los cítricos se consideran plagas cuarentenarias. El objetivo de esta investigación fue el de clarificar la efectividad de substancias utilizadas para limpiar la fruta como jabones, ceras y técnicas como el cepillado mecánico de los frutos (utilizadas singularmente o en combinación) sobre la remoción o mortalidad causada en los ácaros infestando frutos de cítricos. Se hicieron seis bioensayos probando los tratamientos mencionados antes en limón *Citrus limon* (L.) Burm. f. (Sapindales: Rutaceae), usando, *Brevipalpus yothersi* Baker no-virulento como especie modelo. En cada bioensayo todos los estados (huevos, estados inmaduros y adultos) de *B. yothersi* infestando limones, fueron evaluados antes y después de tratamiento. Los resultados indicaron que ninguno de los tratamientos causaba el 100% de reducción de todos los estados de los ácaros, lo cual es requerido en tratamientos de cuarentena. En general, la reducción de ácaros después de un solo tratamiento (jabones, limpiadores, cepillado o encerado solo) no fue significativamente diferente del testigo representado por la inmersión de los limones en agua. Sin embargo, la combinación de varios tratamientos resultó en ~90% de reducción de ácaros, particularmente cuando estos incluían la aplicación de ceras utilizadas para recubrir la fruta. En consecuencia, la combinación de tratamientos incluyendo una solución jabonosa, cepillado mecánico, seguido por en encerado de la fruta, podría ser el tratamiento más efectivo para alcanzar la reducción significativa de todos los estados de ácaros *Brevipalpus* cuando estos están infestando cítricos.

Palabras Clave: virus de la leprosis de los cítricos; reducción de ácaros; frutos de cítricos infestados

The *Brevipalpus* (Acari: Tenuipalpidae) flat mites are highly polyphagous mites with a broad range of hosts, including citrus, grapes, and many ornamental plants (Childers & Rodrigues 2005). Their role as vectors of citrus leprosis virus, CiLV (Bastianel et al. 2006), has greatly increased their worldwide importance as quarantine pests. Symptoms of citrus leprosis include chlorotic lesions at the mite feeding sites on leaves, twigs, and fruit. In the absence of vector control, the local lesions can coalesce, girdle, and kill leaves and twigs, resulting in severe losses of production. CiLV is widespread in the Caribbean and Central and South America; in North America, it has been detected in Mexico (SINAVEF 2012) but not the United States (Childers et al. 2003). Because Mexico is a major supplier of fresh limes (Spreen 2000), including Persian lime *Citrus latifolia* (Tanaka ex Yu. Tanaka) Tanaka and key lime *Citrus aurantifolia* (Cristm.) Swingle (Sapindales: Rutaceae), there is concern that CiLV may enter the United States. Therefore, the United States Department of Agriculture (USDA) National Plant Disease Recovery System has identified citrus leprosis as a critical threat to U.S. agricultural production, particularly sweet orange *Citrus sinensis* (L.) Osbeck (Sapindales: Rutaceae), and has prepared a recovery plan for this high-priority disease (Hartung et al. 2013).

The standard post-harvest procedures for handling commercial citrus fruits include washing and waxing (Porat et al. 2000; Bosquez-Molina et al. 2004), but there is a current effort to find alternative treatments (e.g., environmentally friendly soaps) that can be used effectively against quarantine mites. Several of these treatments have been considered effective in post-harvest situations (Vincent et al. 2003). For instance, coatings with Primafresh 31® (Agro Pro Central

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America), Sta-Fresh 360HS® and Sta-Fresh 600® (Bornnet Corporation), and NatureSeal® (Mantrose-Haeuser, Co.), have been shown to cause approximately 90% mortality of Caribbean fruit fly *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) larvae infesting grapefruits *Citrus paradisi* Mcfad. (Sapindales: Rutaceae) (Hallman et al. 1994). Gould & McGuire (2000) found that coating Persian limes with petroleum-based oils (AMPOL®, Caltex Australia, Sydney, New South Wales; and Sunspray Ultra-Fine Spray Oil®, Sunoco, Philadelphia, Pennsylvania, USA), a natural vegetable oil (Natural Organic oil, Custom Chemicides, Fresno, California, USA), and a soap (Mpede) achieved up to 94% mortality of 2 mealybugs, *Planococcus citri* (Risso) and *Pseudococcus odermatti* Miller & Williams (Hemiptera: Pseudococcidae). This result was considered effective as a post-harvest dip treatment but insufficient to provide quarantine security. Specific data are lacking regarding the effects of these procedures on citrus fruit infested with *Brevipalpus* mites as mitigating measures to reduce the risk of introduction. Therefore, the objective of this research was to evaluate the efficacy of commonly used fruit cleaners, soaps, waxes, and mechanical brushing techniques for removal and mortality of each life stage of *Brevipalpus* mites on citrus, using non-virulent *B. yothersi* Baker as a model.

Materials and Methods

MITE STOCK COLONY

Maintenance of *B. yothersi* populations in the laboratory followed the method of Campos & Omoto (2002) used for rearing of *Brevipalpus phoenicis* (Geijskes) on store-bought lemons *C. limon* (L.) Burm.f. ('Meyer') (Sapindales: Rutaceae). Fruits were washed with distilled water, and after drying, the stylar area was dipped in heated wax, leaving an area of approximately 79 cm^2 to confine the mites. Then, 20 to 30 adult mites, field-collected from infested leaves and branches of *Viburnum odoratissimum* Ker-Gawl. (Dipsacales: Adoxaceae), were transferred per fruit with the aid of a fine brush. Each mite population was kept at 20 to 25 individuals per fruit. The rearing room was maintained at 26 ± 2 °C and 75 to 80% RH, with a photoperiod of 12:12 h L:D. Fruits were renewed every 30 to 35 d.

BIOASSAYS

Products and compounds tested in bioassays were obtained either directly from the manufacturers or from retail stores (Table 1). Six bioassays were conducted at different times of the year, and each bioassay was designated with a number. All stages (eggs, nymphs, and adults) of *B. yothersi* infesting lemons were counted under a dissecting microscope before treatment. Fifteen lemons were dipped in a 5,400 mL aqueous solution containing each product and dose listed in Table 2. The solution was kept under constant manual agitation for 60 s, and lemons were removed afterwards. Water temperature was maintained at 22 °C and pH at 7.0 (verified each time before lemons were placed into a solution). A control treatment consisted of 15 lemons dipped for 60 s in water. To determine if any mites were dislodged into the water after treatment, each solution was divided into 15 parts, sieved individually through a P4 18.5 cm Fisher® filter paper, and mite density and life stages determined under a microscope. Additional treatments for a bioassay were either manual brushing using bottle brushes (25 cm^2) for 1 min or placing each lemon under the brushes of a commercial mite brushing machine (2836 SM, BioQuip Products, California, USA). Mites dislodged were collected in a Petri dish and counted afterwards.

Additionally, some treatments included application of Decco Wax® and Shellac® (Table 1) to the fruits using a paint brush. Each lemon was placed on a drying rack thereafter and held in a climatic chamber at 25 °C and approximately 75 to 80% RH. All *B. yothersi* stages remaining on the lemons were counted under a microscope at 1, 2, 3 and 5 d post-treatment to verify survival and reproduction. Adult and motile immature stages were considered to be dead after treatment if they did not walk when prodded with the tip of a fine brush. Because the effect of treatments on adults and nymphs included dislodging mites from the fruit as well as mite mortality, the data obtained for these 2 stages were expressed as the mean percentage of reduction of mites achieved with each treatment. The data obtained for eggs were expressed as the percentage of eggs dislodged after each treatment. For each bioassay and development stage, data were analyzed separately by 1-way analysis of variance (ANOVA) using a general linear model (SAS v. 9.3 SAS Institute, Inc., Cary, North Carolina, USA); significant ANOVAs were then followed by Fisher least significance difference (LSD) mean separation ($P = 0.05$). Percentage data were arcsine transformed before statistical analysis to correct for non-linearity of percentages.

Results

Application of most soap and foam solutions (Mold Strip, Saf Foam, Fit, Rebel Green, and Veggie Wash) alone, fruit brushing alone, and

Table. 1. List of products used in bioassays of post-harvest treatments against *Brevipalpus yothersi.*

Product	Active materials	Manufacturer
Mold Strip®	Alkaline-based cleaner	HDH Agri-Products Inc., Tavares, FL 32778
SafFoam®	Alkaline concentrated cleaner	HDH Agri-Products Inc., Tavares, FL 3277
Fit® Organic™ Fruit & Vegetable Wash	Water, organic ethanol, organic sunflower oil, organic glycerin, potassium hydroxide, organic grapefruit seed oil	HealthPro Brands Inc., Cincinnati, OH 45242
Rebel Green® Fruit & Veggie Wash	Plant oils, polysorbate-20, grapefruit seed extract, lemon–orange extract	Rebel Green LLC, Milwaukee, WI 53202
Environne®	Oils, polysorbate-20, grapefruit seed extract, lemon–orange extract	Consumer Health Res, Inc., Roseburg, OR 97470
Veggie Wash®	Corn, palm, coconut, citrus oils, sodium citrate, glycerin, grapefruit seed extract	Beaumont Products Inc., Kennesaw, GA 30144
Decco Wax®	Carnauba wax, emulsifier, food grade shellac, silicone, non-ionic antifoam	Decco, Monrovia, CA 91016
Shellac®	Emulsifier, food grade shellac	Shield Brite AP-404, Pace International, Seattle, WA 98101
Clorox®	6.15% sodium hypochlorite (1:10 dilution)	The Clorox Company, Oakland, CA 94612

Table 2. Density reduction (mean % ± SE) of *Brevipalpus yothersi* adults on fruits dipped in soap, foam, and wax solutions with additional fruit brushing.

			% Dislodged and killed			
Treatment	Dose	Mean density before treatment	1 DAT	2 DAT	3 DAT	5 DAT
Bioassay 1						
Mold Strip®	1:3,000	3.3 ± 1.0a	24 ± 9a	27 ± 11a	21 ± 10a	—
Mold Strip® + brushing	1:3,000	3.1 ± 0.6a	36 ± 12a	18 ± 9a	23 ± 8a	—
SafFoam®	1:3,000	0.9 ± 0.4b	14 ± 8a	5 ± 5a	10 ± 7a	—
SafFoam® + brushing	1:3,000	3.9 ± 0.9a	38 ± 12a	10 ± 7a	12 ± 7a	—
Water		3.6 ± 1.5a	20 ± 10a	13 ± 5a	28 ± 1a	—
Bioassay 2						
Mold Strip®	1:2,000	8.7 ± 1.9a	39 ± 10a	26 ± 9a	—	23 ± 10a
Mold Strip® + brushing	1:2,000	5.1 ± 1.1a	48 ± 11a	17 ± 7a	—	24 ± 8a
SafFoam®	1:2,000	8.0 ± 1.4a	36 ± 8a	21 ± 9a	—	28 ± 10a
SafFoam® + brushing	1:2,000	11.2 ± 1.9a	49 ± 8a	41 ± 10a	—	31 ± 11a
Water		13.3 ± 2.6a	43 ± 10a	21 ± 7a	—	15 ± 5a
Water + brushing		8.5 ± 2.0a	57 ± 10a	47 ± 12a	—	12 ± 8a
Bioassay 3						
Fit® Organic™	1:2,000	28.6 ± 8.4a	46 ± 9a	2 ± 2b	42 ± 8a	—
Fit® Organic™ + brushing	1:2,000	22.5 ± 4.8a	39 ± 9a	20 ± 6a	54 ± 9a	—
Water		17.4 ± 5.1a	11 ± 5a	7 ± 4b	20 ± 9a	—
Water + brushing		12.7 ± 2.4a	14 ± 5a	48 ± 1a	19 ± 8a	—
Bioassay 4						
Environne®	1:1,000	6.7 ± 2.6a	41 ± 10a	26 ± 9a	—	20 ± 10a
Environne® + brushing	1:1,000	4.2 ± 1.0a	59 ± 13a	0a	—	13 ± 9a
Rebel Green®	1:1,000	6.3 ± 3.6a	33 ± 11a	27 ± 10a	—	23 ± 11a
Rebel Green® + brushing	1:1,000	2.5 ± 0.7a	66 ± 12a	0a	—	7 ± 7a
Veggie Wash®	1:1,000	6.9 ± 2.1a	34 ± 8a	15 ± 8a	—	28 ± 8a
Veggie Wash® + brushing	1:1,000	3.8 ± 1.0a	75 ± 10a	0a	—	20 ± 11a
Water		3.0 ± 0.7a	33 ± 10a	16 ± 11a	—	27 ± 11a
Water + brushing		4.8 ± 2.4a	59 ± 12a	24 ± 11a	—	10 ± 7a
Bioassay 5						
Environne®	1:1,000	11.5 ± 3.2a	80 ± 7ab	22 ± 9a	9 ± 7a	26 ± 11a
Environne® + brushing	1:1,000	11.1 ± 1.9a	70 ± 8ab	31 ± 11a	27 ± 11a	17 ± 10a
Decco Wax®	1:1,000	15.5 ± 2.5a	87 ± 6ab	33 ± 13a	28 ± 11a	8 ± 7a
Shellac®	1:1,000	16.7 ± 2.7a	91 ± 6a	0a	0a	7 ± 7a
Environne® + brushing + Decco Wax®	1:1,000	14.8 ± 2.9a	90 ± 6a	27 ± 11a	13 ± 9a	0a
Enviroone® + brushing + Shellac®	1:1,000	13.1 ± 2.6a	88 ± 6a	0a	0a	0a
Water		16.1± 0.1a	48 ± 8b	15 ± 6a	28 ± 11a	28 ± 10a
Bioassay 6						
Clorox®	1:1,000	7.3 ± 1.0ab	77 ± 8ab	35 ± 12abc	9 ± 7a	—
Clorox® + brushing	1:1,000	8.9 ± 1.0a	66 ± 19abc	33 ± 11abc	24 ± 10a	—
Environne®	1:1,000	4.0 ± 0.5cd	37 ± 11cde	43 ± 11abc	11 ± 7a	—
Environne® + brushing	1:1,000	1.8 ± 0.5d	13 ± 6e	55 ± 13a	11 ± 5a	—
Clorox® + brushing + Decco Wax®	1:1,000	4.4 ± 0.5bcd	87 ± 9ab	0c	0a	—
Environne® + brushing + Shellac®	1:1,000	1.9 ± 0.5d	100 ± 0a	0c	0a	—
Shellac®	1:1,000	2.6 ± 0.5d	60 ± 13bcd	7 ± 7bc	0a	—
Water		6.1 ± 1.0abc	24 ± 10de	54 ± 12a	7 ± 6a	—

Numbers followed by the same letter in a column were not significantly different ($P > 0.05$). DAT, days after treatment; — evaluations were not carried out on these days.

mixed treatments with soaps followed by brushing did not result in significant reduction of adult mites 1 d after treatment (Table 2; Bioassays 1–4). However, significant reduction ($P = 0.0001$; $F = 5.96$; df = 29, 98) was obtained with 3 fruit treatments in Bioassay 5: application of Shellac alone (91% reduction), dipping in Environne soap solution followed by brushing and Decco Wax application (90% reduction), and dipping in Environne followed by brushing and Shellac coating (88% reduction). In Bioassay 6, there were significant differences among treatments ($P = 0.007$; $F = 1.09$; df = 20, 84), with moderate reductions obtained with Clorox alone (77%) and Clorox plus brushing (66%) and greater reductions obtained following treatments with Clorox plus brushing and Decco Wax (87%) and Environne plus brushing and Shellac (100%). Further reduction in adult densities recorded 2 to 5 d after treatment varied between 2 and 48%, but the results were not statistically significant for most treatments as compared with the untreated controls (water immersion) (Table 2).

For motile immature stages, applications of Saf Foam, Rebel Green, and Veggie Wash (alone or in combination with brushing) did not result in significant reduction of infestation 1 d after treatment (Table 3; Bioassays 1, 2, and 4). Mold Strip plus brushing was effective (79% reduction) at a dilution of 1:2,000 (Bioassay 2; $P = 0.0006$; $F = 2.94$; df = 19, 70), but not at a dilution of 1:3,000 (Bioassay 1). Other treatments effective at reducing immature stages included Fit soap plus brushing (91%) and brushing alone (74%) in Bioassay 3 ($P = 0.001$; $F = 16.82$; df = 12, 42), and Environne plus brushing followed by coating with Decco Wax (59%) or Shellac (60%) in Bioassay 5 ($P = 0.01$; $F = 3.45$; df = 20, 84). In Bioassay 6, moderate reductions were observed following treatments with Shellac alone (79%) and Clorox plus brushing plus wax coating (60%); however, these results were not statistically different from the water control treatment, which had a fairly large percentage of reduction (37 ± 12%). Subsequent mortality of the immature stages 2 to 5 d after treatment was negligible.

Examination of eggs 1 d after treatment indicated that Mold Strip (Table 4, Bioassays 1 and 2) and Fit soap (Bioassay 3) were not effective treatments for dislodging eggs, even when combined with manual brushing. However, percentage of egg reduction was significant for a variety of treatments tested in Bioassay 4 ($P = 0.001$; $F = 3.72$; df = 20, 84), Bioassay 5 ($P = 0.001$; $F = 3.89$; df = 20, 84), and Bioassay 6 ($P = 0.0001$; $F = 4.61$; df = 21, 98). The greatest reduction was obtained with Environne plus brushing and Shellac coating (91%, Bioassay 6), followed by Rebel Green plus brushing (89%, Bioassay 4) and Environne plus brushing and Decco Wax (89%, Bioassay 5).

Table 3. Mortality (mean % ± SE) of *Brevipalpus yothersi* immature stages on fruits dipped in soap, foam, and wax solutions with additional fruit brushing.

			% Mortality at			
Treatment	Dose	Mean density before treatment	1 DAT	2 DAT	3 DAT	5 DAT
Bioassay 1						
Mold Strip®	1:3,000	10.3 ± 3.4ab	33 ± 10a	29 ± 11a	12 ± 7a	—
Mold Strip® + brushing	1:3,000	15.0 ± 3.8a	32 ± 11a	19 ± 8a	8 ± 4a	—
SafFoam®	1:3,000	3.3 ± 0.9b	46 ± 12a	33 ± 11a	23 ± 10a	—
SafFoam® + brushing	1:3,000	16.6 ± 4.9a	44 ± 11a	16 ± 7a	19 ± 9a	—
Water		5.8 ± 1.4b	56 ± 11a	18 ± 6a	38 ± 10a	—
Bioassay 2						
Mold Strip®	1:2,000	35.3 ± 9.6a	38 ± 10bc	23 ± 9a	—	36 ± 10a
Mold Strip® + brushing	1:2,000	32.1 ± 13.0a	79 ± 8a	11 ± 7a	—	7 ± 7a
SafFoam®	1:2,000	8.3 ± 2.3a	16 ± 8c	17 ± 7a	—	3 ± 3a
SafFoam® + brushing	1:2,000	13.6 ± 8.0a	47 ± 10abc	16 ± 7a	—	7 ± 5a
Water		8.8 ± 5.2a	21 ± 9c	11 ± 7a	—	9 ± 5a
Water + brushing		10.8 ± 3.2a	72 ± 8abc	16 ± 9a	—	8 ± 6a
Bioassay 3						
Fit® Organic™	1:2,000	37.8 ± 6.6a	7 ± 3b	41 ± 10a	4 ± 3a	—
Fit® Organic™ + brushing	1:2,000	32.6 ± 6.7a	91 ± 3a	1 ± 1b	38 ± 10a	—
Water		53.4 ± 8.3a	10 ± 5b	2 ± 1b	29 ± 9a	—
Water + brushing		55.4 ± 12.4a	74 ± 7a	25 ± 9a	7 ± 5a	—
Bioassay 4						
Environne®	1:1,000	9.0 ± 3.0a	14 ± 11a	11 ± 6a	—	30 ± 10a
Environne® + brushing	1:1,000	10.0 ± 3.0a	56 ± 12a	13 ± 7a	—	15 ± 9a
Rebel Green®	1:1,000	7.0 ± 2.0a	33 ± 9a	12 ± 4a	—	29 ± 10a
Rebel Green® + brushing	1:1,000	3.3 ± 1.6a	52 ± 13a	6 ± 6a	—	6 ± 6a
Veggie Wash®	1:1,000	7.3 ± 3.0a	29 ± 9a	12 ± 7a	—	26 ± 9a
Veggie Wash® + brushing	1:1,000	9.6 ± 3.3a	52 ± 12a	4 ± 4a	—	20 ± 10a
Water		8.6 ± 2.5a	33 ± 9a	11 ± 7a	—	39 ± 10a
Water + brushing		3.8 ± 1.0a	37 ± 7a	7 ± 3a	—	29 ± 7a
Bioassay 5						
Environne®	1:1,000	14.5 ± 3.2a	7 ± 3c	3 ± 3a	3 ± 3a	7 ± 7a
Environne® + brushing	1:1,000	11.1 ± 2.0a	0c	7 ± 7a	27 ± 12a	10 ± 7a
Decco Wax®	1:1,000	15.5 ± 2.5a	14 ± 10b	7 ± 7a	0a	0a
Shellac®	1:1,000	16.7 ± 2.7a	0c	0a	0a	10 ± 0a
Environne® + brushing + Decco Wax®	1:1,000	14.8 ± 2.9a	59 ± 13a	0a	0a	0a
Enviroone® + brushing + Shellac®	1:1,000	13.1 ± 2.6a	60 ± 13a	13 ± 9a	0a	0a
Water		16.6 ± 3.3a	4 ± 4c	17 ± 9a	5 ± 5a	12 ± 8a
Bioassay 6						
Clorox®	1:1,000	1.5 ± 1.0b	0 ± 0d	31 ± 10a	8 ± 7ab	—
Clorox® + brushing	1:1,000	0.1 ± 0.1b	0d	21 ± 9ab	35 ± 11ab	—
Environne®	1:1,000	12.1 ± 2.0a	43 ± 8bcd	11 ± 4b	26 ± 8ab	—
Environne® + brushing	1:1,000	13.2 ± 2.0a	52 ± 12ab	2 ± 2b	17 ± 8ab	—
Clorox® + brushing + Decco Wax®	1:1,000	1.5 ± 1.0b	60 ± 13ab	0b	0 ± 0b	—
Environne® + brushing + Shellac®	1:1,000	0.3 ± 0.1b	6 ± 6d	0b	0b	—
Shellac®	1:1,000	7.0 ± 1.0ab	79 ± 20ab	0b	0b	—
Water		9.2 ± 1.0a	37 ± 12bcd	9 ± 4b	9 ± 6 ab	—

Numbers followed by the same letter in a column were not significantly different ($P > 0.05$). DAT, days after treatment; — data were not recorded at this time.

Table 4. Number of eggs dislodged after treatment of mite-infested lemons with soaps, waxes, and brushing

Treatment	Dose	Mean density before treatment	% Eggs dislodged 1 DAT
Bioassay 1			
Mold Strip®	1:3,000	7.7 ± 1.9a	23 ± 9a
Mold Strip® + brushing	1:3,000	7.1 ± 1.9a	21 ± 8a
SafFoam®	1:3,000	6.5 ± 1.8a	43 ± 11a
SafFoam® + brushing	1:3,000	5.7 ± 1.5a	37 ± 11a
Water		4.4 ± 1.3a	51 ± 9a
Bioassay 2			
Mold Strip®	1:2,000	24.7 ± 5.2a	22 ± 8ab
Mold Strip® + brushing	1:2,000	20.3 ± 4.1a	39 ± 9ab
SafFoam®	1:2,000	36.7 ± 7.4a	20 ± 6ab
SafFoam® + brushing	1:2,000	34.3 ± 7.3a	34 ± 7a
Water		28.9 ± 7.6a	18 ± 7b
Water + brushing		24.1 ± 4.8a	50 ± 9a
Bioassay 3			
Fit® Organic™	1:2,000	29.6 ± 7.0a	42 ± 11a
Fit® Organic™ + brushing	1:2,000	35.0 ± 8.8a	35 ± 7a
Water		40.6 ± 9.2a	18 ± 8a
Water + brushing		26.8 ± 7.5a	25 ± 8a
Bioassay 4			
Environne®	1:1,000	13.2 ± 2.4a	31 ± 9bc
Environne® + brushing	1:1,000	12.0 ± 2.3a	65 ± 11ab
Rebel Green®	1:1,000	12.5 ± 2.8a	20 ± 7c
Rebel Green® + brushing	1:1,000	11.1 ± 1.9a	89 ± 6a
Veggie Wash®	1:1,000	14.4 ± 3.6a	14 ± 7c
Veggie Wash® + brushing	1:1,000	14.5 ± 3.3a	59 ± 10ab
Water		12.6 ± 3.4a	8 ± 4c
Water + brushing		10.5 ± 3.0a	44 ± 8bc
Bioassay 5			
Environne®	1:1,000	21.1 ± 3.9c	24 ± 8d
Environne® + brushing	1:1,000	21.3 ± 6.7c	31 ± 10cd
Decco Wax®	1:1,000	39.7 ± 9.3ab	84 ± 5a
Shellac®	1:1,000	47.3 ± 5.7a	62 ± 4abc
Environne® + brushing + Decco Wax®	1:1,000	44.0 ± 9.2a	89 ± 5a
Enviroone® + brushing + Shellac®	1:1,000	38.3 ± 6.3abc	64 ± 8ab
Water		25.7 ± 6.1bc	37 ± 8bcd
Bioassay 6			
Clorox®	1:1,000	21.4 ± 7.7a	11 ± 7b
Clorox® + brushing	1:1,000	14.3 ± 3.1abc	28 ± 9b
Environne®	1:1,000	1.4 ± 0.5d	27 ± 11b
Environne® + brushing	1:1,000	10.4 ± 1.8bcd	26 ± 9b
Clorox® + brushing + Decco Wax®	1:1,000	13.7 ± 2.7abc	80 ± 9a
Environne® + brushing + Shellac®	1:1,000	18.6 ± 3.6ab	91 ± 4a
Shellac®	1:1,000	6.0 ± 1.4cd	86 ± 9a
Water		8.2 ± 2.3cd	29 ± 12b

*Numbers followed by the same letter in a column were not significantly different ($P > 0.05$). DAT, days after treatment.

Discussion

Fruit washing, waxing, and brushing are currently among the treatments used for citrus imported from Mexico. The objectives of this study were to determine whether fruit washing combined with brushing and use of soaps could reduce numbers of *B. yothersi* from citrus fruits. In our study, a combination of treatments that involved using the soap Environne (1:1,000 dilution) followed by brushing and coating with Shellac wax caused 88 to 100% reduction in adult density. The general trend observed was that mechanical brushing alone caused 40 to 50% reduction, but the addition of a wax coating improved efficacy up to 60 to 91% reduction.

One day after treatment, the percentage of density reduction obtained for adults ranged between 28 and 85%, whereas the ranges for immature stages and eggs were between 23 and 50% and between 28 and 59%, respectively. The use of soaps and brushing, or soaps by themselves, reduced adult mite densities by only 14 to 46%, as compared with the water control. The concentrations of soaps and foams (1:1,000 dilution) used in this study were actually higher than those typically used in packing houses (1:3,000 dilution), underscoring the poor level of mite control provided by standard fruit washing methods.

Another objective of this study was to determine the efficacy of brushing in mite removal. The results obtained in the different bioassays using only water drenches followed by brushing showed that brushing improved mite removal by only 3 to 27% over the water drench alone. The study also investigated the efficacy of waxing in mite reduction. In general, mite densities were low after waxing was performed in all the bioassays. One outcome of this study was that a combination of different treatments, i.e., soaps followed by brushing and then waxing, resulted in fewer mites left on fruits compared with the water drench, which was considered the control. Consequently, a combination of treatments may be most effective to dislodge or cause a significant reduction of all developmental stages.

In a time of increased awareness among consumers that many of the chemical treatments of fruit and vegetables used to control insects, diseases, and physiological disorders are potentially harmful to humans, there is an urgent need to develop effective, non-damaging physical treatments for insect disinfection and disease control in fresh horticultural products (Fallik 2003). The treatments tested here did not provide 100% reduction of all stages of mites as is required for quarantine treatments; therefore, additional treatments should be evaluated, such as radiation (Hallman 1998), controlled atmosphere treatments (Shijum & Mitcham 1998), and fumigant activity of essential oils (Lim et al. 2011) and ethyl formate (Simpson et al. 2007). These treatments have been shown to provide effective control of tetranychid mites and should be evaluated for efficacy on *Brevipalpus* mites.

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