Effectiveness of Hot-Water Immersion Against Brevipalpus yothersi (Acari: Tenuipalpidae) as a Postharvest Treatment for Lemons

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Abstract

Citrus leprosis is a destructive disease of citrus caused by several viruses (CiLVs) that are quarantine pests in the United States. Brevipalpus yothersi Baker (Acari: Tenuipalpidae) vectors the most virulent strain of CiLV. This mite is present in the United States and could facilitate the spread of the disease if CiLV reaches the country. Postharvest treatments could mitigate B. yothersi on imported commodities from areas where CiLV exists. The current study explores the effectiveness of hot-water immersion as a postharvest treatment against B. yothersi. Lemons were immersed in water at 21, 48, 53, or 63°C for 5, 10, and 15 min. Immersions at 53 and 63°C for all time schedules dislodged over 99% of adult mites. Lemon fruit quality and B. yothersi egg viability after hot-water immersion were also evaluated. Fruit quality significantly decreased in lemons treated at 63°C resulting in decay (grade 3, rejection), while at 53°C there was a quality reduction (grade 2, minimum acceptable market level) compared to lemons immersed at 21°C or nontreated controls (grade 1). None of the eggs hatched when the lemons were immersed in water at 63°C and an average of 1.5% hatched at 53°C for all time schedules. Immersion in water at 53°C for 5 min dislodged 99.71% and 57.14% of adult and immature mites, respectively, and resulted in 98.11% unhatched eggs without significant fruit quality reduction. Hot-water immersion could be a key component in a systems approach to control B. yothersi on imported citrus fruits from countries where citrus leprosis is present.

Key words: citrus leprosis, flat mites, biosecurity, invasive pests, systems approach
In the United States, CiLV-C, CiLV-C2, and CiLV-N are quarantine pests. *Brevipalpus yothersi* and *B. californicus* are naturally present in the United States (Beard et al. 2015) but are still considered pests of concern in areas without established populations. The rapid spread of citrus leprosis throughout all citrus-growing areas in Mexico (Salinas-Vargas et al. 2016) increased concern for this disease reaching the United States because Mexico is a major supplier of fresh citrus. Imports include sweet (*Citrus sinensis* (L.) Sapindales: Rutaceae) and sour oranges (*C. aurantiun* L.), grapefruit (*C. × paradisi* Macf.), clementine (*C. × clementina* Blanco), lemons (*C. limon* (L.)), Persian lime (*C. latifolia* Tan), and key limes (*C. aurantifolia* (Christm.)) (Baldwin and Jones 2012). Although fruit is not considered to be a main pathway for introduction of the disease, the viruliferous *Brevipalpus* mites can follow the fruit pathway. Therefore, postharvest treatments on imported citrus fruit from countries where citrus leprosis is present can potentially control viruliferous vectors.

Individual or combined treatments that provide quarantine security to control pests include pesticide application after harvest, fumigation, hot-water immersion, vapor heat, coatings, irradiation, etc. (Sharp and Heather 2002). Hot-water immersion (Hara et al. 1994, 1997) has been tested and recommended against several insect pests including mealybugs (Gould and McGuire 2000) and fruit flies (Hallman and Sharp 1990, Gould and Sharp 1992). Gould and McGuire (2000) determined that hot-water immersions at 49°C for 20 min is effective to treat Persian limes infested with *Planococcus citri* Riso (Homoptera: Pseudococcidae) and *Pseudococcus ochomerri* Miller & Williams (Homoptera: Pseudococcidae). When star fruit (*Averrhoa carambola* L., Oxalidales: Oxalidaceae) were immersed in hot water at 46-46.4°C for 45 min, 99.9968% of the Caribbean fruit fly larvae (*Anastrepha suspensa* (Loew), Diptera: Tephritidae) were killed (Hallman and Sharp 1990). Guavas (*Psidium guajava* L., Myrtales: Myrtaceae) were disinfested of Caribbean fruit fly larvae after being submerged in hot water at 46.1°C for 35 min and showed only slight fruit quality reduction (Gould and Sharp 1992). Hot-water immersion has also been tested in combination with cold treatment to disinfect Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann), Diptera: Tephritidae), melon fly (*Bactrocera cucurbitae* (Coquillet), Diptera: Tephritidae), and oriental fruit fly (*B. dorsalis* (Hendel)) by fly from papayas (*Carica papaya* L., Oxalidales: Oxalidaceae) were immersed in hot water at 46–46.4°C for 45 min, 99.9968% of the Caribbean fruit fly larvae (*Anastrepha suspensa* (Loew), Diptera: Tephritidae) were killed (Hallman and Sharp 1990). Guavas (*Psidium guajava* L., Myrtales: Myrtaceae) were disinfested of Caribbean fruit fly larvae after being submerged in hot water at 46.1°C for 35 min and showed only slight fruit quality reduction (Gould and Sharp 1992).

Materials and Methods

Mite Stock Colony

*Brevipalpus yothersi* mites were collected from viburnum hedges (*Viburnum odoratissimum* Ker Gawl, Dispacales: Adoxaceae) and Persian lime trees (*C. latifolia*) at the Tropical Research and Education Center, University of Florida. The colony of *B. yothersi* was maintained on store-bought lemons *C. limon* (‘Meyer’). Lemons were washed to remove fruit coatings and residues of pesticides, subsequently infested with 100 adult females, and kept inside plant growth chambers (Panasonic MLR-352H-PA, PHC Corporation of North America, Wood Dale, IL) at 26 ± 2°C and 75–80% RH, with a photoperiod of 12:12 (L:D). Fruit were replaced every 30–35 d.

Effect of Hot-Water Immersion on Adults and Immature Stages of *B. yothersi* on Lemons

A 34.5-liter stainless steel circulating water bath (Thermo Scientific Precision, Fisher Scientific, Pittsburgh, PA) containing 20 liters of distilled water was used. In the first set of experiments we evaluated the efficacy of hot-water treatment against the adult and immature stages of *B. yothersi*. Lemons were washed with distilled water, and after drying, the styal area was dipped in melted paraffin wax (Gulf wax, Gulf oil corporation, Houston, TX), leaving an area of approximately 79 cm² to confine the mites. Then 20–30 adult females were transferred to each lemon and allowed to establish colonies for 30–35 d. Prior to the start of the experiment, adult and immature mites on each lemon were counted under a dissecting microscope immediately before treatment. On average, each lemon had 8 ± 0.47 (mean ± SE) adults and 6 ± 0.55 immatures prior to treatment application. Subsequently, groups of 15 lemons were placed in a wire canister (29 × 67 × 20 cm) and immersed in water at 48, 53, or 63°C for 5, 10, and 15 min. To ensure correct temperature of each treatment, the water temperature was measured at five different locations of the water bath (four corners and the middle) using a thermometer. All combinations of water temperature and immersion time were tested. An additional container with 20 liters of distilled water was kept at 21°C (room temperature) as the control temperature. The immersion temperatures and times were selected based on previous studies with *Citrus* spp. and treatments approved in the USDA treatment manual (Gould and McGuire 2000, Erkan et al. 2005, USDA 2018a). After the treatment, the lemons were allowed to dry for 1 h and all stages of *B. yothersi* remaining on them were counted under a microscope. The experiment was repeated three times (blocks). Each time, only one temperature and a control were tested. Data were analyzed using a generalized linear mixed-effects model (GLMM) with binomial distribution. The proportion of dislodged adults and dislodged immatures were the response variables, while temperature, time of immersion, and their interaction were the fixed factors. The block was a random factor with temperature nested in it. Contrasts among treatments were assessed with the estimated marginal means method of the package ‘emmeans’ of R with a Tukey adjustment of the probabilities (Lenth et al. 2019).
All the analyses were done in R version 3.5.1 (R Development Core Team 2018).

Effect of Hot-Water Immersion on Lemon Quality and the Viability of B. yothersi Eggs

In the second set of experiments, fruit quality and egg viability were evaluated separately. Based on the previous experiments, the 48°C treatment was excluded because it failed to dislodge the adult and immature mites. The experiments were conducted using 4-old harvested lemons (‘Lisbon’) from Santa Paula, CA, that were not treated with fungicides or fruit coating (wax). Fruit quality was assessed by an FDACS/USDA-licensed inspector using the United States Standards for Grades of Lemons (USDA 1999). Lemons graded as a one or two are acceptable by the market, while those graded as a three are rejected. The lemons were randomly assigned in 10 groups of seven. Each group had a different water temperature (21, 53, or 63°C) and a different immersion time (5, 10, or 15 min) assigned. All combinations of water temperature and immersion time were tested including a negative control where the lemons were not immersed into water. After the treatment, the fruit were allowed to dry and subsequently immersed in Carnauba wax (Carnauba Premium, Decco US Post-Harvest, Inc., Monrovia, CA) with Imazalil at 2,000 ppm (Decoziol EC-289, Decco US Post-Harvest, Inc., Monrovia, CA) for 1 s, and allowed to dry again, a common postharvest practice for citrus that prevents water loss and fungal disease development (Altieri et al. 2013). The lemons were placed at the same time in a plant growth chamber at 13°C, with no light and 90% RH. The lemons were graded before the hot-water treatment, and 24 h, 5 d, and 30 d after the treatment. After the last grading, a Brix test was conducted to evaluate whether the sugar content of the lemons was affected by the treatments. This experiment was repeated twice (two blocks) and each time all combinations of temperature and immersion time were tested. The effect of hot-water immersion on fruit quality was analyzed for every assessment (prior to treatment, 24 h, 5 d, and 30 d posttreatment) using linear mixed-effects model (LMM). Grade was the response variable, and temperature, immersion time, and their interaction were the fixed factors, while block was the random factor. The effect of the hot-water immersion on the sugar content of the lemons was evaluated using a LMM with the Brix degrees at the end of the experiment (i.e., 30 d posttreatment) as the response variable, and temperature, immersion time, and their interaction as fixed factors, and block as the random factor. Contrasts among treatments were assessed with the estimated marginal means method of the package ‘emmeans’ with a Tukey adjustment of the probabilities (Lenth et al. 2019). All the analyses were done in R version 3.5.1 (R Development Core Team 2018).

Mite egg viability was assessed using the same type and source of lemons as in the fruit quality experiment. The lemons were washed with distilled water and after drying, the stylar area was dipped in Carnauba wax (Carnauba Premium, Decco US Post-Harvest, Inc., Monrovia, CA), leaving an area of approximately 39 cm² (half of the first experiment) to confine the mites. The fruit were randomly assigned to 10 groups of five. Similar to the fruit quality assessment, each group had a different water temperature (21, 53, or 63°C) and a different immersion time (5, 10, or 15 min) assigned. All possible combinations of water temperature and immersion time were tested including a negative control where the lemons were not immersed into water. Thirty young (2–3 d old) adult B. yothersi females were transferred with a fine brush to the nonwaxed area of the lemons and left to oviposit for 48 h. After the oviposition period the adult mites were removed, and the eggs were counted. On average 46 ± 1.42 (mean ± SE) eggs were present on the lemons prior to the treatment application. Subsequently, the lemons were immersed into water at the different temperatures and immersion times. One hour after the hot-water treatment the eggs remaining on the treated lemons were counted. All lemons (treated and nontreated) were then stored in a plant growth chamber (26 ± 2°C, 75–80% RH, and 12:12 [L:D] h) for 10 d before recording the number of eggs that had not hatched and the emerged larva present. This experiment was repeated three times (blocks). The proportion of dislodged eggs and eggs that did not hatch 10 d after the treatment were calculated. The data were analyzed with a LMM model where temperature, time of immersion, and their interaction were used as fixed factors and block as a random factor. Nonsignificant interactions and factors were removed to find the minimum adequate model. Contrasts among treatments were assessed with the estimated marginal means method of the package ‘emmeans’ with a Tukey adjustment of the probabilities (Lenth et al. 2019). All the analyses were done in R version 3.5.1 (R Development Core Team 2018).

Results

Effect of Hot-Water Immersion Against Adults and Immature Stages of B. yothersi on Lemons

Significantly more adult mites were dislodged with increasing temperature (GLMM: L-ratio = 74.63; df = 3, 14; P << 0.001) and increasing immersion time (GLMM: L-ratio = 17.84; df = 3, 14; P = 0.0001) (Fig. 1) and there was no significant interaction between the two factors (GLMM: L-ratio = 8.23; df = 6, 14; P = 0.22). There was a significant interaction between temperature and immersion time in the proportion of immature stages dislodged from the lemons (GLMM: L-ratio = 16.63; df = 6, 14; P = 0.01) (Fig. 2). Immersion at 53 and 63°C for 5, 10, or 15 min consistently dislodged large proportions of immature B. yothersi (Fig. 2). However, the proportions of dislodged immatures at 21 and 48°C treatments at all time schedules were highly variable and significantly lower than that in the 63°C for 15-min treatment.

Effect of Hot-Water Immersion on Lemon Fruit Quality and the Viability of B. yothersi Eggs

Before the hot-water treatment, lemon grade was similar for all groups (Fig. 3a) assigned to each temperature (LMM: F = 1.23; df = 3, 13; P = 0.3) and immersion times (LMM: F = 0.15; df = 2, 13; P = 0.86). Twenty-four hours after the treatment, there was a significant interaction between temperature and immersion time on lemon grade (LMM: F = 5.47; df = 4, 13; P = 0.004) (Fig. 3b). There were no significant differences in the grade of nontreated lemons and those treated at 21 or 53°C, regardless of the immersion time (Fig. 3b). The fruit quality was significantly reduced when lemons were immersed at 63°C for 5 and 15 min, and slightly less reduced fruit quality was found for those immersed for 10 min at 63°C. Five days after the treatment there was a significant temperature effect on fruit quality; lemons treated at 63°C had significantly lower quality than all other treatments (LMM: F = 121.67; df = 3, 13; P << 0.001) (Fig. 3c). On the last evaluation date, 30 d after the treatment, there was a significant interaction between temperature and immersion time, on fruit quality. Lemons that were treated at 53°C for 5 min or at 21°C for all immersion times did not differ significantly in their grading compared to nontreated fruit (Fig. 3d). Fruit quality was significantly lower in lemons immersed at 63°C for 5, 10, or 15 min, followed by lemons treated at 53°C for 10
There was a significant interaction between temperature and immersion time on the fruit sugar content (LMM: $F = 7.71$; $df = 4, 13$; $P << 0.001$) (Fig. 4). In general, no differences were observed between treated and nontreated lemons. However, sugar content was significantly higher in lemons treated at 63°C for 15 min compared to lemons treated for 5 min at the same temperature.

Significantly more eggs were dislodged after the hot-water treatment at 53 and 63°C in comparison with those treated at 21°C or the nontreated (LMM: $F = 576.35$; $df = 3, 14$; $P << 0.001$) (Fig. 6). Immersion time had no significant effect on the proportion of unhatched eggs 10 d after the hot-water treatment (LMM: $F = 1.99$, $df = 2, 14$; $P = 0.14$).

**Discussion**

After comparing and reviewing all the results, the hot-water treatment at 53°C for 5 min appears to be the most suitable treatment to dislodge most of the mite stages without significant fruit quality reduction. This treatment dislodged 99.71% of the adult mites and 15 min (LMM: $F = 2.85$; $df = 4, 13$; $P = 0.03$) (Fig. 3d). There was a significant interaction between temperature and immersion time on the fruit sugar content (LMM: $F = 7.71$; $df = 4, 13$; $P << 0.001$) (Fig. 4). In general, no differences were observed between treated and nontreated lemons. However, sugar content was significantly higher in lemons treated at 63°C for 15 min compared to lemons treated for 5 min at the same temperature.

Significantly more eggs were dislodged after the hot-water treatment at 53 and 63°C in comparison with those treated at 21°C or the nontreated (LMM: $F = 15.4$; $df = 3, 14$; $P << 0.001$), regardless of the immersion time (LMM: $F = 0.51$; $df = 2, 14$; $P = 0.6$) (Fig. 5). Similarly, significantly more unhatched eggs were recorded when the lemons were immersed in water at 53 or 63°C in comparison with those treated at 21°C or the nontreated (LMM: $F = 576.35$; $df = 3, 14$; $P << 0.001$) (Fig. 6). Immersion time had no significant effect on the proportion of unhatched eggs 10 d after the hot-water treatment (LMM: $F = 1.99$, $df = 2, 14$; $P = 0.14$).

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57.14% of the immatures (Fig. 2), 98.39% of the eggs did not hatch (Fig. 6), and the average lemon grade 30 d after treatment did not differ from the untreated fruit (Fig. 3). Although there was not complete control of the mite population when the lemons were treated at 53°C for 5 min, combination of this treatment with other physical postharvest techniques could result in fruit free of mites. Peña et al. (2015) showed that soap wash in combination with mechanical brushing, following fruit wax application can result in significant reduction in mite populations on lemon fruit. In addition, there is a USDA-approved treatment against *B. chilensis* Baker (Acar: Tenuipalpidae) on limes consisting of soapy water immersion and wax coating (T102-b-1) (USDA 2018a).
Hot-water immersion is a simple, economical, and rapid treatment against insects and mites inhabiting commodities (Vincent et al. 2003). In the United States this treatment is approved for use against the internal feeding larvae of Mediterranean fruit fly and the oriental fruit fly on lychee (Litchi chinensis Sonn., Sapindales: Sapindaceae) and longan (Dimocarpus longan Lour, Sapindales: Sapindaceae). On mangoes (Mangifera indica L., Sapindales: Anacardiaceae) it is approved for treating internal feeding larvae of Mexican fruit fly (Anastrepha ludens (Loew), Diptera: Tephritidae) and on limes it is approved for treating external feeders such as mealybugs and the Chilean false red mite, B. chilensis (USDA 2018a). One drawback of this system, however, is fruit damage when immersed in hot water. For example, a 15–40% shelf life reduction can be expected when star fruit is treated at 46–46.4°C for 45 min (Hallman and Sharp 1990). Dragon fruit (Hylocereus undatus (Haworth), Caryophyllales: Cactaceae) submerged in hot water at 60°C for 60 min experienced significant weight loss in comparison with fruit that was treated at 35°C for the same period (Lum and Norazira 2011). Citrus fruits, however, seem to be positively affected by hot-water immersion. Oranges, lemons, and limes were found to benefit from hot-water immersion, either by controlling pest infestations (Gould and McGuire 2000, Smilanick et al. 2003) or by developing fewer chilling injuries postharvest (Erkan et al. 2005). In our study lemons that were immersed in hot water at 53°C for 5 min did not show a significant fruit quality reduction relative to untreated fruit 30 d posttreatment application (Fig. 3d). It remains unknown whether this treatment can affect the shelf life of the lemons.

The American citrus industry was estimated to be worth approximately $3 billion in 2018, with Florida and California being the major producers (USDA 2018b). A potential incursion of viruliferous B. yonbishi mites could be damaging for the citrus industry in the United States. With Mexico having citrus leprosis while also being
a major supplier of citrus to the United States, there is an increased concern of the potential arrival of the disease through trade of CiLV hosts. Therefore, there is a need to develop treatments to prevent CiLV-infected material as well as viruliferous vectors from arriving.

Brevipalpus yothersi mites acquire citrus leprosis viruses by feeding on infected tissue (Kitajima et al. 2003, Rodrigues et al. 2003, Tassi et al. 2017). Hence, postharvest treatments should target not only actively feeding stages but also eggs that could potentially hatch and feed on CiLV-infected material. Based on the results of this study we propose the hot-water immersion of lemons at 53°C for 5 min as a partially effective treatment against adults and eggs of B. yothersi without a significant impact on fruit quality. Immature stages were only partially controlled by this treatment; thus, a systems approach and additional postharvest treatments (i.e., waxing) may be required to achieve quarantine-level control of these mites from imported commodities. Further studies may be required to determine the compatibility between hot-water immersion and physical postharvest techniques, and to test a larger sample size of mites. Additionally, it would be important to evaluate hot-water immersion on other citrus hosts of Brevipalpus mites and CiLVs.

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