Phytotoxic Response of ‘Dancy’ Tangerine to High-temperature, Moist, Forced-air Treatment for Fruit Fly Disinfection

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Abstract. Early season degreened ‘Dancy’ tangerines (Citrus reticulata Blanco) were size graded and subjected to high-temperature, moist, forced-air (HTMFA) treatments using air at 45, 46, or 48°C for 0, 1, 2, 3, or 4 hours. The phytotoxic response of fruit to the heat treatments was evaluated immediately after treatment and weekly during 3 weeks of cold (4°C) storage. Mortality of nonfeeding, third instar Mexican fruit fly [Anastrepha ludens (Loew)] larvae was estimated for each time-temperature heat treatment combination in water baths that simulated the heating and cooling profiles of heat-treated fruit. Unacceptable phytotoxic symptoms, such as inferior flavor and darkened flavedo tissue, were observed when fruit was treated at 46 or 48°C. Fruit heated with 45°C forced moist air had flavedo color change (%* percent juice yield, soluble solids concentration, and flavor ratings that were statistically similar to ratings for unheated, control fruit. An HTMFA treatment of 3 or 4 hours at 45, 46, or 48°C and subsequent cooling was sufficient to kill 100% of naked larvae in water baths. Market quality of fruit was maintained after a 4-hour HTMFA treatment at 45°C, and 100% mortality of naked larvae occurred after 3 hours at 45°C.

A large volume of the U.S. fresh market supply of ‘Dancy’ tangerines is grown in or marketed through regions infested with the Mexican fruit fly (Leyva et al., 1991; Missiaen, 1981). Tangerines serve as hosts for Mexican fruit fly and, therefore, require disinfection before they can be marketed in fly-free areas. Methyl bromide fumigation is approved for use on tangerines (U.S. Dept. Agr., 1990); however, the food safety and ecological impact of methyl bromide has been under critical review (Danse et al., 1984). Approved, nonchemical quarantine treatments for A. ludens (U.S. Dept. Agr., 1990), such as cold storage (CS) and vapor heat (VH), are not used commercially. A CS treatment of 22 days at 1.7°C (U.S. Dept. Agr., 1990) would likely induce chilling injury in tangerine fruit (Hardenburg et al., 1986) and monopolize 80% of marketable postharvest storage time. The approved VH treatment requires tangerine fruit to be kept 4 h after the center temperature of the fruit reaches 43.3°C during a 6-h treatment (U.S. Dept. Agr., 1990). When grapefruit (Citrus paradisi Macf.) were subjected to a 5-h VH treatment (targeted for Caribbean fruit fly [A. suspensa (Loew)], no phytotoxic response was observed (Miller, 1991a, 1991b). However, the perishable nature and small size of tangerine fruit relative to grapefruit, and the more severe treatment protocol for A. ludens relative to A. suspensa, may explain why VH is not used commercially to disinfest tangerine fruit.

The potential of high-temperature, moist, forced air (HTMFA) as an alternative disinfection treatment has been investigated for various horticultural crops (Couey, 1989; Gaffney and Armstrong, 1990; Gaffney et al., 1990; Kerbel et al., 1987; McGuire, 1991). The HTMFA treatment differs from a VH treatment in that condensation on the fruit surface is prevented by maintaining the dew point temperature of the air chamber 2°C below the coldest fruit surface temperature throughout the duration of the treatment. Fruit moisture loss is minimal, because the relative humidity of the treatment chamber simultaneously increases during the treatment according to the fruit surface temperature. Temperature-sensitive fruits likely will develop less injury when subjected to HTMFA than when subjected to VH, because fruit surface temperatures increase more slowly. Because fruit sensitivity to heat is a primary obstacle to the use of HTMFA for fruit disinfection, phytotoxicity must be evaluated at the time-temperature regimes required to provide 99.997% (Probit 9) mortality of fruit fly eggs and larvae. The purpose of this study was to identify the phytotoxic response of tangerine fruit subjected to HTMFA treatments at various air temperatures and treatment times. This information would then be used to develop an HTMFA quarantine treatment protocol for tangerines that would also retain market quality of the fruit.

Materials and Methods

Insect mortality. Fruit center warming and cooling profiles of small and large fruit monitored during HTMFA treatments and subsequent air cooling were simulated in computer-controlled variable-temperature water baths containing exposed (naked), nonfeeding, third-stage A. ludens larvae immersed in screen containers. This larval stage is the most heat-tolerant stage (M.J. Firko, unpublished data). Control larvae in 23°C water were used to adjust mortality estimates for each HTMFA treatment. Heat profiles to control water bath temperatures were obtained from the mean of four readings (two fruit per HTMFA replication). Water baths equipped for heating and cooling simulated the mean temperature of the fruit center during heating in the forced air chamber and subsequent cooling in air at 23°C. Water temperatures were monitored and controlled with the Water Troll Controlled-Temperature Water Baths computer program (Gaffney, 1990). Temperatures between adjacent 60-sec readings were interpolated with a parabolic function that used previous and subsequent temperatures to calculate set temperature.

Phytotoxicity. Early season ‘Dancy’ tangerines were obtained...
from a commercial grower in Veracruz, Mexico, during Oct. 1990. The harvested fruit were degreened in Mexico under commercial conditions [1 to 5 ppm ethylene for 24 h at 27°C and 80% relative humidity (RH)] and transported (4 h at 23°C) to the U.S. Dept. of Agriculture (USDA) Agricultural Research Service (ARS) Crop Quality and Fruit Insects Research Unit, Weslaco, Texas, in an enclosed van. About 4 days elapsed between harvest and arrival at USDA. The fruit were not waxed or treated with postharvest fungicides.

A multifactorial (3 × 5 × 3) randomized block design with one replication was used to evaluate fruit size, heat-treatment dose, and posttreatment storage duration. Ten small (< 120 g), medium, and large (>161 g) fruit were subjected to a heat treatment at 45, 46, or 48°C for 1, 2, 3, or 4 h. The treated and control fruit were stored at 4 ± 0.7°C and 80% RH for 3 weeks. Quality evaluations were conducted after storing 0, 1, and 3 weeks. All treatment combinations were tested once before replication so pretreatment storage effects would be evenly distributed among the treatments (randomized block design).

The HTMFA chamber design was that of Sharp et al. (1991). All hot air treatments at a particular temperature were completed in a single day. About 12 h before HTMFA treatment, fruit were moved from storage at 4 to 23°C to ensure equal initial pulp temperatures. Four plastic, mesh-bottomed trays containing the fruit to be treated were stacked in columns over the outlet vent inside the forced-air chamber. A tray was removed hourly from the top of the column during the HTMFA treatment to give durations of 1, 2, 3, or 4 h. Control fruit were kept at 23°C throughout the 4 h. The temperature at the center of heat-treated fruit was monitored during cooling in 23°C air until it reached 25°C.

Fruit weight and flavedo color were measured before heat treatment, then after, and weekly during the 3 weeks of storage. Percent weight change after HTMFA treatment and after storage was also determined. Peel color at three marked surface areas on each fruit were taken with a Minolta Chromameter (Model CR-200, Minolta Corp. Ramsey, N.J.) calibrated to a standard white plate under illuminant condition CIE. Color was measured in the L* × b* (L* = white to black, a* = green to red, b* = blue to yellow) color difference mode with the target color as Minolta Color Calibration Plate #CR-A47 0. The percent change in L* (ΔL*), was calculated taking the difference in L* values after heat treatment and before treatment, dividing it by the latter value, then multiplying by 100. Hue angle and saturation index (Little, 1975) were calculated from measured a* and b* values. Two fruit of each size per heat treatment were analyzed before storage and at weekly intervals during storage for soluble solids concentration (SSC), percent titratable acidity (TA), and percent juice yield. The SSC of the tangerine juice was measured with a Reichert Abbe Mark II, temperature corrected, bench-top refractometer. The TA was expressed as percent anhydrous citric acid according to Praschan (1975). Percent juice yield was calculated by dividing the weight of juice extracted (with an electric Oster citrus juicer) by the fruit weight and multiplying by 100. Fruit that developed surface decay during storage were noted and removed from the study.

A 10-member consumer preference panel assessed flavor and odor of fruit segments before and after 3 weeks of storage. Control and heat-treated fruit of different sizes were evaluated as whole fruit and fruit sections at independent randomized stations. A 13-cm line scale, divided into five equal sections, was used to indicate preferences. The line scale was labeled “dislike extremely” at the left end and “like extremely” at the right end. Preferences were quantified by measuring the distance in centimeters from the extreme left of the line scale to the indicated preference mark (ASTM, 1968).

Data for heat-treated fruit were subjected to a factorial analysis of variance (ANOVA) (GLM procedure, SAS Institute, 1988) with fruit size, storage time, air chamber temperature, and treatment duration as main effects. Mean separation of significant main effects was accomplished with Duncan’s multiple range test. A similar statistical analysis was used for heat-treated and control fruit data at a 45°C air-chamber temperature, with fruit size, storage time, treatment duration, and all two-way interactions as main effects. Incidence of fruit decay was analyzed with Pearson chi-square in a 3 × 5 contingency table with chamber temperature and treatment duration as the columns and rows, respectively. Values in the cells were the number of sound fruit.

### Table 1. Percent mortality of A. ludens larvae in water baths that simulated fruit center temperatures of small (<120 g) and large (>161 g) tangerines during high-temperature, moist, forced-air (HTMFA) treatments.

<table>
<thead>
<tr>
<th>HTMFA Temp (°C)</th>
<th>Time (h)</th>
<th>Percent larval mortality (SD)*</th>
<th>Fruit center/maximum temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small fruit</td>
<td>Large fruit</td>
<td>n†</td>
</tr>
<tr>
<td>45</td>
<td>1</td>
<td>803</td>
<td>28.3 (5.9)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>864</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1001</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>924</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td>46</td>
<td>1</td>
<td>859</td>
<td>68.5 (4.8)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>797</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>699</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>674</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td>48</td>
<td>1</td>
<td>649</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>580</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>505</td>
<td>100 (0.0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>465</td>
<td>100 (0.0)</td>
</tr>
</tbody>
</table>

*Heat profile of HTMFA treatments conducted twice.
†Larval survival = successful pupation. Mortality estimates based on two water bath replications.
‡Sample size = sum of two water bath replications.
Results and Discussion

Insect mortality. Mortality of control larvae was low (2.5% ± 1.7%). An HTMFA treatment of 3 or 4 h at 45, 46, or 48 C and subsequent cooling was sufficient to kill 100% of naked larvae in water baths (Table 1). With 46 or 48 C, only 2 h was required for 100% mortality. Small fruit heated somewhat faster than large fruit during the first 2 h of HTMFA treatment. The heating profile for small fruit at 45 C gave 100% fly mortality after 2 h, but some survivors were found with the profile for large fruit under the same conditions. The data indicate that 3 h at 45 C causes 100% mortality of naked larvae. Fruit will need to be heated longer than 3 h, however, because mortality of naked larvae in water is higher than in fruit (M.J. Firko, unpublished data).

Phytotoxicity. Flavedo ΔL* was the only quality characteristic that did not significantly change during the posttreatment storage period (Table 2). It was also the only quality characteristic for which each temperature and treatment duration did not present a similar trend. Flavedo ΔL* was larger for fruit heated at 46 or 48 C than for fruit heated at 45 C. A larger flavedo ΔL* resulted from lower (darker) flavedo L* readings (data not shown). Flavedo ΔL* of fruit heated in 45 C air changed very little (0.8 to 1.0) after 1 and 4 h of treatment compared to the change observed after a 46 C (1.2 to 4.9) or 48 C (1.0 to 15.8) treatment. This trend suggests that the physiological changes responsible for the flavedo ΔL* readings begin to occur after reaching a minimum threshold temperature. Hue angle and saturation intensity of flavedo tissue was not significantly affected by the heat treatments (data not shown).

The temperature of the heat treatment significantly affected the percent of TA (Table 2). The TA also changed significantly during the posttreatment storage period. Percent TA and SSC decreased significantly during storage, indicating that heat-treated fruit retained the ability to use citric acid and six-carbon sugars as respiratory substrates (Ulrich, 1970; Whiting, 1970). However, the percent of TA in tangerines heated at 48 C was significantly higher than that of tangerines heated at 45 or 46 C, and a significant decrease in percent TA was observed after 1 and 4 h of heat treatment. Higher TA at higher temperatures yet lower TA over time illustrate: two opposing influences of the heat treatment. The rate of cellular respiration probably increases as heat moves from the fruit surface into the fruit center during the heat treatment until some maximum temperature at which respiratory enzymes are denatured and catalytic activity is lost.

Palatability of citrus increases directly with the ratio of SSC:TA. A reduction in this ratio is, therefore, undesirable. The SSC:TA ratio was higher in fruit that had been stored for 3 weeks (19.8) than in fruit before storage (14.9), and this elevated ratio is most likely responsible for the superior flavor ratings of stored fruit.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Flavedo ΔL* (%a)</th>
<th>Titratable acidity (MS × 10^2)</th>
<th>Soluble solids concn (MS × 10^3)</th>
<th>Flavor rating</th>
<th>Juice yield (%)</th>
<th>Wt loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage (ST)</td>
<td>3 (1,2)</td>
<td>6.3</td>
<td>27.0&quot;</td>
<td>567.1&quot;</td>
<td>3.3&quot;</td>
<td>981.4&quot;</td>
<td>182.7&quot;</td>
</tr>
<tr>
<td>Size (SZ)</td>
<td>2</td>
<td>8.3</td>
<td>0.4</td>
<td>414.2&quot;</td>
<td>1.8&quot;</td>
<td>133.4&quot;</td>
<td>10.2&quot;</td>
</tr>
<tr>
<td>Temp (T)</td>
<td>2</td>
<td>461.5&quot;</td>
<td>3.5&quot;</td>
<td>1.3</td>
<td>2.1&quot;</td>
<td>332.0&quot;</td>
<td>1.5&quot;</td>
</tr>
<tr>
<td>Time (T)</td>
<td>3</td>
<td>191.9&quot;</td>
<td>2.2&quot;</td>
<td>14.3</td>
<td>0.9&quot;</td>
<td>34.4</td>
<td>0.1</td>
</tr>
<tr>
<td>T × T</td>
<td>6</td>
<td>108.0&quot;</td>
<td>0.3</td>
<td>10.3</td>
<td>0.2</td>
<td>19.7</td>
<td>0.3</td>
</tr>
<tr>
<td>T × T × ST</td>
<td>33 (11,22)^t</td>
<td>11.0</td>
<td>0.7</td>
<td>28.6&quot;</td>
<td>0.13</td>
<td>30.3&quot;</td>
<td>0.4&quot;</td>
</tr>
<tr>
<td>T × T × SZ</td>
<td>22</td>
<td>24.7</td>
<td>0.9</td>
<td>12.9</td>
<td>0.11</td>
<td>8.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Error</td>
<td>72 (24,47)^t</td>
<td>15.3</td>
<td>0.6</td>
<td>9.9</td>
<td>0.12</td>
<td>1.43</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Means

Storage (wks)
- 0: NE, 0.61 a, 9.1 a, 2.9 a, 53.7 a, 0.8 a
- 1: 3.4, 0.50 b, 8.7 b, NE, 58.2 b, 3.7 b
- 2: 4.1, 0.43 c, 8.2 c, NE, 63.5 c, 4.9 c
- 3: 4.2, 0.42 c, 8.3 d, 3.4 b, 65.1 c, 6.1 d

Size
- Small: 3.3, 0.5, 8.3 a, 2.9 a, 58.6 a, 4.1 a
- Medium: 4.4, 0.5, 8.6 b, 3.2 b, 60.0 a, 3.7 b
- Large: 4.1, 0.5, 8.8 c, 3.4 c, 61.9 b, 3.8 b

Temp (C°)
- 45: 0.8 a, 0.47 a, 8.5, 3.5 a, 63.1 a, 3.8 a
- 46: 2.9 b, 0.48 a, 8.5, 2.3 b, 58.1 b, 3.7 a
- 48: 7.9 c, 0.52 b, 8.6, 2.3 b, 59.2 b, 4.1 b

Time (h)
- 1: 1.0 a, 0.50 a, 8.6, 3.4 a, 60.4, 3.9
- 2: 2.3 ab, 0.50 a, 8.5, 3.2 ab, 59.0, 3.8
- 3: 4.6 b, 0.50 a, 8.6, 3.1 bc, 59.8, 3.9
- 4: 7.2 c, 0.45 b, 8.5, 2.9 c, 61.3, 3.8

^tMeans based on ratings from 10 judges for each evaluation, with 1 = dislike extremely and 6 = like extremely.
^df = degrees of freedom.
( , ) df for flavor and flavedo ΔL*, respectively.
** Not evaluated and significant at P ≤ 0.05 and 0.01, respectively.
Tangerines heated in 45°C air were rated significantly superior in flavor to tangerines heated at 46 or 48°C (Table 2). Fruit treated at 45°C also had a higher SSC : TA ratio (18.0) than fruit treated at 46 or 48°C (17.7 and 16.5, respectively). However, flavor ratings of fruit heated for 1 h were significantly superior to fruit heated for 4 h in spite of an increased SSC : TA ratio from 17.2 after 1 h of heat treatment to 18.8 after 4 h of heat treatment. Preference panelists informally reported an off-flavor in fruit treated at 46 or 48°C, one that became more pronounced the longer the fruit was treated. It seems likely that physiological changes resulting in off-flavor development also respond to a minimum temperature threshold. The temperature of the heat treatment significantly affected the amount of juice obtained from a fruit and percent weight loss (Table 2). Percent juice yield also changed significantly during the posttreatment storage period. The significant increase in percent juice yield during the first 2 weeks of storage indicates that heat-treated fruit retain the ability to break down central parenchyma cells within juice vesicles, as was reported to occur during storage (Burton, 1982). Percent juice yield was highest in fruit heated at 45°C.

The air chamber temperatures and treatment time durations evaluated in this study did not significantly affect the incidence of decay during the 3 weeks of storage (c² = 1.3, P = 0.996, data not shown). Decay was observed in 26 out of 90 (29%) control fruit. Fruit heat treated at 48°C for 1 to 4 h had two decayed fruit out of 120 fruit. Fifteen and 19 decayed fruit were observed, respectively, among the 120 fruit heat treated at 45 and 46°C for 1 to 4 h. An increase in flavedo ΔL* was the only observable symptom of heat injury.

Air at 45°C appears to be the maximum heat treatment at which minimal phytotoxic symptoms appear. Fruit heated with 45°C forced moist air had statistically similar flavedo ΔL*, percent juice yield, SSC, and flavor ratings as unheated control fruit (Table 3). Heated fruit had significantly lower percent TA and significantly higher percent weight loss than unheated control fruit (time = 0). Heated fruit had ≈ 0.1 percent less TA and lost ≈ 0.5% more moisture than control fruit. Heat treatment at 45°C may elevate the SSC : TA ratio and result in enhanced fruit palatability.

Results from this research indicate that an HTMFA treatment at 45°C for at least 3 h likely will ensure 100% insect mortality and maintain the market quality of degreened ‘Dancy’ tangerines. Researchers have reported an increased tolerance to heat treatments after curing at 16°C (Houck, 1967). Therefore, degreening may have conditioned the fruit to withstand the heat treatments. Further research on the response of ‘Dancy’ tangerine that has not been degreened before heat treatment is warranted. The use of hot, moist, forced air as a quarantine treatment is promising because it does not require use of potentially toxic chemicals, does not impair the market quality of the fruit at optimal time and temperatures, and leaves no chemical residue on the fruit.

### Literature Cited


