Fruit Age and Gibberellic Acid Effect on Epicuticular Wax Accumulation, Respiration, and Internal Atmosphere of Navel Orange Fruit

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Additional index words. Citrus sinensis, 'Washington' navel, rind, postmaturation, senescence, gas exchange, conductance

Abstract. Relationships among fruit age, epicuticular wax, weight loss, internal atmosphere composition, and respiration were investigated in mature 'Washington' navel orange fruit (Citrus sinensis (L.) Osbeck). Fruit epicuticular wax, internal CO2, and internal C2H4 increased, whereas as the season advanced, weight loss during storage and respiration decreased. Concomitantly, fruit conductance to CO2 was reduced. GA3 (10 ppm) application prior to fruit color break reduced the increase in epicuticular wax and thereby delayed the decrease of CO2 conductance, resulting in less of an increase in internal CO2. GA3-treated fruit were not consistently different for other parameters evaluated.

Chemical name used: 1α,2β,4α,4β,10β)-2,4α,7-trihydroxy-1-methyl-8-methylene gibb-3-ene-1,10-dicarboxylic acid 1,4α-lactone (gibberellic acid).

As early as November, 'Washington' navel orange fruit matures in California. Following maturation the rind softens and market value of the fruit is reduced due to the development of certain rind disorders (8). GA3 reduces the incidence of these rind disorders, when applied as a preharvest spray, by delaying senescence on the tree and after harvest (8). Moreover with the aid of (2,4-dichloro-phenoxo)acetic acid (2,4-D), a fruit-drop-reducing agent (21), citrus growers commonly store their fruit on the tree for a long period of time with little loss or damage to the fruit.

Recent research has shown that GA3 applied to navel oranges prior to or at the time of fruit color break significantly reduces the rate of accumulation of fruit epicuticular wax and maintains the crystalline structure of the wax during the senescence stage, in contrast to 2,4-D-treated or untreated fruit (12). Studies of surface wax deposits on plant leaves (16, 17, 20) showed that the accumulation of waxes in or near the epistomatal chamber constitutes a barrier to gas diffusion and exchange by conifer needles. Hanover (15) also suggested the 2 factors most likely affecting the emission of volatiles from plant leaves are internal concentrations of volatiles and the amount of surface wax. Epicuticular waxes are also known to increase cuticular resistance and reduce transpiration rates (3, 14, 19). Furthermore, it has been shown (2) that postharvest weight loss of 'Valencia' orange is reduced by increased quantities of epicuticular wax.

In 1920, Magness (18) proposed that internal O2 and CO2 concentrations of a plant tissue are determined by: 1) the rate of oxygen use and CO2 production within the tissue; 2) the permeability of the tissue epidermal coverings to gas exchange; and 3) the differences in pressures of the gases inside and outside the tissues. Eaks and Ludi (11) showed the internal atmosphere of citrus fruit to be affected by temperature, washing, and waxing. Certain packinghouse practices increased CO2 concentrations within the fruit of citrus (25). In particular, fruit waxing was shown to increase CO2 and decrease O2 concentrations inside the fruit (4, 5, 9, 24). The effect of fruit waxing on...
respiratory activity of citrus is rather controversial. Vines et al. (24) showed that increasing wax thickness on citrus fruit surface had no effect on the evolution of CO$_2$, whereas Ben-Yehoshua (5) demonstrated a reduction in the respiratory activity of Valencia orange fruit as a result of waxing. Coating apples with wax emulsion increased the resistance of the skin to diffusion of gases (22). Coating reduced internal O$_2$, increased internal CO$_2$, and reduced respiratory activity.

Because fruit physiology is significantly affected by the above-mentioned factors, we examined the influence of gibberellic acid treatment and of fruit age on the accumulation of epicuticular waxes vs. weight loss, respiration, and the internal atmosphere of navel oranges during the postmaturation and senescence stages of development.

**Materials and Methods**

**Plant material.** Twenty-four 18-year-old ‘Washington’ navel orange trees on ‘Rubidoux’ trifoliate rootstock [Poncirus trifoliata (L.) Raf.], planted in sandy loam soil with a spacing of 4.5 × 7.2 m and with furrow irrigation, were the source of all fruit material used in this study. The grove was located near Visalia in the San Joaquin Valley, Calif. Fruit samples were collected during the postmaturation and senescence phase (Jan.–Apr. 1985).

**Treatment application and experimental design.** On 4 Oct. 1984 (2 weeks prior to fruit color break), half the trees received the isopropyl ester of 2,4-D at a concentration of 16 ppm acid equivalent and served as the control; the other half received both 2,4-D (16 ppm) and GA$_3$ at a rate of 10 ppm. Trees were arranged in a randomized, complete block design with six 2-tree replications per treatment, and the treatment solutions were applied as entire tree sprays to the point of runoff.

**Epicuticular wax.** Samples consisting of 20 fruit were harvested from each of the 6 replications by collecting random fruit from all sides of each tree. These fruit were then measured for fruit surface area using Turrell’s tables (23). Epicuticular wax extraction was performed using the chloroform dip method, as outlined previously (12).

**Respiration rate.** The respiration rate was estimated by monitoring the evolution of CO$_2$ from steady-state chambers of about one liter capacity which were maintained at 20°C. Each chamber contained 2 fruit and 12 chambers were used per treatment. Thus, the set-up consisted of 24 chambers.

The rate of CO$_2$ production was monitored with a calibrated Model 215 Beckman infrared nondispersion CO$_2$ analyzer connected to a Leeds and Northrup recorder and equipped with a switching system to sequence the exiting gas from each fruit chamber and the air background to the analyzer. The air stream, with a flow of 8 liters·hr$^{-1}$, metered by calibrated capillaries through the chambers, was freed of CO$_2$ by bubbling it through a 2 N NaOH solution and was then humidified by bubbling it through water. The readings were taken once daily for a week, and respiration rates were calculated as ml CO$_2$·kg$^{-1}$·hr$^{-1}$.

**Internal atmosphere.** The composition of the fruit internal atmosphere was examined during the first day and a week after harvest. Fruit were held at 20°C and 50% to 60% RH during the experimental period. After removal of the fruit button, the needle of a one ml gas-tight syringe was inserted into the central core of the fruit, and 1-ml gas samples were taken (one for CO$_2$ and O$_2$ determination and another for ethylene determination). Sampled fruit were discarded. Twenty fruit per replication per treatment were used at each sampling.

Percentages of internal O$_2$ and CO$_2$ were determined using a Varian Aerograph thermo-conductivity gas chromatograph equipped with a dual-column system. The CO$_2$ was separated on a 76 cm × 6 mm copper column packed with 50–80 mesh Poropak T, and oxygen was separated on a 3 m × 6 mm copper column packed with 60–80 mesh molecular sieve 5A. Oven and detector temperatures were 54°C and 140°C, respectively. Helium was the carrier gas with a 50 ml·min$^{-1}$ flow rate. Filament current was 145 milliamperes.

Internal ethylene concentration was evaluated on a 1-ml gas sample using a Varian Aerograph flame ionization gas chromatograph equipped with a 2 m × 3 mm copper column packed with 60–80 mesh activated alumina. Oven and detector temperatures were 80°C and 170°C, respectively, and N at a flow rate of 50 ml·min$^{-1}$, was used as the carrier gas.

Comparison of peak heights with those of standard gases was used to determine the fruit internal concentration of the different gases.

**Fruit conductance to CO$_2$.** Conductance was estimated in a manner similar to previous applications of Fick’s law (6, 7) to plant organs as follows:

$$G_{\text{gas}} = \frac{J_{\text{gas}}}{[\text{gas}]_{\text{in}} - [\text{gas}]_{\text{out}}}$$

where $G_{\text{gas}}$ is conductance (distance/unit time), $J_{\text{gas}}$ is flux rate of the gas (volume/unit fruit surface area/unit time), and $[\text{gas}]_{\text{in}}$ and $[\text{gas}]_{\text{out}}$ are CO$_2$ concentrations inside the fruit and in the respiratory chamber at steady state, respectively.

**Fruit weight loss.** Weight loss was determined on samples of 10 fruit per replicate after 1 week in storage at 20°C and 50% to 60% RH.

**Results and Discussion**

Results are shown as a 6-part figure plus a table. When reference is made to a particular part of the figure, the reader should look at the table for statistical guidance.

Fruit surface area was not affected by GA$_3$ treatment (data not shown), but by 31 Jan., 17 weeks after GA$_3$ was applied, the quantity of wax per unit of fruit area was higher on control than on GA$_3$-treated fruit (Fig. 1A). Furthermore, the rate of wax accumulation during the 9-week sampling period was faster on control than on fruit from GA$_3$-treated trees. These results are in agreement with a previous report (12).

In concert with the continued accumulation of surface wax from 31 Jan. through 4 Apr., the loss of fruit weight during 7 days of storage decreased substantially for fruit harvested later in the season (Fig. 1A). GA$_3$ treatment influenced weight loss to only a minor degree. Because fruit harvested late in the season had reduced rates of respiration (Fig. 1E), and because Ben-Yehoshua (5) found that water loss accounted for about 90% of the weight loss of fruit during storage, we have concluded that the major factor responsible for less weight loss from samples harvested later in the season was reduced water loss due to increased quantities of epicuticular wax. Thus, if postharvest decay organisms are controlled, navel oranges harvested relatively late in the season should have a longer shelf life than fruit harvested earlier in the season. Regardless, the weight loss/wax relations we found in nature are consistent with those reported by Albrigo (2) and with reports (4, 5) that the application of wax emulsions to citrus fruit reduce weight loss and improve keeping quality.

Time of harvest had a major effect on the internal atmosphere of navel oranges as shown by measurements made one day after
Fig. 1. The influence of fruit age, GA3, and storage on epicuticular wax (A, solid lines), weight loss (A, broken lines), internal CO2 (B), internal O2 (C), internal C2H4 (D), respiration rate (E), and conductance to CO2 (F). Relative values were plotted using 31 Jan. 0 ppm GA3 values for the first day of storage (solid lines) as 100%, except for weight loss where the 31 Jan. 0 ppm GA3 value for 7 days of storage (broken lines) represents 100%. Absolute values for 100% were 85 μg·cm⁻² for wax, 8.3% for weight loss, 0.69% for CO₂, 20.8% for O₂, 24 parts/billion for C2H4, 36 ml CO₂·kg⁻¹·hr⁻¹ for respiration, and 5.6 cm·hr⁻¹ for conductance to CO₂. Notations in Fig. 1A apply to Fig. 1 A through F.

Table 1. Statistical information regarding the 'Washington' navel orange data shown in Fig. 1.

<table>
<thead>
<tr>
<th>Factor</th>
<th>0 vs. 10 ppm GA3</th>
<th>Change among dates⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day storage</td>
<td>7 day storage</td>
</tr>
<tr>
<td></td>
<td>31 Jan.</td>
<td>5 Mar.</td>
</tr>
<tr>
<td>Epicuticular wax</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Weight loss</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Internal atmosphere</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>CO₂</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>O₂</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C₂H₄</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Respiration rate</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Conductance to CO₂</td>
<td>**</td>
<td>**</td>
</tr>
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</table>

⁶For one day after harvest. Similar statistical differences were obtained after 7 days in storage across the 3 harvest dates.

NS, *, **, ***Nonsignificant (NS) or significant at 5% level (*), 1% level (**), or 0.1% (*** ) level.
NA indicates no data were available for analysis.
harvest (Table 1). In fruit from control and GA$_3$-treated trees, additional “tree-storage” time increased CO$_2$ and C$_2$H$_4$ concentrations (Figs. 1B and 1D). Although there was a statistically significant effect of time on the O$_2$ component of the internal atmosphere one day after harvest (Table 1), we view the O$_2$ change as too minor (Fig. 1C) to be of physiological significance.

During postharvest storage, additional increases in CO$_2$ and C$_2$H$_4$ were observed (Figs. 1B and 1D). In view of the steady-state storage conditions and the concomitant decrease in respiration (Fig. 1E), such increases would be difficult to explain if it were not for the fact that fruit conductance to CO$_2$ decreased substantially during storage as shown in Fig. 1F. An increase in gaseous diffusion due to peel drying during storage was demonstrated by Ben-Yehoshua (4, 5).

Data presented in Fig. 1F also show that conductance to CO$_2$ decreased as the harvest season advanced and that conductance was lower for control than for GA$_3$-treated trees. Furthermore, data presented in Table 1 for fruit harvested in April and stored for 7 days showed that the quantity of wax per unit surface area increased during storage. Whether this increase was due to continued deposition of wax, to a reduction in surface area due to water loss, or to a combination of both factors is not clear. However, it seems clear that conductance to CO$_2$ decreased as the quantity of epicuticular wax increased. The same conclusion probably holds for O$_2$ and C$_2$H$_4$.

Fruit waxing as a packinghouse treatment was shown to increase CO$_2$ and reduce O$_2$ inside citrus fruit (4, 5, 9, 11). The respective concentrations of these gases are markedly affected by the thickness of the wax coating (4). Furthermore, Ben-Yehoshua (5) demonstrated that the flavedo portion of the orange rind was the primary resistance site to CO$_2$ and O$_2$ diffusion, and our results further suggest that the cuticle and its epicuticular waxes, particularly, might be the site of highest resistance to diffusion of gases in the fruit. Indeed, an increase in resistance to gaseous diffusion upon waxing was reported on apples (22). Studies have also shown that waxing of oranges increased their internal C$_2$H$_4$ concentration (5).

Fruit respiration decreased appreciably as sampling date advanced and during storage (Fig. 1E — similar results were obtained for days 2–6, thus daily data are not shown). Such a decline in respiration of citrus fruit was reported by others (1, 10). Whereas fruit from the GA$_3$-treated trees consistently had a reduced quantity of epicuticular wax and CO$_2$ concentration in the internal atmosphere, there were no consistent differences in respiration rates between GA$_3$-treated and untreated fruit (Table 1) nor in concentrations of O$_2$ or C$_2$H$_4$ of the internal atmosphere of control and treated fruits (Table 1). Perhaps the similarity in respiration rates indicates that GA$_3$ had no major impact on whole fruit respiration and that navel orange epicuticular wax has an effect on permeability to CO$_2$ greater than the other 2 gases. Thus, the moderating effect of GA$_3$ on quantity [and perhaps quality (13)] of wax deposited was sufficient to produce measurable effects on internal CO$_2$ concentrations, but insufficient to cause significant changes on internal O$_2$ and C$_2$H$_4$ concentrations.

In conclusion, during postmaturation and senescence, the quantity of epicuticular wax of navel orange fruit increased significantly. This increase was associated with a reduction in weight loss during storage, significantly increased CO$_2$ and C$_2$H$_4$ concentrations, and reduced respiration rates. Although we saw statistically significant reductions of internal O$_2$ for “day 1” fruit as the postmaturation season progressed, we view these minor changes as physiologically insignificant. GA$_3$ seemed to moderate the increase in epicuticular wax as well as CO$_2$ changes of the internal atmosphere of the fruit. However, internal O$_2$ and C$_2$H$_4$ concentrations of navel orange fruit were not consistently affected by GA$_3$ treatment.

**Literature Cited**


Ice Nucleation and Supercooling in Freeze-sensitive Peach and Sweet Cherry Tissues

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Abstract. Ice nucleation temperatures were measured in peach [Prunus persica (L.) Batsch ‘Redhaven’] and sweet cherry (Prunus avium L. ‘Bing’) flowers, fruits, and stems by differential thermal analysis and by controlled freezing in test tubes and of droplets. The mean nucleation temperatures of peach and sweet cherry flowers were —4° to —6°C, while those of 0.5-cm stem segments were —6° to —7°. The highest ice nucleation activity in peach flowers appeared to be associated with the pedicels. A logarithmic function described the relationship between stem weight and ice nucleation temperature, with 1.5-g (16-cm) stem segments freezing as high as —3°. Apparently, stem tissue contained a lower concentration of ice nucleators active above —5° than did the floral tissue. Homogenization of the tissues reduced their ice nucleation activity. Structural integrity may be necessary for the manifestation of optimal activity. Ice nucleation-active (INA) Pseudomonas syringae bacteria were isolated from the surfaces of peach and sweet cherry floral tissues only occasionally and then at low populations. The absence of epiphytic INA bacteria from these tissues suggested that ice nucleation was promoted by intrinsic ice nucleators within the plant. These intrinsic ice nucleators appeared to be active at temperatures similar to INA bacteria. Although these nucleators may be less numerous than bacterial ice nuclei on heavily colonized plant surfaces, their presence nonetheless suggests that control of INA bacteria or inhibition of the ice nucleation activity of INA bacteria alone may be insufficient to prevent ice nucleation and freezing injury of freeze-sensitive flowers and fruits during spring frosts in the orchard.

As Prunus flower buds deacclimate in the spring, they become sensitive to freezing injury following ice formation. This transition from a freeze-resistant to freeze-sensitive state occurs rapidly following the emergence of the tips of the petals through the calyx (4, 9). Currently, the only strategy employed to prevent freezing injury to freeze-sensitive flowers and fruits in commercial orchards is to maintain the temperature of these tissues above that required for ice nucleation. This temperature control is accomplished by selecting frost-free sites, or by using orchard heaters, wind machines, or sprinklers.

During spring frost episodes, ice nucleation is initiated by heterogenous ice nucleators. Ice nucleation is dependent upon whether an ice nucleator is present and active under ambient conditions. It also depends upon the probability that a subcritical nucleus of water molecules will surmount the free energy-of-formation barrier to become a critical nucleus capable of catalyzing ice formation (13). Before ice nucleation can occur, however, water must supercool (13). Therefore, enhancement of supercooling to lower the ice nucleation temperature is a potential method of protecting freeze-sensitive floral and fruit tissues.

While many ice nucleators are active at —10°C or lower (30), few have been identified that are active at temperatures above —10°. Strains of the epiphytic bacteria Pseudomonas syringae (van Hall) (5, 7, 15, 17, 19, 22, 24, 32), Erwinia herbicola (Löhnis) Dye (17–19, 33), and Pseudomonas fluorescens Migula (25) have been found to be effective ice nucleators at temperatures above —5°. The presence of these ice nucleation-active (INA) bacteria on almond, avocado, bean, citrus, corn, pear, pumpkin, soybean, and tomato has been associated with frost injury of these plants (1, 5, 15, 16, 18–21, 33). Frost injury was proportional to the logarithm of the INA bacterial populations present on the leaf surfaces (20). Frost injury of annual plants with low populations of the INA bacteria was avoided by supercooling to as low as —8° to —10° (1, 5, 15, 18–20, 22).

A survey showed that INA strains of these bacteria are present on many plant species from widely separated areas of the United States (17). INA P. syringae populations of up to 106 cells/g fresh weight have been found in commercial almond, apple, apricot, peach, pear, and sweet cherry orchards in California, Oregon, and Washington (7, 21). While low populations of INA bacteria were found during the warm, dry summer months and in the winter, higher levels were isolated from developing buds and flowers in some orchards during the cool, moist spring (7).