PEACH AND NECTARINE INKING IN CALIFORNIA

Carlos H. Crisosto, R. Scott Johnson, Kevin R. Day, Bob Beede, and Harry Andris

After three years of study, we have demonstrated that physical injury and contamination are essential for inking development. Abrasion damage releases anthocyanin/phenolic pigments, which are located in the skin cells, allowing the reaction of these pigments with the heavy metal contaminants. We found that iron, copper, and aluminum were the most deleterious contaminants of those studied in inducing inking on abraded fruit. Approximately 10 ppm iron was enough to induce inking at the physiological fruit pH (~3.5). This contamination can occur within 15 days before harvest, during harvest or packing operations. Foliar nutrient, fungicide, and insecticide preharvest sprays may act as sources of contamination for inking development depending on the preharvest application interval. We completed our inking research by developing safe preharvest application intervals which yielded low inking incidence for iprodione (Rovral), triforine (Funginex), vinclozolin (Ronilan), benzimidazole (Benlate), and certain foliar nutrients (containing heavy metals).
Introduction

Inking on peach and nectarine fruits has become an increasingly frequent problem in the last decade in California, Washington, Georgia, South Carolina, and Colorado. Inking or skin discoloration is also a problem in other production areas in the world including Italy, Australia, Argentina, and Chile. Inking symptoms appear as discolored brown and black spots, and are restricted to the skin (Picture 1). Although inking affects only the fruit's cosmetic appearance, this disorder causes considerable losses to the peach and nectarine industry each year. Through our previous anatomical studies, we learned that the type of physical injury associated with inking is abrasion. The damaged skin cells, where the anthocyanin/phenolic pigments are located, were collapsed while the underlying fleshy cells (mesocarp cells) remained intact. Unfortunately, abrasion injury frequently occurs during harvest and hauling operations, and it is very difficult to eliminate. As a follow up to our previous work, we decided to determine where inking damage occurs during postharvest handling operations, and if physical damage and/or exogenous contamination are related to its development. This information is fundamental to understanding inking development and for generating recommendations to reduce inking incidence for the stone fruit industry.

Methods

Inking occurrence

Inking occurrence during commercial harvest and postharvest handling was recorded on Flavorcrest, Elegant Lady, and Henry peaches grown in the Traver area, Tulare County (an area with a history of inking). Samples were collected on three harvest dates for each cultivar and at three handling points during harvest and transport: 1) directly from the tree
and field-packed; 2) from bins, after bin filling and transport in the orchard to the loading point, approximately 4 miles; and 3) from bins arriving at the packinghouse (after handling and transporting to the packinghouse). Four replications of 18 fruit were taken for each cultivar for each treatment. Fruit samples were picked at random from three trees selected previously and marked. Fruit were tracked during routine harvest and collected at the three locations noted. After collection, fruit samples were carefully placed in tray packs, padded, and packed in the orchard or packinghouse before being transported to the University of California, Kearney Agricultural Center (KAC) for subsequent inking evaluation.

**Effects of pH and metallic ions**

Fruit of >Flavorcrest=, >Elegant Lady=, and >O=Henry= peaches, and >May Glo= and >Flaming Red= nectarines were randomly picked from orchards at or near the KAC at commercial maturity according to ground color. Fruit were carefully handled to avoid physical damage. Twenty fruit from each of five randomly selected trees (replications) per cultivar were separated into two main treatments (50 fruit each), unwashed (UW) and washed (W). Washed fruit were submerged in tap water for five minutes, rinsed with distilled water (dH2O), and placed on clean paper towels to air-dry. The purpose of the washing treatment was to remove metallic ions and/or other contaminants from the fruit surface. Each treatment was applied either to non-abraded fruit (NA) or abraded fruit (A). The abrasion was induced with a rotatable automatic toothbrush and a fruit holder (Picture 2). The fruit holder was a modified apple peeler, which allowed forward and backward movement of the fruit during reversible rotations. During abrasion, the fruit was hand-rotated forward and then backward (one cycle) while the automatic toothbrush head abraded the skin (the cycle was done three times with initial toothbrush head
position 0.5 cm apart from the previous one to maximize uniform abrasion). The hand rotation speed was kept as consistent as possible for all fruit samples.

In each subtreatment (NA and A), fruit were randomly sampled for preparation of skin discs. Six skin discs were used for each of the different pH and ion applications. The discs were sampled from the red-colored surface of the fruit, either non-abraded or abraded, using a cork borer (44 mm² inner area). The discs were randomly placed on a piece of cardboard covered with a paper towel. Each disc was identified by the layout order during the following experiments. After measuring the color with a Minolta Colorimeter CR 200 in the L*a*b* color notation system (C illuminant, calibrated with standard white plate, and 0° viewing angle), the skin discs were placed in the treatment solutions (30 ml) and any air bubbles formed on the skin disc surface were removed by stirring gently. For pH treatments, solutions of pH 3, 4, 5, 6, 7, 8, and 9 were prepared with phosphate buffer (0.1M). For ion treatments, solutions of sodium, aluminum, ferrous iron, copper, tin, and zinc ions were prepared in a final ion concentration of 100 ppm in dH2O. For iron concentration treatments, ferrous iron solutions were prepared in ion concentrations of 10, 25, 50, 75, 100, and 200 ppm. Ion compounds used were all in chloride salt form for uniformity and all solutions were prepared daily. Solutions of 100 ppm bicarbonate or nitrate were also used. The control solution was dH2O and an untreated check was used for abraded and non-abraded subtreatments. After a 15 minute incubation period, the discs were taken out, blotted with Kimwipes, and air-dried for one minute, then the color was measured again. Discoloration was expressed by the relative change in the color value a*, as Δa*, since it amply reflected the visual darkening on the skin discs. The Δa* was calculated as the difference between the a* before treatment and the a* after incubation. The higher value of Δa* reflected a darker discoloration.
Preharvest chemical sprays

> Fantasia = and > Flaming Red = nectarines, and > Kern Sun =, > Flavorcrest =, > Elegant Lady =, > O = Henry =, > Summer Lady = and > Cal Red = peaches were chosen for experiments. Fungicides and foliar nutrients commonly used by the California stone fruit industry were selected and applied to entire trees according to label instructions.

At least eight trees per cultivar were used as replicates for each treatment, except for > Kern Sun = and > Cal Red = where twenty-five trees per replicate were used and four rows were left as a buffer between treatment-replicates (approximately 6 acre plot). All of the fungicide and foliar nutrient sprays were applied using an air-blast sprayer (approximately 100 gallons per acre). Z.I.P. (2 qts./acre/100 gallons) was applied 1, 18, and 22 days before harvest (DBH); Rovral (0.5 lb/100 gallons) and Funginex (12 oz/100 gallons) were applied 7, 3, and 1 (DBH); Benlate (2 lb/100 gallons) and Ronilan (0.5 lb/100 gallons) were applied 4 and 14 DBH, respectively. Fruit located in the outer canopy were harvested at commercial maturity.

Harvesting was done using picking bags, dumping in wooden bins, and transporting on trailers to the KAC, F. Gordon Mitchell Postharvest Laboratory. Four boxes of approximately 60 fruit each (volume filled) were hand packed from each tree (replicate). These boxes were labeled, room cooled, and kept in cold storage for later inking evaluation.

Analysis of pesticides for heavy metals

Samples from each spray solution used in our experiments were sent out for commercial analysis for iron, copper, aluminum, and tin.
Inking evaluation

In all experiments, evaluation of inking was done by placing fruit samples in a room controlled at 68°F (20°C) and 80% RH for 2-3 days before inking evaluation. Inking was determined by two methods: 1) percentages of individual fruit presenting inking symptoms (incidence), and 2) an aggregated inking index (AII) based on measurements of total fruit surface area affected by inking (intensity). AII was measured using a 0.9 cm diameter loop; a larger discolored area was counted as two or more, accordingly. The percentage of cull fruit was calculated based on the California Quality Standards (U.S. Dept. of Agriculture, 1987), which states that any fruit presenting a discolored area \( \geq 0.9 \) cm in diameter should be rejected.

Results and Discussion

Inking occurrence during handling

Average incidence of inking increased dramatically with fruit handling after harvest (Fig. 1). High inking levels were detected on fruit sampled during harvest as well as before and after fruits were transported to the packinghouse. Inking incidence on fruit picked directly into tray packs and transported gently to KAC was 42%, 42%, and 29% for Flavorcrest=, Elegant Lady=, and O=Henry=, respectively. Inking incidence before and after transport to the packinghouse was nearly 100%. High cull levels, up to 40%, were measured after transport within and out of the orchard on Flavorcrest=, Elegant Lady=, and O=Henry= peaches. Fruit picked and packed directly in the orchard had 10% culls.

Responses of skin discs to pH solutions
Dark discoloration did not develop on skin discs of non-abraded fruit either unwashed or washed when exposed to pH solutions from 3 to 9 in all peach and nectarine cultivars tested (Fig. 2). On abraded skin discs from unwashed and washed fruit, the redness was intensified after incubation in a buffer of pH 3, especially on those from the cultivars >May Glo=, >Flavorcrest=, >O=Henry= and >Elegant Lady=. Treatment in buffers of pH 4 and 5 did not markedly change the Δa* of skin discs in any of the cultivars examined. On skin discs from >Flavorcrest=, >Elegant Lady=, and >O=Henry= peaches, some discoloration started to occur in a buffer of pH 6. In buffers of pH 7, 8, and 9, dark discoloration of various degrees developed on the abraded skin discs of all of the cultivars (Fig. 2). The washing treatment induced significant dark discoloration only on abraded >May Glo= and >Flaming Red= nectarines. Abrasion treatment significantly increased development of dark discoloration in all of the tested cultivars (data not shown).

A significant interaction between washing and abrasion on dark discoloration formation was determined. The UW-NA and W-NA combinations showed no dark discoloration formation, however, the abraded fruit with or without washing displayed high levels of dark discoloration. The W-A treatment yielded the highest dark discoloration formation in all of the cultivars. This may be explained due to an increase in abrasion susceptibility by immersing fruit in water prior to our low level abrasion treatment. Disappearance of the dark discoloration caused by high pH buffers was noticed a few hours after the skin discs were removed from the buffers. An experiment was conducted to monitor the post-treatment change in skin color using skin discs from washed fruit of >Cal Red= peach and >Flamekist= nectarine. The results indicated that the dark discoloration caused by high pH gradually disappeared after about three to five hours leaving red to light brown spots (data not shown). Thus, exposing fruit to clean
Responses of skin discs to metallic ions

On skin discs from NA fruit, regardless of being washed or not, no dark discoloration was observed after any metallic ion 100 ppm solution treatments in all of the cultivars except Copper on >May Glo= and >Flaming Red= (Fig. 3). On skin discs with or without washing, ions of iron, aluminum, and copper caused evident discoloration (Fig. 3). The discoloration caused by iron and aluminum ions was black. Of the metallic ion solutions tested, iron caused the most severe discoloration. Sodium, stannous, or zinc ion solutions had little effect on the $\Delta a^*$ of the skin discs (Fig. 3), as did bicarbonate and nitrite solutions (data not shown). With iron, skin discs from fruit of >May Glo= and >Flavorcrest= developed more discoloration than those of >Elegant Lady=, >O=Henry=, and >Flaming Red=. The skin discs of >Flavorcrest= and >O=Henry= fruit showed more discoloration than other cultivars when treated with aluminum. The dark discoloration caused by iron and aluminum did not fade for at least four days (data not shown). Thus, hydrocooling abraded fruit with water containing heavy metals may be inducing inking.

Response of skin discs to iron concentration

Since iron was the most effective ion in causing skin discoloration, a concentration effect was investigated. Iron solutions of 10 to 200 ppm did not cause discoloration on skin discs of NA fruit with or without washing treatment of any cultivars after 15 minutes incubation (Fig. 4).

With abraded discs, an iron concentration as low as approximately 10 ppm caused dark
discoloration in all the cultivars tested (data not shown). The \( \Delta a^* \)s on skin discs of W-A fruit were generally higher than those of UW-A fruit. Iron concentrations of >50 ppm resulted in no significant change in the \( a^* \) value.

**Preharvest sprays**

>Kern Sun= peach inking intensity was highest on fruit from the Benlate 3 DBH and Rovral 1 and 3 DBH treatments (Table 1). The Rovral 7 DBH, Ronilan 8 DBH, and Funginex 1, 3, and 7 DBH treatments did not induce any commercially important inking development on peach fruit from >Kern Sun=. Non-abraded fruit did not present inking symptoms in any of these treatments. Fruit from the commercial treatment (Rovral applied at bloom time) did not exhibit any commercially significant inking (Table 1). The role of fungicides acting as contaminants in inking incidence has been demonstrated in our previous trials. Fruit from the Rovral 3 DBH, Funginex 1 and 3 DBH treatments were not commercially affected. Z.I.P. applied 22 DBH did not induce inking on peaches but did induce inking when applied within 18 DBH (Table 1).

**Pesticide chemical analysis**

High iron, aluminum, and copper, and low tin concentrations were detected in most of the pesticides analyzed in these studies (Table 2). High iron (1,930-2.5 ppm) and aluminum (3,920-<5.0 ppm) concentrations were measured in Imidan 50WP, Topsin, Benlate, Rovral, Dipel 2x, and Rally. Foli-Cal, Funginex, Rally, and Topsin had copper concentrations ranging from 1,960-6.95 ppm. Tin levels were usually below 5 ppm in most of the chemicals analyzed.
Recommendations

Based on these experiments the following recommendations are suggested to help growers reduce inking problems.

1. Reduce fruit abrasion damage.
   A. Handle fruit gently.
   B. Avoid long hauling distances.
   C. Keep harvest containers free of dirt.

2. Reduce contamination of fruit.
   A. Keep harvesting equipment clean.
   B. Avoid dust contamination on fruit.
   C. Check water quality for heavy metal (Fe, Cu & Al) contamination
   D. Do not spray foliar nutrients containing heavy metals within 20 DBH (unless there is a deficiency).
   E. Tentative preharvest application intervals for the following fungicides and foliar nutrients were developed:
      Foliar nutrients containing heavy metals = 20 DBH, Benlate = 12 DBH, Rovral = 7 DBH, Funginex = 3 DBH and Ronilan = 1 DBH.

3. In case of a possible inking situation with peach and/or nectarine, delay packaging for 48 hours to detect fruit inking damage during grading.

4. As a short-term solution, we suggest chemical manufacturers (foliar nutrients, fungicides and insecticides) develop preharvest application intervals to avoid inking.

5. As a long-term solution, we suggest chemical manufacturers attempt to identify and remove
remove possible sources of contamination from products that may contribute to inking.

References


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FIGURE CAPTIONS

Fig. 1. Influence of harvest operations on percentage with inking and percentage of culls of Flavorcrest, Elegant Lady, and O=Henry fruit collected at harvest: 1) directly from the tree and field-packed (tree), 2) after bin filling and transport in the orchard to the loading point (orchard), and 3) after handling and transport to the packinghouse (packinghouse). Each value represents an average of three cultivars using four replications of 18 fruit each. Vertical bars represent SE.

Fig. 2. Development of dark discoloration of peach and nectarine fruit skin discs in various phosphate buffer pH solutions. Treatments: UW = unwashed, W = washed, NA = non-abraded, and A = abraded. Vertical bars represent SE.

Fig. 3 Dark discoloration of peach and nectarine fruit after 15 min incubation in metallic ion solutions (dH2O, pH 3.5). Treatments: UW = unwashed, W = washed, NA = non-abraded, and A = abraded. Vertical bars represent SE.

Fig. 4 Change in color (Δa*) of skin discs from peach and nectarine fruit in response to different iron concentrations of solutions. Treatments: UW = unwashed, W = washed, NA = non-abraded, and A = abraded. Vertical bars represent SE.
Table 1. Influence of fungicide sprays on 'Kern Sun' peach inking incidence.

<table>
<thead>
<tr>
<th>Treatments*</th>
<th>DBH</th>
<th>Inking intensity (mm²)H</th>
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<tbody>
<tr>
<td>Rovral I (bloom only)</td>
<td>-</td>
<td>6.5</td>
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<tr>
<td>Rovral</td>
<td>1</td>
<td>68.80</td>
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<td>Rovral</td>
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<td>37.94</td>
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<tr>
<td>Funginex</td>
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<td>Funginex</td>
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<td>2.62</td>
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<tr>
<td>Funginex</td>
<td>7</td>
<td>7.86</td>
</tr>
<tr>
<td>Benlate</td>
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<td>78.51</td>
</tr>
<tr>
<td>Ronilan</td>
<td>1</td>
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<td>Ronilan</td>
<td>8</td>
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<tr>
<td>Z.I.P.</td>
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<tr>
<td>Z.I.P.</td>
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<td>77.0</td>
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<tr>
<td>Z.I.P.</td>
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<td>30.0</td>
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<td>Water</td>
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<td>LSD 0.05</td>
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<td>20.8</td>
</tr>
</tbody>
</table>
Table 1. Footnotes

* Air-blast (100 gallons/acre).

H Fruit with areas equal to or greater than 63.6 mm² are rejected according to California quality standards.

I Rovral spray at bloom.
Table 2. Heavy metal concentrations of selected chemicals used commercially by the stone fruit industry.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Aluminum</th>
<th>Copper</th>
<th>Iron</th>
<th>Tin</th>
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<td>Benlate</td>
<td>3,690.0</td>
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<td>Rovral</td>
<td>55.2</td>
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<td>29.3</td>
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<td>Funginex</td>
<td>&lt;5.0</td>
<td>18.4</td>
<td>&lt;2.5</td>
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<tr>
<td>Ronilan DF</td>
<td>&lt;10.0</td>
<td>&lt;1.0</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
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<td>Topsin M 70WP</td>
<td>1,740.0</td>
<td>9.6</td>
<td>1,930.0</td>
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<td>Rally 40W</td>
<td>3,920.0</td>
<td>7.0</td>
<td>864.0</td>
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<td>Imidan 50WP</td>
<td>2,410.0</td>
<td>&lt;1.0</td>
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<td>Dipel-2X</td>
<td>30.6</td>
<td>&lt;1.0</td>
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<tr>
<td>Omite 30</td>
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<td>N.D.</td>
<td>N.D.</td>
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* mg/Kg of chemical.

H None detected.

I Not tested.