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Development of Dark Skin Discoloration on Peach and Nectarine Fruit in Response to Exogenous Contaminations

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Abstract. **Dark skin discoloration development on peach and nectarine cultivars was investigated in response to exogenous pH and metallic ions. The influence of skin abrasion and washing in combination with exogenous contaminants was studied in a factorial design experiment by using skin discs. Only abraded skin discs with and without washing developed discoloration after being exposed to high pH and different metallic ion concentrations. Among the metallic ion contaminants studied (Fe, Al, Cu, Sn, Zn, and Na), iron was the most effective in causing dark skin discoloration at physiological pH (3.5). Iron concentrations ≥10 ppm induced dark discoloration on abraded fruit skin. Dark discoloration development produced by exposing the skin tissue to pH levels >6 was reversible, whereas the dark discoloration induced by iron and aluminum remained stable.**

Skin discoloration, or inking, is one of the major postharvest cosmetic defects on fresh market peaches and nectarines because it affects the visual appearance and, therefore, the marketability of the fruit. The disorder has been reported in all major national production areas (Crisosto et al., 1991, 1992, and 1993; Denny et al., 1986; Hopfinger and Frecon, 1986; Ing, 1992; Phillips, 1988). The discolorations appear as spots or stripes of various shapes in black, blue, brown, and other colors. The disorder is commonly observed in the packinghouse and after transit and storage (Phillips, 1988; Ridley et al., 1976; Van Blaricom and Webb, 1965), but also during harvesting and hauling operations (Crisosto et al., 1993).

Skin discoloration development is associated with physical injury occurring during fruit handling (Crisosto et al., 1993) and transportation (Phillips, 1988). A recent anatomical study, using light microscopy and Scanning Electron Microscopy (Crisosto et al., 1993), demonstrated that the physical injury associated with discolored skin spots was abrasion. The epidermal cells in the discolored spots were broken but those in nondiscolored skin spots were intact, while the fresh tissue cells (hypodermis and mesocarp) underneath the epidermis were intact in both cases. A common phenomenon following physical injury to plant tissue is browning due primarily to phenolic oxidation resulting from the mixing of phenolics and polyphenoloxidases upon the collapse of cellular compartmentation (Mellenthin and Wang, 1974; Rouet-Mayer et al., 1990). Phenolic oxidation is likely the cause of the brown skin discoloration found on injured peach and nectarine fruit.

The dark discoloration (black, blue, purple spots) probably emanates from nonoxidation reactions involving anthocyanins (Cheng et al., 1992; Crisosto et al., 1991; Denny et al., 1986), which are abundant in the skin cells of peach and nectarine fruit (Hsia et al., 1965; Van Blaricom and Senn, 1967). One type of the non-oxidation reactions involves the transformation of the molecular structure of anthocyanins at high pHs (Timberlake and Bridle, 1980). The color of anthocyanins depends on the pH of the solution. The pigments exist mainly in the red-colored flavylium salt form at low pH (1.0). As the pH rises, the pigments gradually transform into the colorless carbinol pseud-bases from pH 4 to 5 (Jurd and Asen, 1966). Further increases in pH lead to the development of purple color and above pH 7, blue color, due to the formation of the blue quinoidal base. Another type of the non-oxidation reaction that can cause dark discoloration is the formation of metallo-anthocyanin complexes at physiological pH (Bayer et al., 1966; Jurd and Asen, 1966; Osawa, 1982). Anthocyanins such as cyanidin-3-glucoside can react with metallic ions to give derivatives of blue and other colors. The extent of the metallo-anthocyanin formation also depends on the ion species.

As micro-environmental conditions of a high pH and metallic iron often exist during postharvest fruit handling, understanding the fruit's response to different pHs and metallic ions is important to reduce the skin discoloration disorder. Several studies of the anthocyanins' response to exogenous pH and metallic ions have been done using solution systems (Asen et al., 1969; Bayer et al., 1966; Jurd and Asen, 1966), but none have reported using fruit skin tissue.

The objective of this research, conducted with fruit skin discs as a model system, was to investigate the effects of abrasion in combination with washing on the development of fruit skin discoloration under controlled exposure to metallic ions and a range of pH.

Materials and Methods

Plant materials. Fruit of 'Flavorcrest', 'Elegant Lady', and 'O'Henry' peaches and 'May Glo' and 'Flaming Red' nectarines were randomly picked from the orchard of the University of California Kearney Agricultural Center at commercial maturity according to ground-color maturity chip. Some characteristics of the fruits are presented in Table 1. Fruit were carefully handled to avoid physical damage.

Effects of pH and metallic ions. 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 5 min, rinsed with distilled water (dH_2O), and placed on clean paper towels to air-dry. The purpose of the washing treatment was to remove metallic ions and/or other contaminants on the fruit surface.

Each treatment was applied either to nonabraded fruit (NA) or

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Table 1. Peach and nectarine fruit characteristics at commercial maturity.

Variety	Skin pH	Fruit SSC (%)	Fruit firmness[z] (N)	Skin anthocyanins[y] (mg·g^{-1} DW)
May Glo	3.6	9.8	55.7	1.2
Flavorcrest	3.8	11.0	55.9	0.9
Elegant Lady	4.0	12.2	45.9	1.0
O'Henry	4.0	9.6	43.4	1.0
Flaming Red	4.1	10.2	54.5	1.0

[z]Flesh firmness measured with an UC firmness tester having a 8-mm tip.
[y]In equivalent of cyanidin-3-glucoside; DW = dry weight.

abraded fruit (A). The abrasion was induced with a rotatable automatic toothbrush and a fruit holder. 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 nonabraded or abraded, using a cork borer (44 mm^2 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 gentle stirring. The pH and ion solutions were selected based on preliminary results from experiments carried out during the 1991 growing season. For pH treatments, solutions of pH 3, 4, 5, 6, 7, 8, and 9 were prepared with phosphate buffer (0.1 m). For ion treatments, solutions of sodium, aluminum, ferrous iron, copper, tin, and zinc ions were prepared in final ion concentration of 100 ppm in dH_2O. 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 dH_2O and an untreated check was used for abraded and nonabraded subtreatments. After 15 min, the discs were taken out, blotted with Kimwipes, and air-dried for ≈1 min, and the color was measured again. Discoloration was expressed by the relative change in color value hue a*, assigned Δa*, since it amply reflected the visual darkening on the skin discs. The Δa* was calculated by subtracting the $a^*_{bef\text{-}aft}$ of the treated skin discs with that of the control skin discs. The $a^*_{bef\text{-}aft}$ was the difference of the measured a* before and after incubation. A higher value of Δa* reflected a darker discoloration after incubation.

Fruit analysis. Flesh firmness was measured using a Univ. of California firmness tester with an 8-mm tip (Western Industrial Supply, San Francisco). Skin from opposite cheeks of each fruit was removed and flesh firmness was calculated as an average of two determinations per fruit. A wedge from each fruit within a replication was removed and combined into a composite sample from which juice was extracted with a hand press and filtered through cheesecloth. The soluble solids content was then determined with a temperature-compensated refractometer. Fruit skin pH was determined with a pH meter using a supernatant prepared by homogenizing the peeled skin strips in distilled water (2 ml·g^{-1}) and centrifuging for 10 min at 20,000× *g*. The peeled skin strips had been scraped to remove as much of the flesh as possible.

For estimation of skin anthocyanin and mineral levels, a composite sample was collected from a skin sample with skin peeled from five fruit. Three composite samples were collected for each cultivar and used for analysis. The skin was peeled and the flesh tissue was scrapped off just before freezing in liquid nitrogen and freeze-dried. Anthocyanins in the freeze-dried skin were extracted with methanol containing 0.01% HCl. The skin material was pale in color after three extractions. The combined extracts were condensed with a Speed Vac (model RC1010, Jouan, Winchester, Va.) and centrifuged as above to remove suspended materials. Anthocyanin content in the extracts was determined by using a spectrophotometer (model Spectronic 21; Bausch & Lomb, Rochester, N.Y.) and the pH differential method (Daravingas and Cain, 1968) using wavelengths 512 nm and 700 nm and a molar extinction coefficient of 26,900. Measurements were triplicated and expressed as milligrams of cyanidin-3-glucoside per gram dry weight of fruit skin, using molecular weight of 449 ($C_{21}H_{21}O_{11}$). Fruit mineral analysis was done by the DANR Analytical Laboratory of the University of California.

Statistical analysis. Mean differences were analyzed by F test (LSD) using analysis of variance procedures (ANOVA) of the Statistical Analysis System (SAS) for the personal computer program (SAS Institute, 1992).

Results

Responses of skin discs to pH solutions. Dark discoloration did not develop on skin discs of nonabraded fruit either unwashed or washed when exposed to pH solutions from 3 to 9 in all peach and nectarine cultivars tested (Fig. 1). 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', and 'O'Henry'. Treatment in a

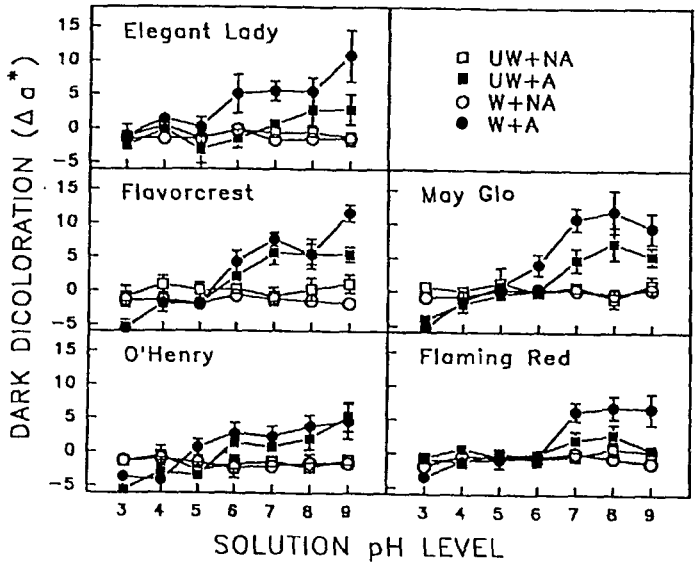

Fig. 1. Development of dark discoloration of peach and nectarine fruit skin discs under various phosphate buffer pH solutions. Treatments: UW = unwashed, W = washed, NA = non-abraded, and A = abraded. Vertical bars represent SE.

buffer of pH 4 and pH 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. 1).

Washing treatment induced significant dark discoloration only on abraded 'May Glo' and 'Flaming Red' nectarine. Abrasion treatment significantly increased development of dark discoloration in all of the tested cultivars (Table 2).

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 A 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. 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 'Lancey' peach and 'Flamekist' nectarine. The results showed that the dark discoloration caused by high pH gradually disappeared after about 3 to 5 h leaving red to light brown spots (data not shown).

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 solution treatments in all of the cultivars (Fig. 2).

On A skin discs with or without washing, ions of iron, aluminum, and copper caused evident discoloration (Fig. 2). The discoloration caused by iron and aluminum ions was black and, of the ions tested, iron caused the most severe discoloration. Sodium, stannous, or zinc ion solutions had little effect on the Δa* of the skin discs (Fig. 2), 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 4 days (data not shown).

Response of skin discs to iron concentration. Since iron was the most effective ion in causing skin discoloration, a concentration

Table 2. Relationship between washing and abrasion treatments on pH-related fruit skin dark discoloration of peach and nectarine cultivars.

Treatment	Dark discoloration (Δa*[z])				
	MG[y]	FC	EL	O'H	FR
Washing × abrasion					
Unwashed + nonabraded	0.6	0.6	−0.8	−1.6	0.2
Unwashed + abraded	5.8	5.4	2.0	2.6	1.8
Washed + nonabraded	0.8	−1.5	−1.6	−1.8	−0.7
Washed + abraded	10.7	8.2	4.1	3.5	6.6
Significance					
Washing	*	NS	NS	NS	*
Abrasion	*	*	*	*	*
Washing × abrasion	*	*	*	*	*

[z]Δa* = Dark color development, higher value indicates darker color. Pooled data were used (treatments of pH 7, 8, and 9).
[y]MG = May Glo; FC = Flavorcrest; EL = Elegant Lady; O'H = O'Henry; and FR = Flaming Red.
[NS, *]Nonsignificant or significant at $P = 0.05$.

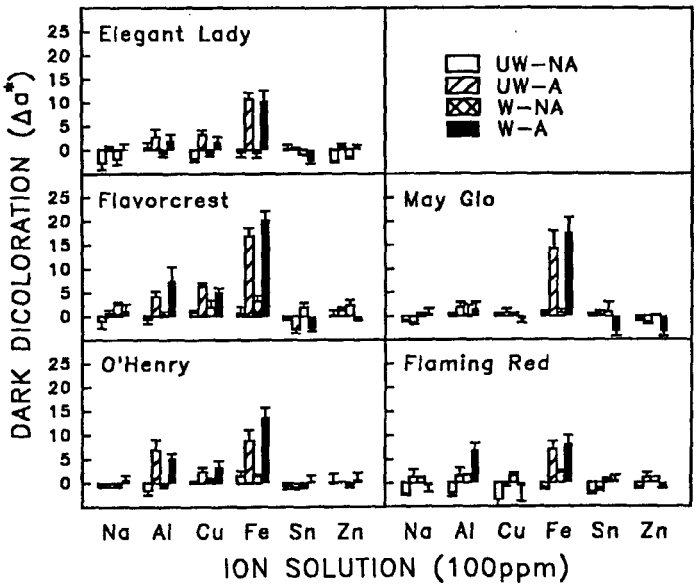

Fig. 2. Dark discoloration of peach and nectarine fruit after 15 min incubation in metallic ion solutions (dH_2O, pH 3.5). Treatments: UW = unwashed, W = washed, NA = non-abraded, and A = abraded. Vertical bars represent SE.

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 for all cultivars after 15 min incubation (Fig. 3). With A discs, an iron concentration as low as 25 ppm caused dark discoloration in 'May Glo', and 'Elegant Lady', 'Flavorcrest', 'O'Henry', and 'Flaming Red' (Fig. 3). The Δa*s on skin discs of W–A fruit were generally higher than those of UW–A fruit (Fig. 3). Iron concentration >50 ppm resulted in no significant change in a* value and thus the Δa* values of skin discs treated with 50 to 200 ppm iron solutions for each cultivar were pooled and used for treatment comparison of discoloration response to iron. Washing treatment induced significant dark discoloration on 'Flavorcrest', 'Elegant Lady', and 'Flaming Red', but not on 'May Glo' and 'O'Henry'. Abrasion treatment always increased dark discoloration on either unwashed or washed fruit. There was no significant

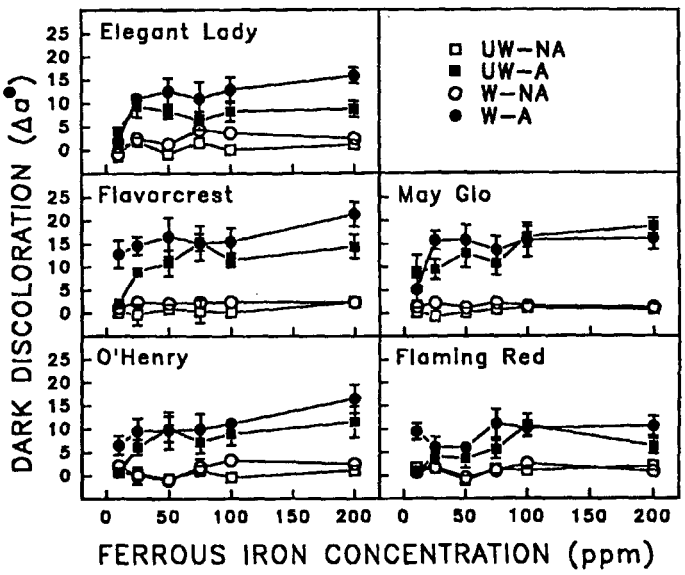

Fig. 3. Responses of skin discs in Δa* to different iron concentration solutions in peach and nectarine fruit. Treatments: UW = unwashed, W = washed, NA = non-abraded, and A = abraded. Vertical bars represent SE.

interaction between washing and abrasion treatments (Table 3).

The levels of dark discoloration caused by iron treatments (Fig. 3) were higher than those caused by high pH (Fig. 1) treatments.

Fruit analysis. There were no significant metallic ions (Fe, Al, Cu, etc.) concentration differences between skin samples of UW and W fruit in all of the cultivars (data not shown). Skin of washed peaches and nectarines had ≈50 ppm and ≈60 ppm iron and aluminum, respectively, on a dry-weight basis.

Discussion

This study has demonstrated that, under controlled laboratory conditions, abrasion in combination with exogenous iron contamination is a prerequisite for dark skin discoloration. Without skin abrasion, no dark discoloration was observed on fruit skin disks exposed to solutions of high pH or to metallic ions. It has been reported that abrasion or rubbing injures epidermal cells (Crisosto et al., 1993), in which vacuoles release anthocyanins and other phenolics, causing skin discoloration on peach and nectarine fruit. Abrasion is probably the major contributor to the physical injuries associated with the discoloration reported on fruit after transit (Phillips, 1988) and during packinghouse operations (Ridley et al., 1976). Discoloration caused by dropping a solution of 0.1 *N* potassium hydroxide on a peach surface (Denny et al., 1986) might be explained by the ability of the alkali to destroy the fruit cuticle, penetrate the epidermal cells, raise the pH of the cell sap, and lead to the formation of the anthocyanin-blue quinoidal base in the cell sap.

The absence of naturally occurring exogenous metallic ion contaminants in the fruit samples in our study explains why the dark discoloration development was not observed in the UW–A treatments. Nonetheless, the fact that exogenous iron concentrations ≥25 ppm in combination with abrasion induced dark color discoloration in all of the tested cultivars indicates the important role of the exogenous contaminants in dark skin discoloration. Iron and aluminum accumulation on the fruit would depend on preharvest sprays, dust, timing of the spray applications in relation to harvest, and rainfall before harvest. Although this disorder is induced during the harvesting operation (Crisosto et al., 1993), it is detectable later during the packinghouse operations.

We observed in our study that W fruit tended to suffer and develop more dark discoloration, suggesting that the physical properties of the skin could influence the fruit's susceptibility to abrasion and subsequent discoloration. Thus, differences in initial tissue water status may explain the inconsistency of washing treatment to promote dark discoloration among the cultivars.

Table 3. Relationship between washing and abrasion treatments on iron-related fruit skin dark discoloration of five peach and nectarine cultivars.

	Dark discoloration (Δa^{*z})				
Treatment	MG[y]	FC	EL	O'H	FR
Washing × abrasion					
Unwashed + nonabraded	0.7	1.5	0.5	0.3	0.9
Unwashed + abraded	14.7	13.0	7.9	3.5	6.1
Washed + nonabraded	1.5	2.8	2.5	1.7	1.6
Washed + abraded	15.3	17.1	13.1	11.8	9.5
Significance					
Washing	NS	*	*	NS	*
Abrasion	*	*	*	*	*
Washing × abrasion	NS	NS	NS	NS	NS

[z]Δa^* = Dark color development, higher value indicated darker color. Pooled data were used (treatment of 50, 75, 100, and 200 ppm iron).
[y]MG = May Glo; FC = Flavorcrest; EL = Elegant Lady; O'H = O'Henry; and FR = Flaming Red.
[NS,*]Nonsignificant or significant at $P = 0.05$.

Our results support the hypothesis that anthocyanins and other phenolics are the major contributors to dark discoloration when peach and nectarine tissue are exposed to high pH, and/or high iron and aluminum ion concentrations under physiological pH. The color changes of abraded skin discs in pH solution between 3 and 9 were similar to that of pure cyanidin-3-glucoside (Jurd and Asen, 1966). The absence of anthocyanins and lack of dark discoloration in the flesh tissue underneath the skin discs also supported our hypothesis. Further supporting evidence comes from the fact that the dark discoloration induced by high pH was reversible, which is a characteristic of anthocyanins. In many studies (Bayer et al., 1966; Jurd and Asen, 1966; Osawa, 1982), combining anthocyanins with iron or aluminum in aqueous solutions has formed metallo-anthocyanin complexes producing a dark discoloration. Involvement of anthocyanins as phenols in this dark discoloration disorder is also indicated by the observation that they commonly appear on the red surface area (blush) of the fruit, as observed by us and by others (Denny et al., 1986, Hopfinger and Frecon, 1986). However, the possible involvement of other phenolics, such as chlorogenic acid and catechin as well as other metabolites such as organic acids, on dark skin discoloration should not be omitted. The role of these phenolics and iron under fruit epidermal cell conditions (pH = 3.5–5.0) on dark skin discoloration incidence should be studied.

The amount of anthocyanin in most of the cultivars' skin cells seems to be sufficient for the discoloration reaction because we did not find any correlation between levels of anthocyanins and the dark discoloration levels among cultivars in previous work (Crisosto et al., 1991, 1992). For this reason, we believe that the amount of anthocyanins in different cultivars is not a limiting factor in dark discoloration development. The cultivars used in this study did not differ in skin anthocyanin content (Table 1).

Reversion of dark discoloration may result from a re-acidification of the wounded skin tissues from surrounding living cells. Since the blended skin tissues had a pH of 4, at which the anthocyanins should be colorless, it is also possible that the return of redness in the abraded tissue may be a copigmentation reaction (Asen, 1976; Jurd and Asen 1966). The fact that the dark discoloration caused by iron and aluminum did not fade for at least 4 days after it developed indicated that the complexes formed by anthocyanin, other phenolics, and these metal ions were stable under the microenvironmental condition in the skin tissue. Therefore, dark skin discoloration caused by iron or aluminum contamination will persist during fruit transportation and storage. During our 2-year survey of hydrocooling and dumping tank water quality, we never found iron concentrations >2 ppm in several packinghouses in the San Joaquin Valley, California (unpublished data).

In summary, abrasion injury is the prerequisite cause of skin dark discoloration on stone fruit in the presence of high pH or high concentrations of iron or aluminum. Physical characteristics of the skin tissues may play a role in the development of dark discoloration by affecting the skin's susceptibility to abrasion injury.

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