Updating Peach, Nectarine and Plum Inking and/or Skin Discoloration Development Information

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Abstract

Our several years results seem to indicate that peach and nectarine skin discoloration, also called inking disorder, is triggered by the combination of physical damage during harvesting-hauling combined with 'post-harvest stresses'. Our previous seasons results indicate low energy physical injury (abrasion) combined with specific metal contamination, will induce development of this damage on the fruit. Abrasion damage releases anthocyanin/phenolic pigments, which are located in the skin cells, allowing the development of inking damage when cells are exposed to these 'stressful' situations. During the latest season, we have corroborated our previous results by demonstrating that low energy physical injury combined with metal contamination or specific fungicides will cause skin discoloration of peaches and nectarines.

However, a high level of a new skin discoloration damage incidence has been observed recently. As the symptoms are a little bit different than the traditional field inking previously reported, we have called this blemish, which is frequently high in white flesh cultivars, as "skin burning disorder". One unique characteristic of this blemish, contrary to our traditional field inking, is that the incidence increases after packing, handling, and especially cooling. Our new findings show that other post-harvest stresses different to exposure to heavy metal contamination such as exposure to high air velocity during forced air cooling (FAC) or high water pH during washing-brushing, will induce the development of this skin burning on some cultivars, when combined with previous physical damage. Although sometimes the symptoms of the traditional field inking and this new reported skin burning disorder can be similar, we will make a distinction between both skin damage types depending on their origin from now on. The skin burning disorder seems to be induced by the combination of physical injury (abrasion) with high pH sanitation water and/or high speed forced air cooling carried out during the post-harvest period.

To reduce skin burning incidence, physical damage during pre- and post-harvest periods must be minimized, at the same time that metal contamination, exposure to high pH solutions, and exposure to high speed forced air cooling should be avoided in susceptible cultivars.

Objectives

- Identify new sources of heavy metal contamination as a precursor for inking development.
- Determine minimum heavy ions concentration (Fe, Cu and Al) necessary to trigger the metallo-pigment reaction and formation of dark spots.
- Screen pre-harvest for potential inking precursors and post-harvest additives (such as chlorine, ozone, fungicide, detergents, etc.) for inking development.
- Study pigment composition and antioxidant capacity on the metallo-pigment inking reaction.
- Attempt practical ways to prevent inking and even reverse inking damage.
- Test other potential causes of inking development in the California industry.

This information will be fundamental to understanding inking and burning disorder development and triggering, and for generating recommendations to eliminate and/or reduce tree fruit industry losses due to inking and skin burning reported worldwide.

Introduction

Inking has become an increasingly frequent skin disorder on peach and nectarine fruits for several decades in different production areas such as California, Washington, Italy, Australia, Argentina and Chile (Crisosto et al., 1999; Denny et al., 1986). Inking or skin discoloration is characterized as discolored brown-and-black spots, and the damage is restricted to the skin. Although inking affects only the fruit's cosmetic appearance, the disorder causes economic losses to the peach and nectarine industries each year because blemished fruit are not marketable (Cheng and Crisosto, 1994; Crisosto et al., 1993).

Abrasion injury is one of the major precursors of inking (Crisosto et al., 1993). The other precursor for inking development is the metallo-anthocyanin pigments released from damaged skin cells, where they are located, that collapsed while the underlying fleshy cells (mesocarp cells) remained intact (Crisosto et al., 1993). Our previous work indicated that the presence of metallic ions such as iron (Fe), copper (Cu) and aluminum (Al) were also an important precursor for inking development (Crisosto et al., 1999). Therefore, abrasion and heavy metal contamination need to be avoided. However, it is well worth noting that other factors or chemical compounds that we have not identified may also be involved in inking formation. Abrasion damage releases phenolic pigments such as anthocyanin, chlorogenic acid, etc., which are located in the skin cells, allowing the reaction of these pigments with iron and/or aluminum metal contaminants (Arakawa and Ogawa, 1994; Cheng and Crisosto, 1997; Crisosto et al., 2000). However, after different seasons of observations, we hypothesize that different practices during post-harvest handling, packaging and cooling process, such as contact to high pH sanitation water, waxes and fungicides, or cell dehydration due to forced air cooling, could also be important factors in inking development on peaches and nectarines, and therefore need to be studied. Since many new foliar-nutrient, fungicide, and insecticide chemicals have become available for the tree fruit industry in the last decade, we screened many of them for iron and aluminum concentrations and its role in inking development. Among them, we identified several new chemicals that have high concentrations of iron and/or aluminum that may be involved in the inking formation.

Recently, a high level of a new inking incidence type has been observed during our interactions with cooperators and discussion with visitors (Fig. 2). As the symptoms are a little bit different than the traditional field inking (Fig. 8) previously reported, we have called this blemish which is frequently high in white flesh cultivars as "skin burning disorder". One unique characteristic of this blemish, contrary to our traditional field inking, is that the incidence increases after packing, handling, and especially cooling. In fact, it was brought to our attention that most of the damage was observed on the exposed part of the fruit above tray cavity and no damage symptoms occurred under the price-look-up sticker. Sometimes the symptoms of the traditional field inking and this new reported skin burning disorder can be similar and difficult to differentiate, though. From now on, we will use different terminology to refer to different types of damages:

1. **Inking**
    a. For the traditional inking developed in the field and observed at fruit harvesting
2. **Skin Burning**
    a. For the skin damage developed after packing and handling, and due to exposure to high pH and/or high forced air cooling velocity, combined with pre- and/or post-harvest abrasion

Forced-air cooling (FAC) is a widely used method for cooling fruits, vegetables, or cut flowers. Cooling is achieved by forcing cold air through containers and past individual pieces of product. One of the disadvantages of FAC is that it causes excessive water loss in some commodities (Thomson et al., 2002). Because air flow past a product sweeps released moisture from the air surrounding the product and speeds moisture loss, FAC may not be appropriate for some wilting-sensitive products (Thomson et al., 2002). Cold room (CR) produces the least moisture loss when compared to horizontal forced air cooling (HFC) and vertical forced air cooling (VAC) (Edeogu et al., 1997). In this work we investigate whether the stress produced by the FAC on the fruit surface could be a triggering factor for the development of the skin burning disorder.

The remarkable economical impact of this problem for the fruit industry encourages further detailed research on answering new questions related to inking and burning formation.

Material and Methods

1. **Role of Metal Contamination and High pH on the Inking/Burning Development**

    Peaches and nectarines from different cultivars and sources were exposed to different pH solutions for inking and/or burning development according to our previous work (Cheng and Crisosto, 1994, 1995, 1997). White and yellow flesh cultivars with low and high acidity were used in this test. Cultivars were selected based on previous information and feedback from research committee.

    Each treatment was applied to non-abraded fruit and abraded fruit. The fruit was abraded with a rotatable automatic toothbrush. During abrasion, the fruit was hand-rotated forward and then backward while the automatic toothbrush head abraded the skin. The hand-rotation speed was kept as consistent as possible for all fruit samples. Intensity of abrasion was also tested in one of the experiments, abrading the fruit from 0 to 5 times.

    After 15 minutes of incubation on the tested solution, fruit were removed from the solution and allowed to air-dry for about 15 minutes. Effect of contact time with the solution was tested in one of the experiments, leaving the fruit immersed in the different ion or pH applications for 1, 15, 30 and

45 minutes. For pH treatments, solutions of pH 7, 7.5, 8, 8.5, 9, 9.5 and 10 were prepared with phosphate buffer (0.1 M). For ion treatments, solutions of ferrous-iron and aluminum were prepared in a final concentration of 1, 10 and 100 ppm. All solutions were prepared daily. The control treatment was dry fruit, in contact with no solution (air).

In each sub-treatment (abraded and non-abraded) six skin disks were randomly marked with a silver marker in the skin surface of each fruit to measure color change after the contact with the solution. Each disk was identified by the layout order during the following experiments. Before and after the treatment, color was measured 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). Discoloration was expressed by the relative change in the color value L* (lightness), since it reflects the darkening on the fruit skin. The change in L* was calculated as the difference between the L* before the treatment and the L* after incubation. The higher L* difference reflected a darker discoloration.

2. **Role of Forced Air Cooling on the Skin Burning Development**

The effect of the cooling-hauling process on burning disorder development was studied using 'Snow Princess', 'Autumn Snow' and 'Autumn King' white flesh peach cultivars. Fruit were harvested at commercial maturity according to ground color.

With the help of cooperators, we executed several large tests using different white flesh cultivars and post-harvest treatments. In order to test the effect of different handling operations over the incidence of inking on the white flesh peaches, we collected fruit at five different points during post-harvest handling:

1. Directly from the tree and field-packed
2. From totes at arrival at packing house after transporting from the orchard to the packing house
3. At three different points at the packaging line
    a. Short dry
    b. Long dry
    c. Long wet treatments

After sampling, fruit were carefully transported to the cold storage for the different cooling treatments. After the cooling operation, fruit were gently transported to Kearney Agricultural Center (KAC) to carry out the burning incidence evaluations. Burning incidence was defined as percentage of fruit showing well defined inked (light brown to black) areas larger than an aggregated area of 63 square millimeters. Potential burning areas (dark yellow to light brown) were also scored.

To quantify burning damage on the fruit, we scored visually every piece of fruit as potential damage or burning damage. Percentage of damaged fruit per treatment was then calculated.

Results and Discussion

1. **Role of Metal Contamination and High pH on the Inking/Burning Development**

Our previous work indicated that the presence of metallic ions such as iron (Fe) and aluminum (Al) were important precursors for inking development. At that time, we found that at least ~10 ppm Fe was enough for metallo-anthocyanin formation that results in inking development (Crisosto et al., 1999). In this season, we tested the effect of different concentrations of aluminum and ferrous-iron on the developing of inking disorder in different peach and nectarine cultivars.

Our results showed that solutions of aluminum and ferrous-iron at concentrations of 1 ppm may increase the inking burning damage compared to the control fruit. The darkening of the fruit skin was always more intense when fruit was previously brushed (Fig. 1), which indicates one more time the importance of previous abrasion (brushing) as the first step to the development of inking disorder. On the other hand, our results showed that the damage intensity triggered by iron and aluminum depends on the cultivar, which indicates that there are some cultivars more susceptible to inking damage than others. This result agrees with the whole inking investigation carried out by our team during this and previous seasons.

Post-harvest fungicides as Scholar® (fludioxonil), Bumper® (propiconazole), Mentor™ (propiconazole), and Elevate® (fenhexamid), were also evaluated for potential involvement in inking, since different sources of chemicals (additives, pesticides and foliar nutrients) used in commercial peach production may act as sources of contamination for inking development due to their confirmed heavy metals content (Crisosto et al., 1999).

Results showed that, in general, Mentor™ and Elevate® induced skin darkening on the fruit skin for the four different peach cultivars tested (Fig. 2), when compared with untreated fruit (air). On the other hand, Bumper® induced lighter skin staining (Fig. 6), inducing a little bit different symptoms than the black staining induced by Mentor™ and Elevate®. In the case of Scholar®, the effect varied depending on the cultivar tested. Scholar® treated fruit resulted in lighter skin stains after treatment in fruit from 'Grand Pearl', 'Fay Elberta' and 'Snow Princess', whereas darker skin staining was observed in 'JH Hale'. These preliminary results show that fungicides may act as a source of contamination for inking development after abrasion caused in other moments of the harvesting and handling process, and therefore should be controlled to minimize their negative effects.

Dark discoloration (black, blue, purple spots) probably emanates from non-oxidation reactions (metallo-pigments) involving anthocyanins, chlorogenic acid, and other phenolics, which are abundant in the skin cells of peach and nectarine fruit (Crisosto et al., 1999). Other types of the non-oxidation reactions involve the transformation of the molecular structure of anthocyanins at high pHs. The color of these pigments depends on the pH of the solution. It is known that pigments exposed to pH higher than 7.0 may trigger pigment changes from bright yellow-red to dark (inking). Since during commercial packing, fruit are exposed to high pHs at the washing and waxing steps, we studied how different pH solutions affect burning development. As explained above, we will use the term "burning skin disorder" for the damage which occurred during the post-harvest and handling operation, in order to differentiate it from the traditional inking damage which occurred in the field.

Our results showed that pHs over 8.0-8.5 induced dark staining (burning) in the different cultivars tested (Fig. 3 and 4). Extremely darker skin staining was observed after the fruit was exposed to pH 9.0, 9.5 and 10.0 solutions in susceptible cultivars. It is remarkable to note that the inking damage appeared in the abraded fruit, whereas no significant damage was observed in non-abraded fruit, even after exposure to the highest pH solutions. This result confirms our hypothesis that both burning and inking skin disorders are triggered by the combination of abrasion and posterior damage, namely specific metal contamination, exposure to high pH or dehydration due to FAC. The results also showed different susceptibility from cultivars to the development of skin burning triggered by exposure to high pH. 'Autumn Snow' showed the higher susceptibility to high pHs among the three different cultivars tested, showing intense dark staining after exposure to pHs higher than 9 (Fig. 3).

Abrasion intensity was a critical issue in the development of inking and/or burning disorder (Fig. 5). Darkening of fruit skin was found after the combination of intense abrasion of fruit skin with exposure to high pH (9.0) or ferrous-iron solutions. Non-abraded fruit showed none or much less inking and/or burning damage than abraded fruit for all the treatments and cultivars tested. However, no consistent differences were found among different intensity abraded fruit (Fig. 5). The explanation of this result could be that once the fruit skin is damaged, the collapse of the skin cells leads to the release of phenolic pigments that will trigger inking damage, when exposed to different 'post-harvest stresses' as high pH, heavy metals or dehydration. Therefore, no big differences are found at different levels of abrasion intensity, since the skin cells are already damaged and phenolic pigments are ready to react even with lesser damage.

On the other hand, contact time with high pH solution (pH 9.0) did increase the intensity of burning damage observed in the fruit from different cultivars (Fig. 6). Skin darkening increased simultaneously with the contact time with the solution in 'Snow King', 'Summer Sweet' and 'Snow Princess' white flesh peach cultivars (Fig. 6). No effect of contact time was found on 'Sweet Dream' fruit, however, probably due to its lower susceptibility to the development of skin burning induced by exposure to high pHs.

2. Role of Forced Air Cooling on the Skin Burning Development

Fruit gently picked-packed directly from the tree and /or totes did not develop high incidence of white flesh skin burning disorder after forced air cooling (FAC) for any of the three tests carried out with any of the three different white flesh cultivars (Fig. 7, 9 and 10). These results could be explained since abrasion damage was reduced in those treatments, and, as has been previously demonstrated in other works (Cheng and Crisosto, 1994, 1995; Crisosto et al., 1993), abrasion is the first step for inking and/or burning development. Therefore, fruit must be handled gently, avoiding long hauling distances and keeping harvest containers free of dirt to reduce abrasion damage and consequently, potential burning development.

White flesh skin burning disorder can be reduced and/or avoided on fruit that was subjected to any packing line system by using room cooling (RC) rather than FAC (Fig. 7, 9 and 10). Fruit that was subjected to any standard packing line system combined with FAC had a high incidence of this problem (Fig. 7). Post-harvest packaging treatments such as short dry and long dry packaging slightly reduced burning disorder incidence even when fruit was FAC after packaging. Post-harvest packaging treatments such as short dry, long dry and long wet packaging line did not make any difference on skin burning disorder when fruit was RC after packaging. In this commercial operation pH was kept at ~7.5. Fruit packed using perforated liners still showed high incidence of the problem when FAC. Fruit packed using pallet solid wrap showed low incidence of the problem when FAC, but the final fruit temperature after cooling period was undesirable during this test. Protection of fruit from dehydration during harvesting-storage-transportation-retail handling will reduce the development of this blemish.

When we compared the burning skin damage found in 'Autumn King' after different harvesting-cooling processes (Fig. 9), we could see that harvesting directly in totes reduced the burning incidence within any cooling system used, since abrasion is reduced when compared with regular packaging. In agreement with the results found for 'Autumn Snow', the most burning damaged fruit was found in FAC packed fruit, where the combination of skin abrasion with FAC dehydration resulted again in critical cosmetic damage. It is worthy to note the different percentage of burning damaged fruit between Room Cooling Packed and Room Cooling Totes. This result could be explained by the higher abrasion damage that packed fruit were subjected to previous to the cooling process. This result may show the importance of abrasion in the development of the burning discoloration. It is also noticeable that the percentage of damaged fruit from the Room Cooling Packing treatment on Autumn King cultivar (Fig. 9) was higher than the percentage of damaged fruit with a similar treatment (Long Wet Room Cooling) when performed on Autumn Snow cultivar (Fig. 7). This result could be explained by a different susceptibility of both cultivars to the skin burning disorder, or also by the different rate of physical damage induced in the packing process of both tests. However, in both tests, the abrasion produced during packing together with the stress induced by FAC resulted to be an explosive combination for the development of the skin burning disorder on both white flesh peach cultivars. Another interesting result was the lower percentage of damaged fruit observed when fruit was subjected to warm forced air compared to the regular FAC treatment. This result shows that the temperature of the air used for the FAC operation influences the extent of the stress induced on the fruit skin cells.

In order to gain a better understanding on how air velocity influences the development of the skin burning damage, we subjected regular packed 'Snow Princess' fruit to four different treatments (Fig. 10): warm (no cooling), room cooling (no forced air), and two different air velocity FAC (1.3 CFM/lb and 40 CFM/lb). Both air velocity FAC treatments showed higher percentage of damaged fruit than warm and room cooled fruit, in agreement with the previous results obtained with 'Autumn Snow' and 'Autumn King' cultivars. Besides, it could also be seen that higher air velocity caused the highest burning damage on the fruit skin, increasing from 60% of damaged fruit when FAC was at 1.3 CMF/lb to 100% of damaged fruit when FAC was at 40 CFM/lb (Fig. 10). Therefore, dehydration which occurred in the fruit skin cells due to high velocity cold air seems to be a factor to avoid for minimizing burning damage on sensitive white flesh peach cultivars. Anyway, future work in this direction needs to be done to fully understand the role of temperature and air velocity on the triggering of the white flesh burning disorder.

Based on our current work, it looks like the white flesh skin burning disorder is triggered by the combination of physical damage during harvesting-hauling combined with different 'post-harvest stresses'. Post-harvest stresses such as fruit exposure to high air velocity during forced air cooling (FAC) and/or high water pH during washing-brushing will induce development of this damage on the fruit. The fact that fruit picked-packed directly from the tree and/or totes did not develop high incidence of white flesh skin burning disorder after FAC pointed out that physical damage is a necessary factor to trigger this skin blemish problem as proved for traditional field inking disorder. In other field and laboratory tests, when fruit is exposed to high pH, skin burning disorder will develop in areas that have been physically damaged during harvesting-hauling. This may explain why fruit from the dry (no exposure to water-chlorine) packingline treatments had lower white flesh skin burning disorder than fruit that was wet during the packing operation.

Conclusions

The results found in this work, support well the approach on packaging these inking and/or burning susceptible white flesh peach cultivars without brushing and chlorine rinse to avoid skin damage during brushing and chlorination. Moreover, our results highlight the importance of reducing to the minimum the physical damage or abrasion on the fruit surface during pre- and/or post-harvest operations.

By the end of this season, our research team has a much better understanding of the 'white flesh skin burning disorders' and traditional inking problems, but we still have a lot of work to go into the mechanisms that cause this skin discoloration in depth, and hence being able to find practical ways to predict and eradicate these problems from our California stone fruit industry.

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Tables and Figures

Table 1. Different post-harvest handling treatments performed on 'Autumn Snow'.

Treatments	Treatment description	Sub-Treatment
Tree	Fruit was picked and packed directly from trees.	Room Cooling (RC)
Tree	Fruit was picked and packed directly from trees.	Forced Air Cooling (FAC)
Tote	Fruit picked and packed directly from totes at arrival at packinghouse.	Room Cooling
Tote	Fruit picked and packed directly from totes at arrival at packinghouse.	Forced Air Cooling
Short Dry packaging (SD)	Fruit was placed on the packingline after the washing-brushing-waxing, and water and wax were not used during their operation.	Room Cooling
Short Dry packaging (SD)	Fruit was placed on the packingline after the washing-brushing-waxing, and water and wax were not used during their operation.	Forced Air Cooling
Long Dry packaging (LD)	Fruit was placed on the packingline at dumping, but water and wax were not used during their operation.	Room Cooling
Long Dry packaging (LD)	Fruit was placed on the packingline at dumping, but water and wax were not used during their operation.	Forced Air Cooling
Long Wet packaging[z] (LW)	Fruit was placed on the packingline at dumping and water, chlorine and wax were used.	Room Cooling
Long Wet packaging (LW)	Fruit was placed on the packingline at dumping and water, chlorine and wax were used.	Forced Air Cooling
Long Wet Box Liner packaging (LWBL)	Fruit was packed using a perforated box liner and fruit was placed on the packingline at dumping where water, chlorine and wax were used.	Room Cooling
Long Wet Box Liner packaging (LWBL)	Fruit was packed using a perforated box liner and fruit was placed on the packingline at dumping where water, chlorine and wax were used.	Forced Air Cooling
Long Wet Full Pallet Solid Wrap packaging (LWFPSW)	Fruit was placed on the packingline at dumping and water, chlorine and wax were used. Then full pallet was wrapped using a solid liner prior to FAC.	Forced Air Cooling

[z] Water pH during brushing was maintained at 7.4-7.6 by using Muriatic acid and monitored by a pulse ORP system.

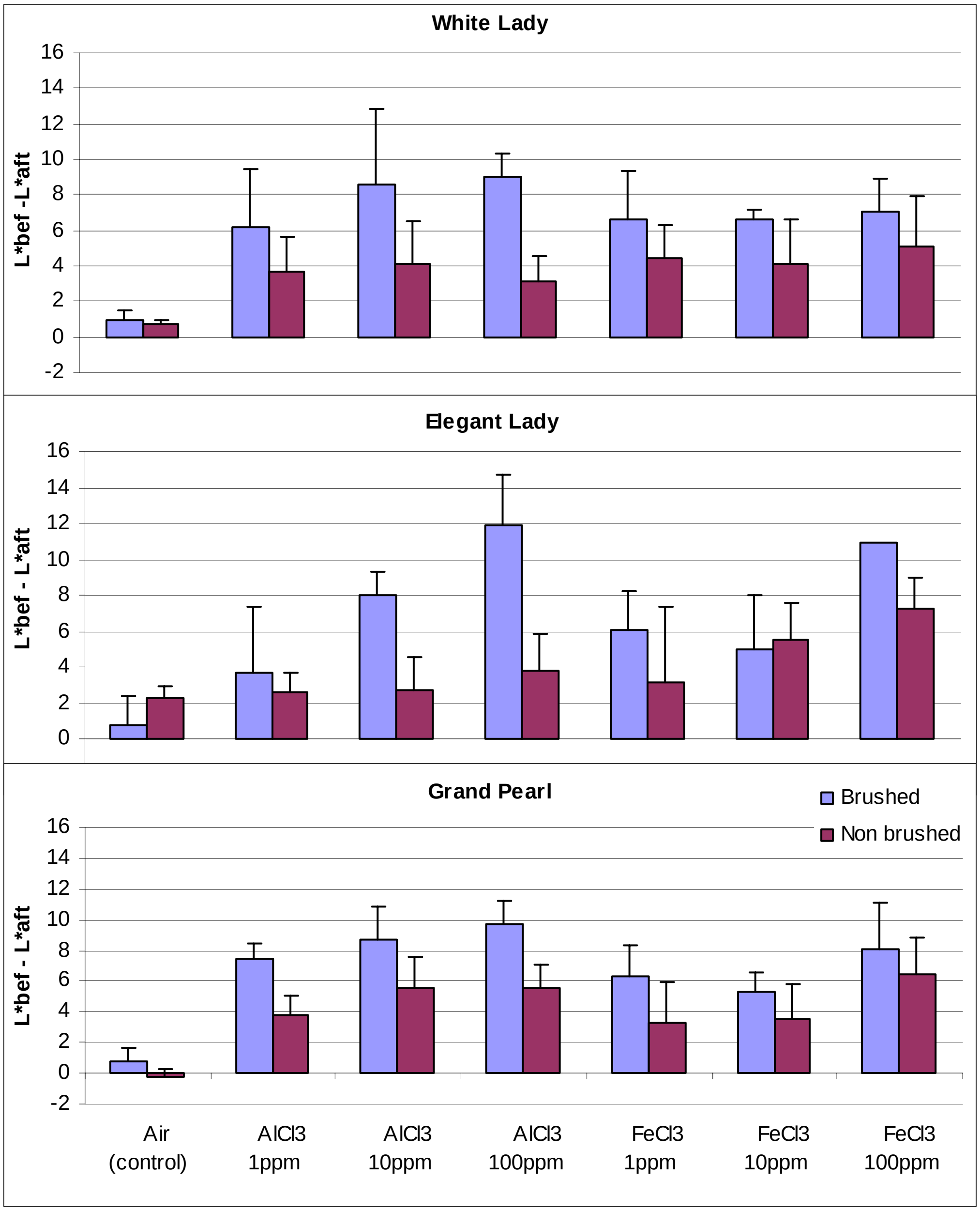

Figure 1. Increase of darkness (decrease of lightness, L*) on the fruit skin surface after exposing the fruit to different ferrous-iron and aluminum ($AlCl_3$) solutions. Air was used as control treatment in this experiment.

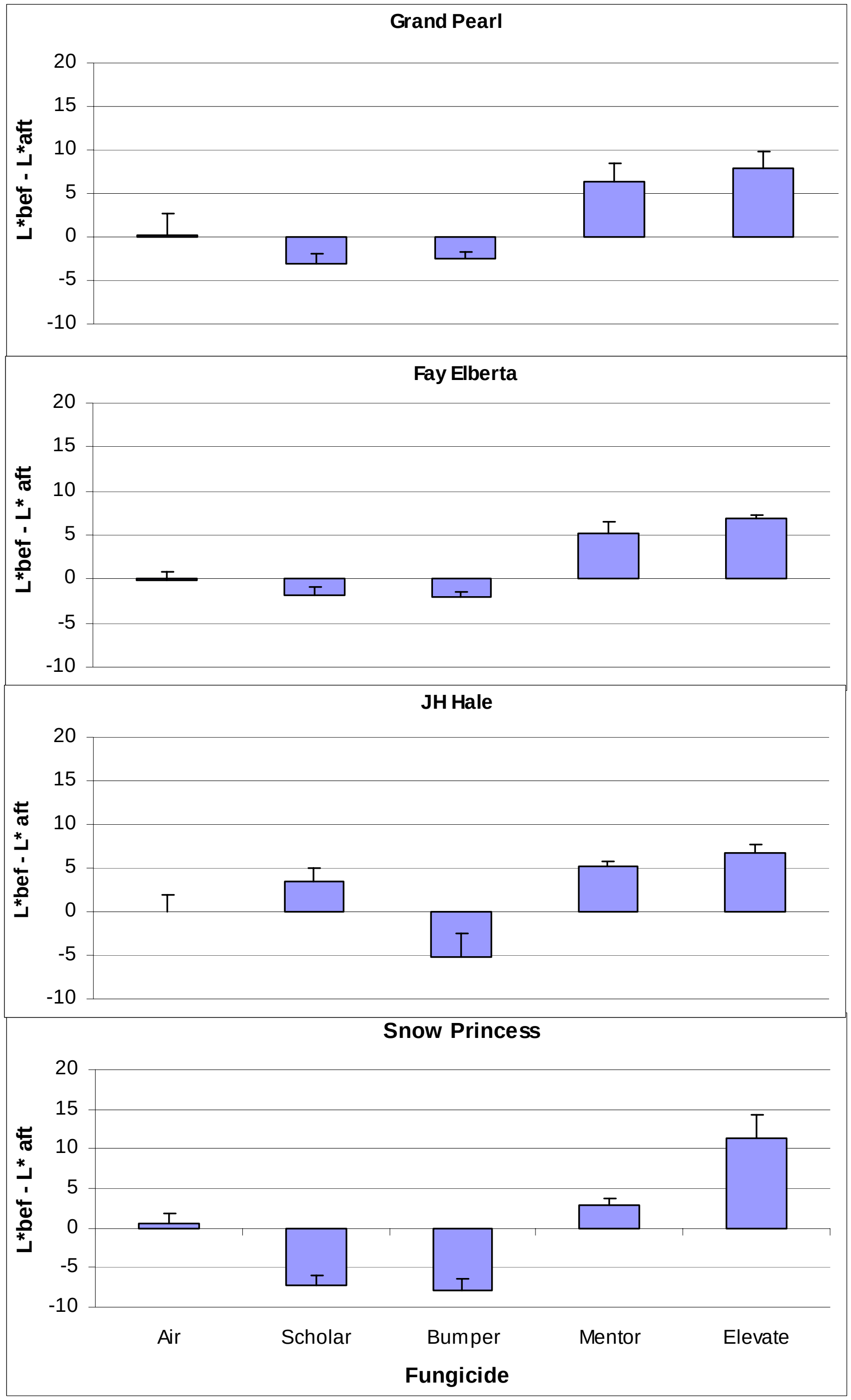

Figure 2. Effect of contact time with different fungicides (Scholar, Bumper, Mentor and Elevate) on the fruit skin darkening. Air was used as control treatment in this experiment.

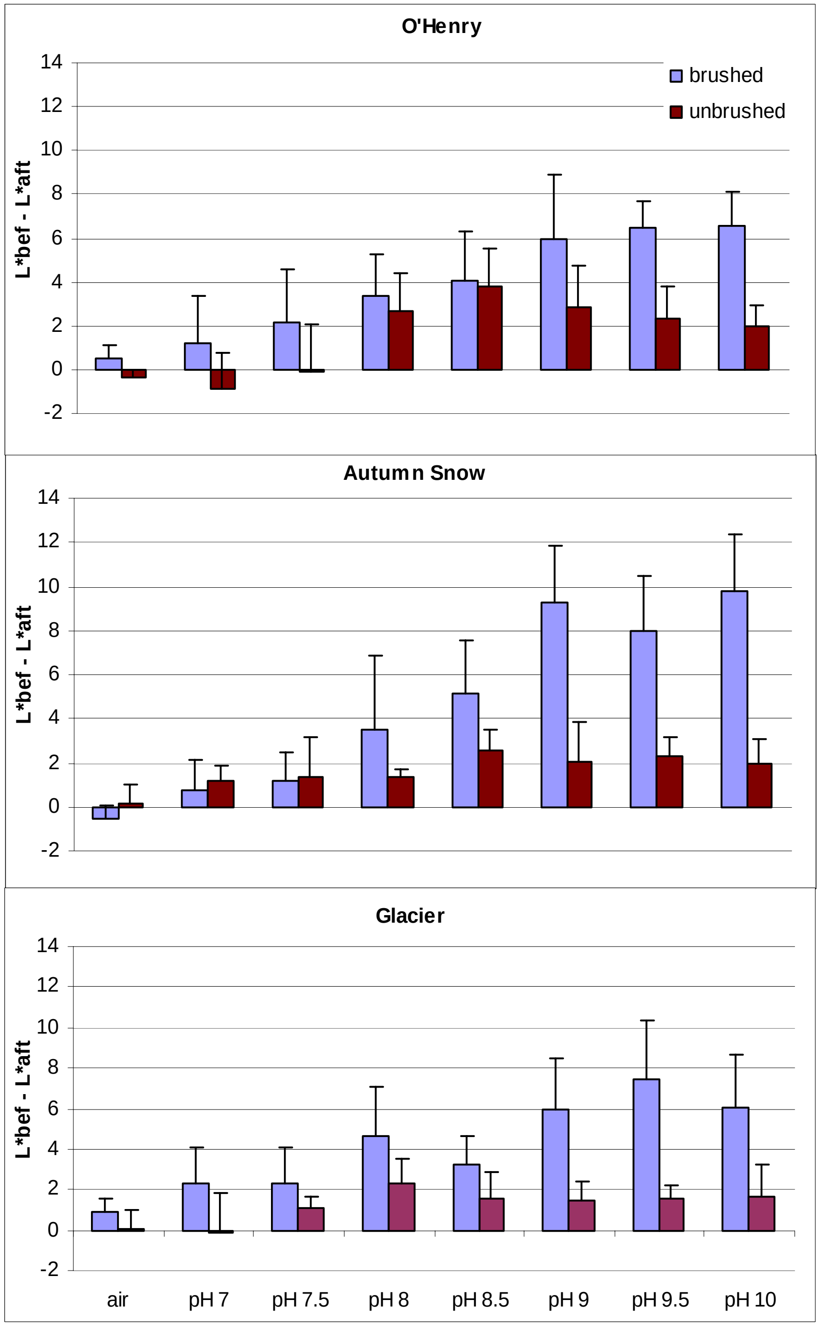

Figure 3. Increase of darkness (decrease of lightness, L*) on the fruit skin surface after exposing the fruit to different pH solutions. Air was used as control treatment in this experiment.

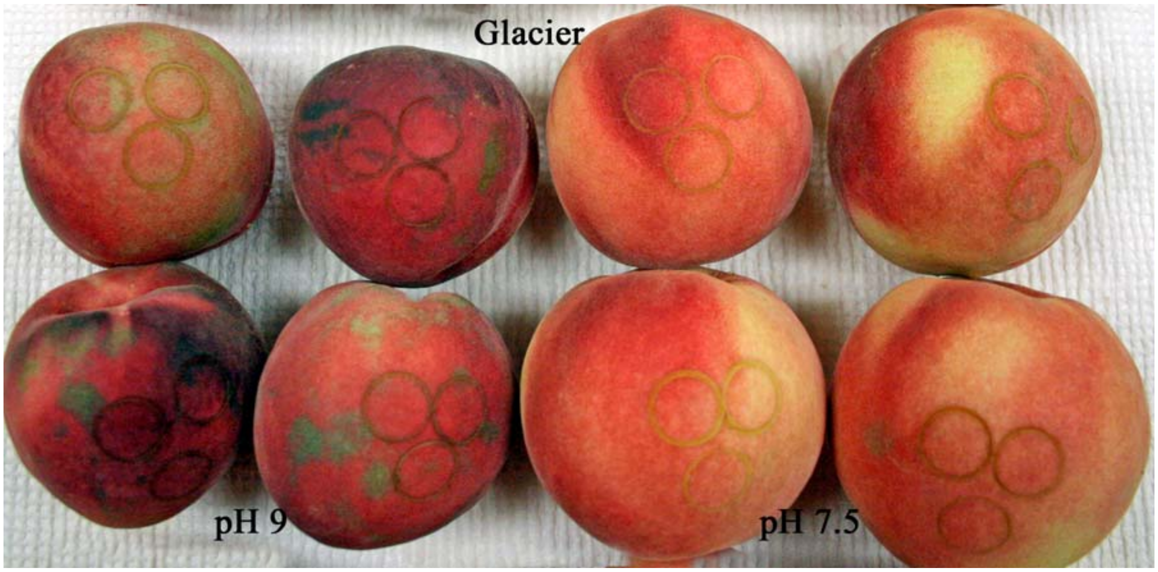

Figure 4. Burning skin disorder development on 'Glacier' fruit after 15 minutes in contact to pH 9 (left) and pH 7.5 solutions (right).

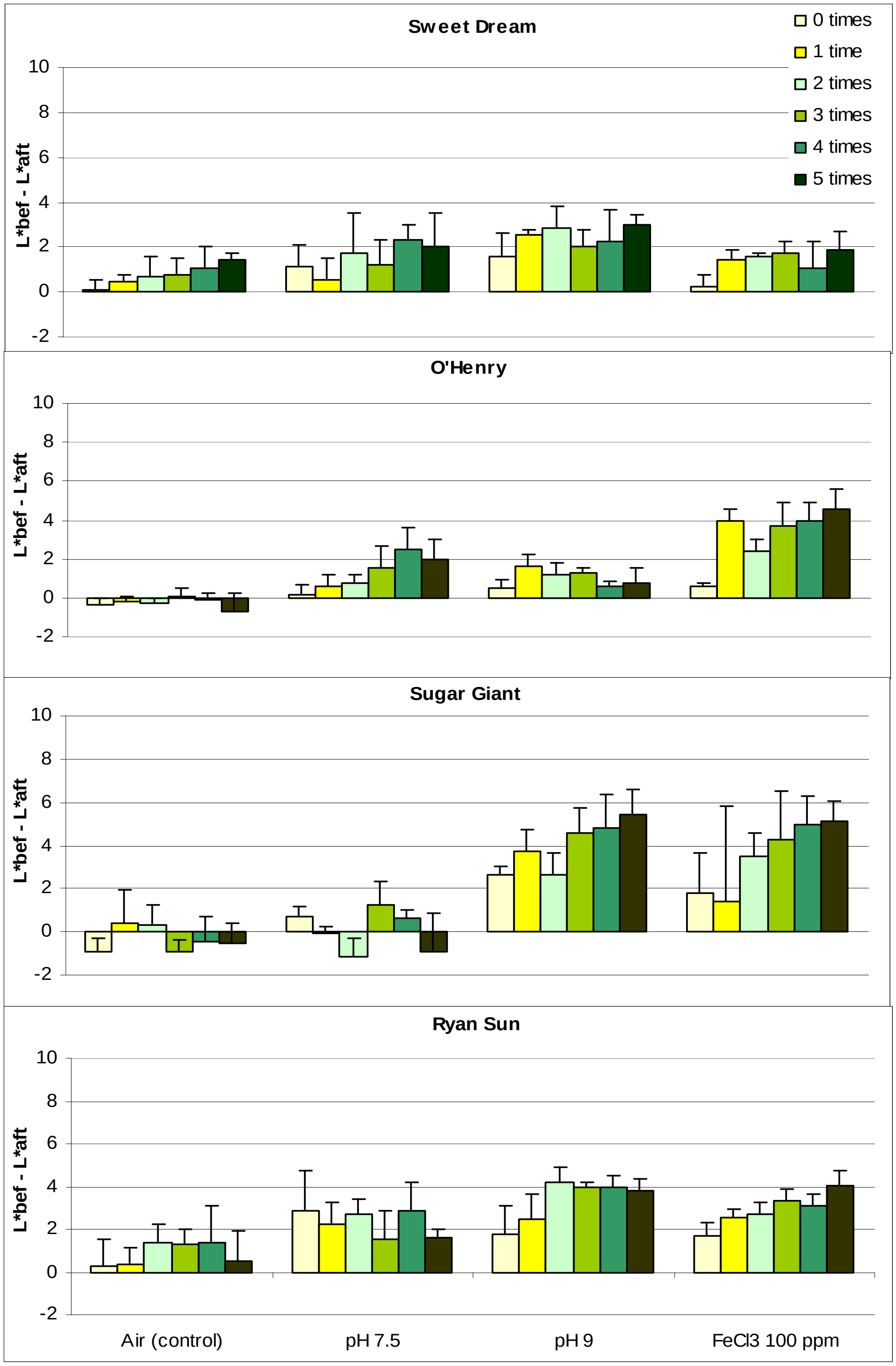

Figure 5. Effect of abrasion intensity on change of color (increase of darkness) on the fruit skin surface after exposing the fruit to air (control), pH 7.5, pH 9 and $FeCl_3$ solutions. Fruit was brushed (abraded) from 0 to 5 times. Air was used as control treatment in this experiment.

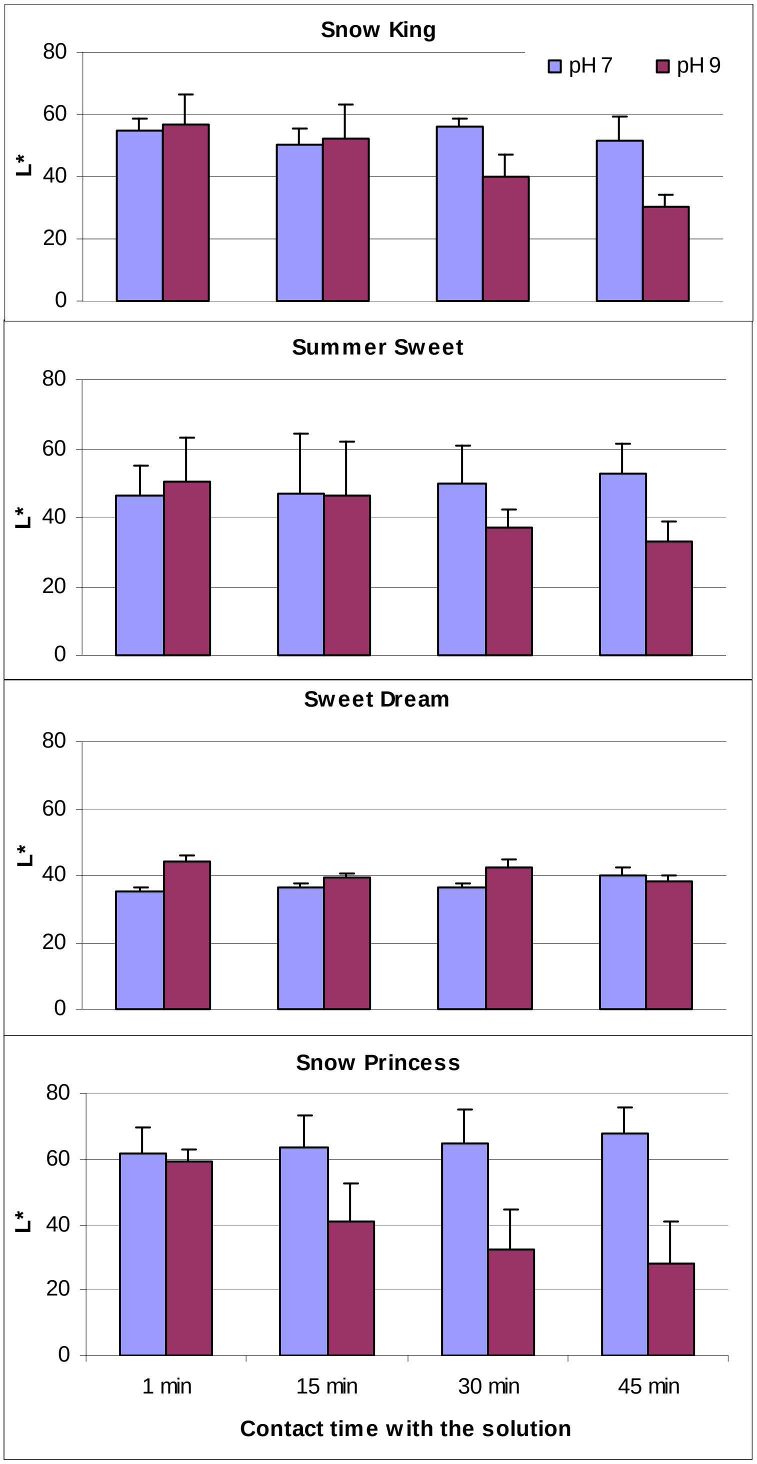

Figure 6. Effect of contact time with different pH solutions on the fruit skin darkening. Fruit was left in contact with the solutions for 1, 15, 30 and 45 minutes.

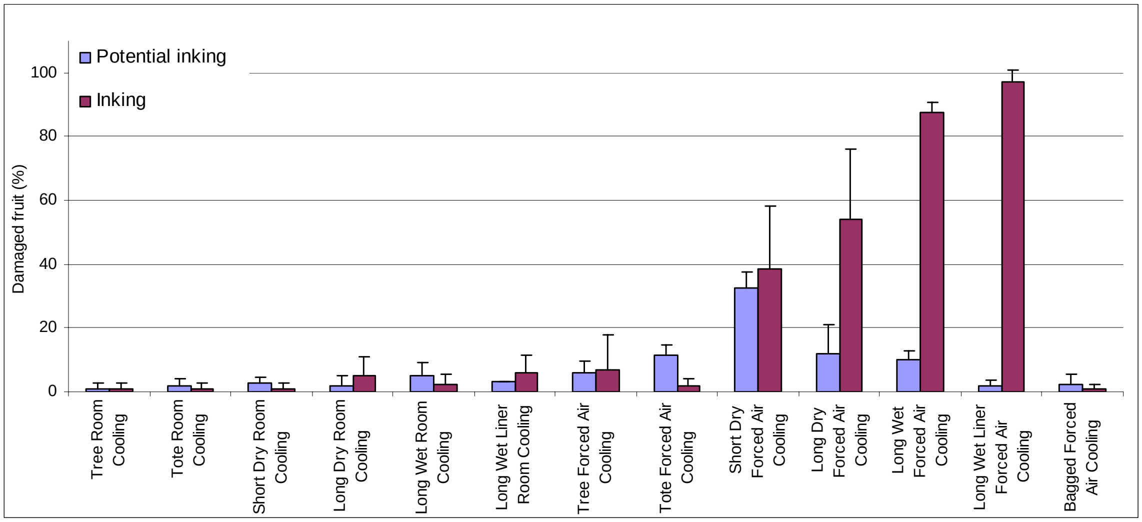

Figure 7. Incidence of skin burning disorder affected by different post-harvest handling on 'Autumn Snow'.

Figure 8. Incidence of 'skin burning disorder' on 'Autumn Snow' white fleshed peach.

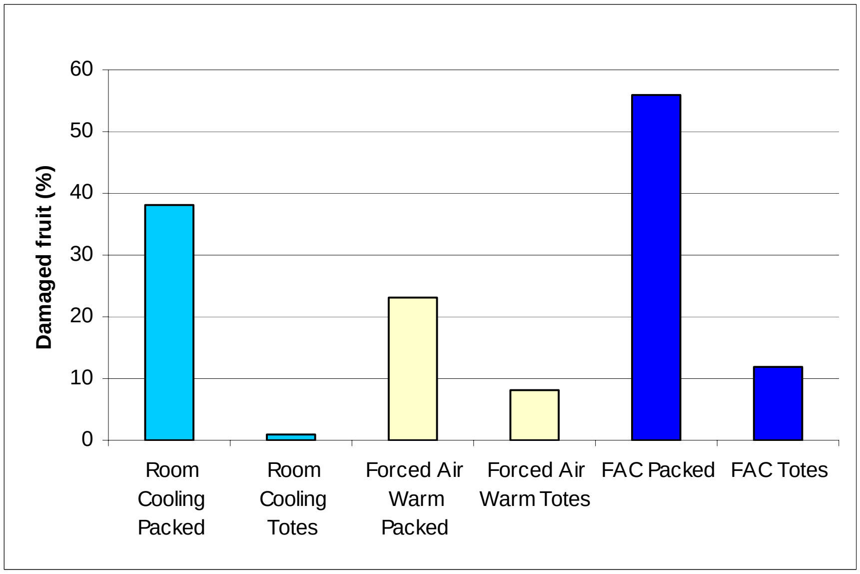

Figure 9. Incidence of skin burning disorder affected by different post-harvest cooling-handling on 'Autumn King' white flesh peach cultivar. Abbreviations: FAC, forced air cooling.

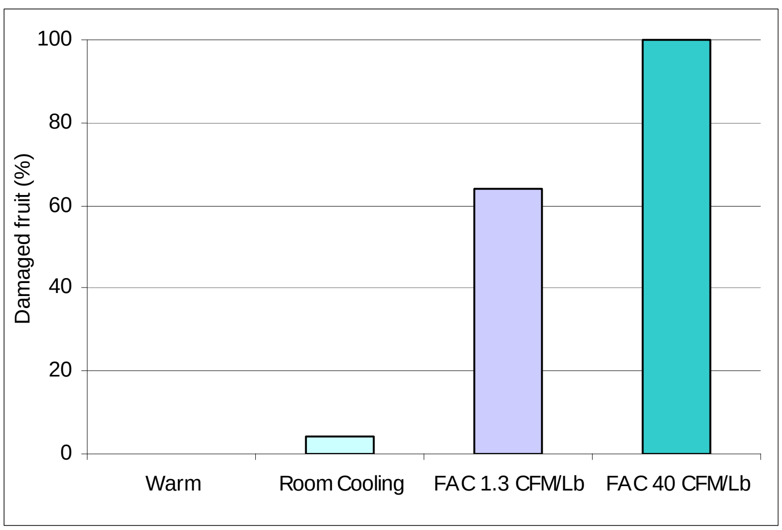

Figure 10. Incidence of skin burning disorder affected by different post-harvest cooling-handling on 'Snow Princess' white flesh peach cultivar. Abbreviations: FAC, forced air cooling; CFM, cubic feet per minute.