Updating Peach and Nectarine Inking and Skin Burning Development Information

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Abstract

Inking skin discoloration is one of the major postharvest cosmetic defects on fresh market peaches and nectarines. Results from previous seasons confirmed that abrasion and pre-harvest metal contamination are precursors of inking formation (field inking) (Cheng and Crisosto, 1994; Crisosto et al., 1999). Therefore, abrasion damage and heavy metal contamination need to be avoided. Abrasion damage releases phenolic pigments which are located in the skin cells, allowing the reaction of these pigments with iron, aluminum, and/or copper metal contaminants at fruit pH. 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 heavy metals concentrations. Among them, we identified several new chemicals that have high concentrations of iron and/or aluminum that may be involved in the inking formation.

Last season we reported a new skin discoloration disorder that we named skin burning. This disorder is triggered by the combination of physical damage during the harvesting operation combined with packaging 'postharvest stresses'. A unique characteristic of this blemish contrary to the traditional field inking, is that the incidence increases during packing and especially cooling. Our new findings indicate that other postharvest stresses different to exposure to heavy metal contamination, such as exposure to high air flow during forced air cooling (FAC) or high pH water during washing-brushing, will induce the development of this skin burning on the fruit 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, triggers and ways to reduce their incidence are different, and therefore, we will make a distinction between both skin damage types depending on their origin.

Because of this new situation, we dedicated a lot of our time and resources to understand this new skin disorder. An open house discussion at KAC and a newsletter issue were used to update our San Joaquin Valley clientele regarding this new problem during last season. The remarkable economical impact of this problem for the California fruit industry encourages further detailed research on answering new questions related to inking and skin burning formation.

Objectives

- Identify new sources of heavy metal contamination as precursors for field inking development
- Determine the relationship between pigment composition and the susceptibility to develop skin burning discoloration
- Determine the relationship between the cuticle thickness and their susceptibility to physical damage
- Understand how the skin burning discoloration disorder is triggered during packaging operations
- Evaluate rate of cooling and skin burning discoloration disorder development
- Evaluate packaging and skin burning discoloration development

This information will be fundamental to understand inking and burning disorder development and triggering, and to generate recommendations to eliminate and/or reduce California tree fruit industry loses due to inking and skin burning.

Material and Methods

Determination of heavy metals in pre- and post-harvest chemicals
Different sources of chemicals (additives, fungicides, pesticides and foliar nutrients) used in commercial peach production were collected and original samples (unaltered) were prepared for Fe, Cu and Al determination. The analyses were performed by the UC Davis Analytical Lab (http://anlab.ucdavis.edu/), based on the modified protocol described by Sah and Miller (1992) (Crisosto et al., 2008).

Phytochemical analysis
Peaches and nectarines from different cultivars, white and yellow fleshed, and low and high acidity were collected from different sources to eliminate source as a variable to influence skin burning susceptibility. Cultivars were selected based on feedback from the Californian peach and nectarine industry. Three replications of approx. 100 g of skin tissue of each cultivar-source combination were frozen immediately in liquid nitrogen and stored at -80°C until the phenolics biochemical determinations were carried out.
The ground frozen skin tissue was extracted with 80% methanol (v/v). The hydroalcoholic skin extract was used for HPLC analyses, total phenolics content, antioxidant capacity and absorption spectrum assays as described in Cantín et al. (2009a).

Cuticle isolation
Fruit skin disks were removed with a cork borer and as much of the underlying fleshy tissue as possible trimmed away. The disks were incubated in a digestion solution at 35 °C for 24 h with continuous shaking in a warm bath. After the mesophyll and epidermal

tissues had completely digested away, the isolated cuticles were rinsed in distilled water, allowed to air dry, and then weighed to the nearest µg. Cuticle density was calculated as its weight divided by the area of the disk (Crisosto et al., 1993).

Effect of forced air cooling and packaging on skin burning development

In order to study the effect of forced air cooling (FAC) and packaging on skin burning development, we evaluated the rate of skin burning on the fruit after using different packaging and cooling methods: room cooling, forced air cooling (FAC), and hydrocooling. Different air flows (0.25 cfm/lb, 0.50 cfm/lb and 1.0 cfm/lb) were tested for FAC. For the FAC experiments we used a FAC tunnel in which the air flow can be regulated depending on the fruit load. We also tested different types of packaging (vented or non-vented boxes, and fruit individually packed in plastic bags) on the skin burning development.

This season's findings

New sources of heavy metal contamination as precursors for field inking development

In this season, different pre- and post-harvest additives, foliar nutrients, fungicides, miticides and insecticides used in commercial peach production were screened for potential inking precursors as Fe, Cu and Al (Table 1). Our previous work indicated that ~10 ppm Fe and ~100 ppm Cu or Al solutions were enough to induce metallo-anthocyanin formation in isolated skin disks that results in inking development (Crisosto et al., 1999).

There were huge differences of the amount of Fe, Al and Cu contained by the different unaltered chemicals analyzed (Table 1). Regarding Fe, Delegate® (4,085 ppm) showed the highest concentration by far, followed by Altacor® (802 ppm) and Entrust® (490 ppm), Serenade A50® (260.1 ppm), Success® (104.4 ppm) and Envidor® (13.9 ppm). The rest of the screened chemicals had Fe contents lower than 10 ppm. However, all the chemicals had very low heavy metal content, below the recommended values, once the calculations were done according to the label spray dilutions.

Nevertheless, our previous experiments on *in vivo* tissue showed that solutions of ferrous-iron, aluminum and copper at concentrations <10 ppm applied directly to the fruit, may increase the inking damage compared to the control fruit on susceptible cultivars (Cantín et al., 2009b). On the other hand, our results showed that the damage intensity triggered by iron and aluminum highly depends on the cultivar. These indications show that there are some cultivars more susceptible to inking damage than others.

Therefore, although all the chemicals analyzed in this work showed a heavy metal concentration below the maximum recommended value on label suggested spray dilutions, it does not mean that those unaltered chemicals with heavy metals have not any influence on the development of inking disorder on the fruit. A study of the heavy metal residues that these chemicals leave in the skin of the fruit should be carried out in the future in order to elucidate their influence in the inking damage.

Relationship between skin pigment composition and skin burning susceptibility

After our previous results showed differences in skin burning susceptibility among different peach and nectarine cultivars, we studied their skin pigment composition looking for correlations between their skin phenolic profile and their skin burning susceptibility. Considerable variation was found in the amount of individual skin phenolic compounds, antioxidant capacity (AOC) and browning prtential among different cultivars, as previously reported (Cantín et al., 2009a).

Skin burning susceptibility was significantly higher for nectarine than for peach fruit (Table 2). This result could be explained by the significantly higher contents of some specific phenolics compounds (hydroxycinnamate, anthocyanin and flavonol) found in the skin of nectarine when compared to the peach fruit (Table 2). Conversely, no significant differences in skin burning susceptibility were found between yellow and white fleshed fruit (Table 2).

Significant differences were found between skin burning low/non-susceptible cultivars and the very susceptible cultivars for their phenolics profile (Fig. 1). The concentration of all the phenolic compounds identified in the fruit skin extract was significantly lower in the low/non-susceptible peach and nectarine cultivars than those found in the very susceptible ones. Additionally, low or non-susceptible cultivars had significantly lower TPC, AOC and BP than fruit from susceptible and very susceptible cultivars. These results show that cultivars with higher amounts of phenolic compounds, TPC, AOC and/or BP in their fruit skin cells, tend to be more susceptible to the development of skin burning when exposed to triggering conditions. This could be explained by the higher amount of phenolic compounds available to undergo potential structural transformations triggered by high pH, which will ultimately lead to skin burning discoloration development. This is an important result to consider, since the demonstrated beneficial effects of antioxidant compounds on health are making the antioxidant capacity of fruits an important trait to boost in current peach and nectarine breeding programs, and this could be causing a higher tendency to develop skin burning damage.

Cuticle thickness and susceptibility to physical damage

Significant variability was found on the cuticle thickness, measured as density, among the different peach and nectarine cultivars evaluated (Table 3), ranging from 2.56 µg/mm^2 in the case of 'Summer Sweet' to 15.12 µg/mm^2 in 'Sugar Giant'. In general, peaches (average 8.98 µg/mm^2) had denser cuticles than nectarines (average 7.01 µg/mm^2), although differences were not statistically significant. No significant differences were found between yellow and white fleshed fruit. These results agree with previous results by Crisosto et al. (1993). That study also reported that early season cultivars had less dense cuticles than later season cultivars.

The differences in the cuticle thickness could affect the fruit weight losses during cooling and storage on different peach and nectarine cultivars. However, the percentage of fruit weight loss after the cooling process (room cooling or FAC) observed for different peach and nectarine cultivars were very small (always lower than 2% after 24 h of cooling at 0°C), and they were not related to their cuticle thickness (Table 4). This result indicates that there is not a statistically significant correlation between cuticle thickness and weight loss during the cooling-storing process and therefore cuticle thickness does not explain the differences in skin burning or inking susceptibility. At the same time, no differences were found on the percentage of fruit weight loss when comparing room cooling and FAC (Table 4), which means that the skin burning damage is not directly related to the total fruit weight lost during the cooling process. We believe that there must be other detrimental processes different from fruit weight loss, such as skin abrasion or cell disruption occurring in the fruit during the FAC operation which induces the development of skin burning.

Effect of cooling and packaging on skin burning development

Our previous results showed that 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 (Cantín et al., 2009b). Also, it was demonstrated that fruit packed using pallet solid wrap showed low incidence of the problem when FAC. Besides, it was also seen that higher air flow caused the highest skin burning damage on the fruit. During this season, we designed several experiments in order to fully understand the role of temperature, air velocity, and packaging on skin burning development.

This season's experiments corroborated the negative influence of high velocity FAC on the development of skin burning. Higher skin burning incidence was observed in the fruit of 'Summer Sweet' and 'Glacier' after cooling at FAC 1.0 cfm/lb, compared to what happened when RC (Fig. 2 and 3). This result was observed for most of the cultivars tested what indicates that RC should be the elected cooling method for skin burning susceptible cultivars.

Different susceptibilities to the development of skin burning after FAC 1.0 cfm/lb and on the skin damage evolution during storage were also observed among the different peach and nectarine cultivars (Fig. 4). These results agree with our previous findings on the variability of skin burning susceptibility, and highlight the importance of adjusting the postharvest handling methods to the specific cultivar depending on its skin burning susceptibility.

Effect of different air flow on skin burning development

After having proved the negative effect of high velocity FAC on the skin burning development, we examined the effect of FAC at different air velocities. As shown in Fig.

5 for 'Summer Sweet', low skin burning incidence was observed when the fruit was RC or FAC at 0.25 cfm/lb after 1 and 2 weeks of subsequent cold storage. However, much higher skin burning incidence was observed after FAC at 0.5 cfm/lb and 1.0 cfm/lb. Similar results were observed for other cultivars, although the skin burning incidence and the evolution during storage varied depending on the cultivar. These results point out that the air flow used in the FAC operation, is a decisive factor on the skin burning damage developed in the fruit skin, and it also indicates that air flows higher than 0.25 cfm/lb enhance the skin burning in susceptible cultivars.

Recommendations

In general, to reduce field inking and skin burning incidence, physical damage during pre- and post-harvest periods must be minimized. At the same time of metal contamination, exposure to high pH solutions, and exposure to high velocity forced air cooling should be avoided in susceptible cultivars. Due to the remarkable importance of these skin disorders for the California fruit industry, we will be executing further detailed research in order to answer new questions and to give the best guidelines to minimize these problems.

How to reduce field inking incidence:

- Reduce fruit abrasion damage by treating fruit gently and avoid long hauling
- Reduce fruit contamination by keeping picking containers dirt free and clean; avoid dust contamination on fruits
- Check your water quality for contamination with heavy metals (Fe, Cu & Al)
- Test your pesticides for presence of heavy metals (Fe, Cu & Al) early in the season. [Growers need to know the composition of the chemicals commonly used in their tree fruit pre-harvest and post-harvest operations and understand how they may affect inking incidence]
- Do not spray foliar nutrients or pre-harvest fungicides containing Fe, Cu, or Al within 21 days of predicted harvested
- In orchards where inking is a problem, delay packing for ~48 hours so you will be able to remove fruit with field inking before placing fruit in the box

How to reduce skin burning incidence:

- Minimize physical damage or abrasion on the fruit surface during pre- and/or post-harvest operations
- Handle fruit gently, avoid long hauling distances and keep harvest containers free of dirt
- In a standard packing operation, washing water pH in the brushing-washing or hydrocooling operation should be continuously maintained around 6.5-7.0 [The installation of automated systems (ORP) to monitor and/or adjust active/effective chlorine and pH levels is critical to increase disease control effectiveness and decrease potential skin burning development]

- Based on our current results, we recommend dry packing (without brushing and a chlorine rinse) for the very susceptible peach or nectarine cultivars
- Avoid the fast cooling air velocities for the skin burning susceptible peach or nectarine cultivars For susceptible cultivars, we suggest cooling the fruit by room cooling, without forced air

Tables

Table 1. Specific heavy metal concentrations (Al, Cu and Al) in different unaltered chemicals used in the tree fruit industry.

Source	Fe (ppm)	Cu (ppm)	Al (ppm)
Agrimek	<3.0	<0.5	<20
Altacor	802	1.5	14380
Decco 241 cleaner	<3.0	<0.5	<20
Decco 251	<3.0	<0.5	<20
Decco 295	<3.0	<0.5	<20
Decco 550 organic	<3.0	<0.5	<20
Delegate	4085	4.8	69930
Entrust	490	<1.0	7980
Envidor	13.9	<0.5	<20
Latron B1956	<3.0	<0.5	<20
Onager	<3.0	<0.5	<20
Scholar	<3.0	<0.5	<20
Serenade A50	260.1	1.2	550
Success	104.4	<0.5	510
Tilt	<3.0	<0.5	<20
Vigorcal	<3.0	0.8	<20

Table 2. Skin burning susceptibility, skin phenolics profile, total phenolics content (TPC), antioxidant capacity (AOC) and browning potential (BP) associated with fruit type (peach/nectarine) and flesh color (yellow/white) qualitative traits, analyzed in 21 different peach and nectarine cultivars.

Quality trait	Skin burning susceptibility* (1-3)	CA (μg/g FW)	NCA (μg/g FW)	C3G (μg/g FW)	Q3R (μg/g FW)	Q3Glu (μg/g FW)	Q3Gal (μg/g FW)	Catechin (μg/g FW)	TPC (μg GAE/g FW)	AOC (μg TEAC/g FW)	BP (ΔA420/h)
Peach	1.9 b	114.1 b	18.7 b	141.1 b	56.8 b	42.3 b	12.4 b	34.5 a	1540.2 a	3551.3 a	0.5 a
Nectarine	2.3 a	220.5 a	54.3 a	287.7 a	191.5 a	137.4 a	33.3 a	28.7 a	1591.3 a	3372.1 a	0.5 a
Yellow	2.0 a	155.5 a	34.7 a	266.6 a	135.2 a	88.9 a	29.6 a	30.2 a	1588.1 a	3386.1 a	0.4 b
White	2.1 a	174.7 a	35.1 a	104.7 b	87.9 b	79.6 a	25.0 a	34.8 a	1520.2 a	3616.3 a	0.7 a

*Skin burning susceptibility scored on a scale of 1 (non-susceptible) to 3 (very susceptible). For each pair of traits (peach/nectarine, yellow/white fleshed), in each column, means with the same letter are not significantly different according to *t* test ($P \leq 0.01$). Abbreviations: CA, chlorogenic acid; NCA, neochlorogenic acid; C3G, cyanindin-3-glucoside; Q3R, quercetin-3-rutinoside; Q3Glu, quercetin-3-glucoside; Q3Gal, quercetin-3-galactoside; GAE, gallic acid equivalents; TEAC, Trolox equivalent antioxidant capacity.

Table 3. Cuticle thickness (?g/mm^2) of different peach and nectarine cultivars.

Cultivar	Fruit type	Flesh color	Cuticle thickness (μg/mm^2)
Brittney Lane	peach	yellow	11.43
Candy Pearl	nectarine	white	4.32
Country Sweet	peach	yellow	10.64
Diamond Ray	nectarine	yellow	8.65
Elegant Lady	peach	yellow	3.82
Glacier	peach	white	8.83
Grand Pearl	nectarine	white	12.07
Honey Blaze	nectarine	yellow	6.35
Honey Fire	nectarine	yellow	6.90
Honey Kist	nectarine	yellow	3.96
Ice Princess	peach	white	9.17
Ivory Princess	peach	white	9.05
Johny White	peach	white	7.92
July Flame	peach	yellow	12.22
Kay Sweet	nectarine	yellow	7.13
Rich Lady	peach	yellow	9.73
Ruby Diamond	nectarine	yellow	5.04
Snow Princess	nectarine	white	6.68
Spring Snow	peach	white	9.05
Sugar Giant	peach	white	15.12
Summer Sweet	peach	white	2.56
Sweet Dream	peach	white	4.53
Vista	peach	yellow	7.92
White Lady	peach	white	9.48
Zee Lady	peach	yellow	12.22

Table 4. Percentage of weight loss observed in different peach and nectarine cultivars after room cooling or FAC 1.0 cfm/lb for 6 h, and storage at 0°C for 24 h, 1 week or 2 weeks.

Cultivar	Weight Loss (%) after 24 h		Weight Loss (%) after 1 w		Weight Loss (%) after 2 w	
	room cooling	FAC 1.0cfm	room cooling	FAC 1.0cfm	room cooling	FAC 1.0cfm
Diamond Ray	1.6	1.1	2.9	2.3	4.0	4.6
Elegant Lady	1.2	1.1	3.7	2.3	5.9	5.1
Glacier	1.4	1.3	3.6	3.6	5.9	6.0
Grand Pearl	1.5	0.9	2.4	1.9	3.5	3.1
Ice Princess	0.8	0.8	1.6	2.5	3.2	4.8
Ruby Diamond	1.6	1.5	2.5	2.5	4.6	4.5
Snow Princess	1.1	1.1	3.5	3.3	6.1	5.7
Sugar Giant	1.2	0.9	2.8	2.6	4.2	5.0
Sugar Giant	1.1	0.7	1.7	2.1	3.6	3.2
Summer Sweet	1.3	1.8	2.3	3.0	3.8	4.3
Sweet Dream	1.3	1.1	2.7	2.8	4.2	4.5
White Lady	0.8	0.7	2.4	1.6	4.6	3.7
Zee Lady	1.1	0.9	2.3	2.4	3.7	4.4

Figures

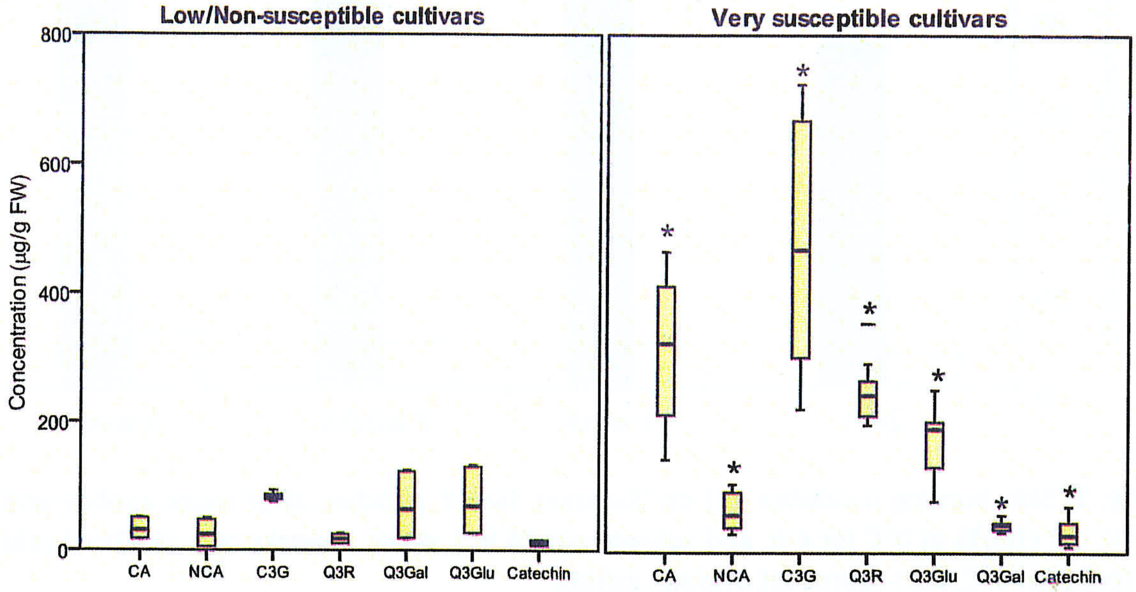

Fig. 1. Range and distribution of skin phenolic compounds on the skin of 21 peach and nectarine cultivars low/non-susceptible (left) and very susceptible (right) to skin burning. The horizontal line in the interior of each box is the median value. The height of each box is equal to the interquartile distance, indicating the distribution for 50% of the data. *Represents significant differences for each phenolic compound between low/non-susceptible and very susceptible cultivars at P ≤ 0.01. Abbreviations: CA, chlorogenic acid; NCA, neochlorogenic acid; C3G, cyaniding-3-glucoside; Q3R, quercetin-3-O-rutinoside; Q3Glu, quercetin-3-O-glucoside; Q3Gal, quercetin-3-O-galactoside.

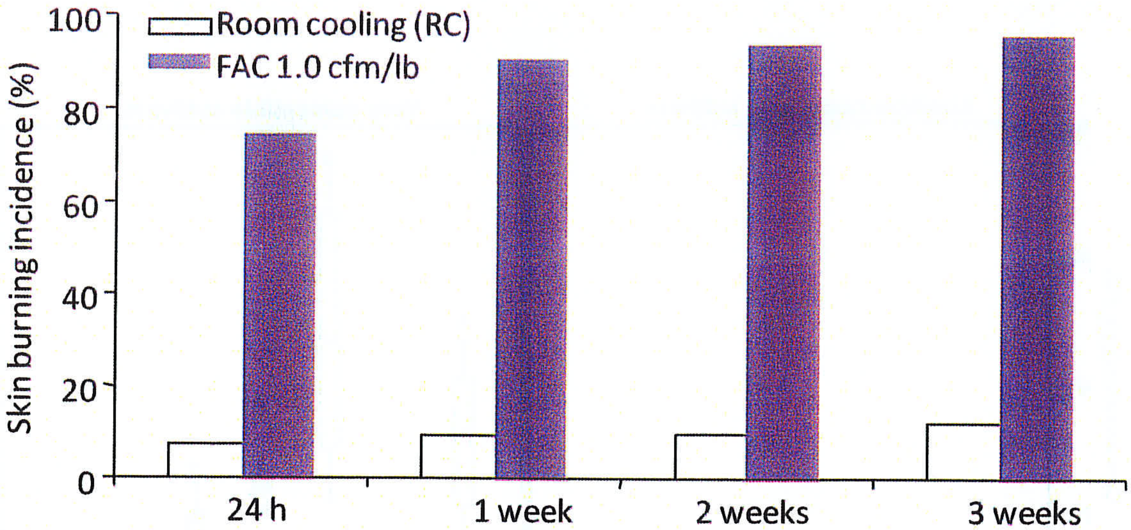

Fig. 2. Skin burning incidence (%) on 'Summer Sweet' peaches after room cooling and FAC 1.0 cfm/lb at 0°C for 6 h, and subsequent 24 h, 1 week, 2 weeks or 3 weeks of cold storage at 0°C. Bars represent average values.

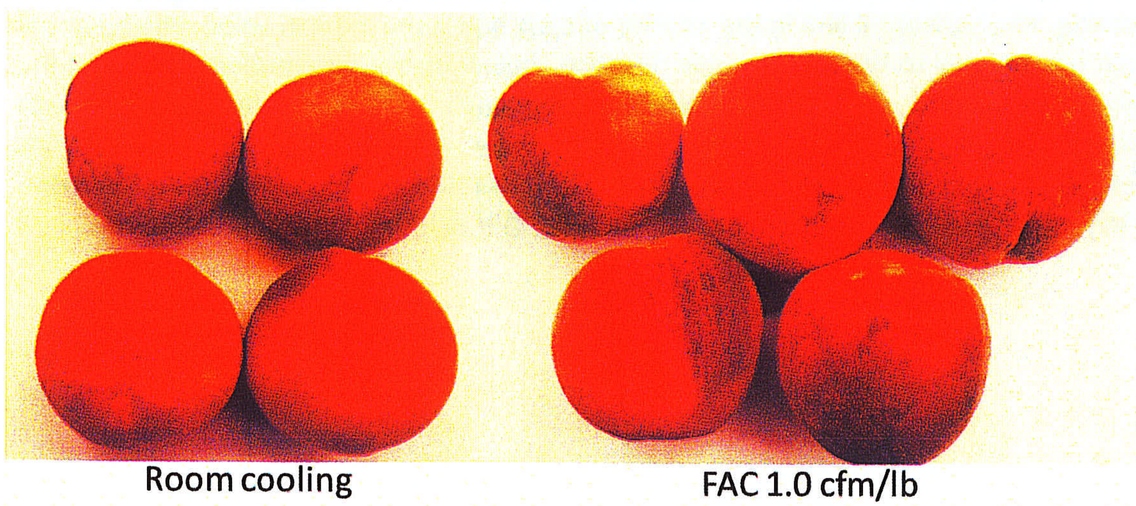

Fig. 3. 'Ice Princess' peaches after room cooling (left) and FAC 1.0 cfm/lb (right) at 0°C for 6 h, and subsequent 1 week of cold storage at 0°C.

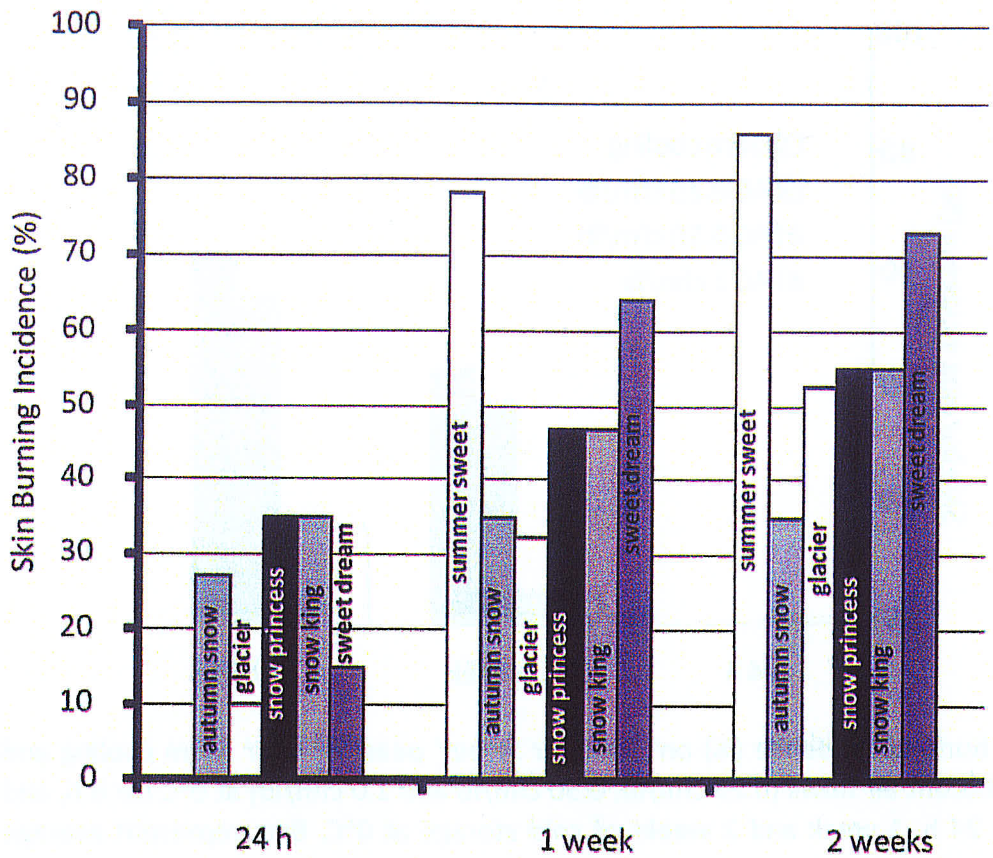

Fig. 4. Susceptibility to skin burning incidence (%) on different peach and nectarine cultivars after FAC 1.0 cfm/lb at 0°C for 6 h, and subsequent 24 h, 1 week or 2 weeks of cold storage at 0°C. Bars represent average values.

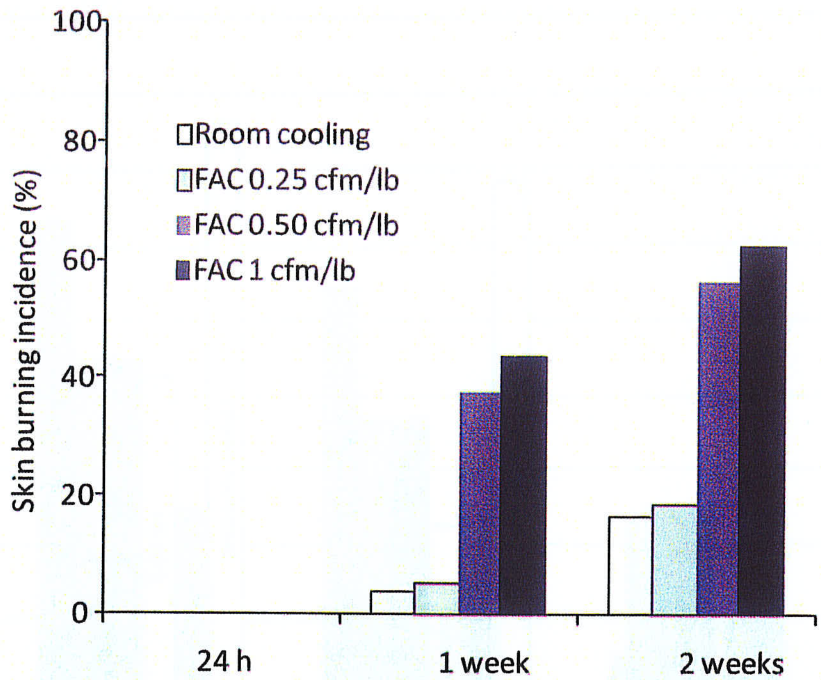

Fig. 5. Skin burning incidence (%) on 'Summer Sweet' peaches after room cooling and FAC with different air flows (0.25 cfm/lb, 0.50 cfm/lb and 1.0 cfm/lb) at 0°C for 6 h, and subsequent 24 h, 1 week and 2 weeks of cold storage at 0°C. Bars represent average values.