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

**PROJECT LEADER:** Dr. Carlos Crisosto
**COOPERATORS:** Harold Dou, Ryan Hessler, Kevin Day, and Ebenezer Ogundiwin

## ABSTRACT

During this year's work, our studies have corroborated our previous work that demonstrated that low energy physical injury (abrasion) combined with specific metal contamination (iron, aluminum, and copper) causes skin discoloration, also called inking, on peaches and nectarines. 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, aluminum, and/or copper metal contaminants at fruit pH. Since many new foliar-nutrients, fungicides, and insecticides have become available for the tree fruit industry in the last decade, we screened many of them for iron, copper and aluminum 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. Their metallo-anthocyanin reaction activity was also confirmed under controlled laboratory conditions. We examined in detail 12 commercial instances in which inking occurred. Among them only two cases out of 12 were not related to contamination of known inking precursors due to preharvest sprays. The hypotheses that low titratable acidity and/or pH, pigment composition, air pollution or potential contamination during postharvest handling may be important factors in inking development, are also being studied. Development of preharvest intervals is being proposed as a way to reduce inking incidence for these chemicals.

## INTRODUCTION

Inking has been a major skin disorder on peach and nectarine fruits for several decades (Crisosto et al., 1998). Recently, inking has frequently been reported in the stone fruit production area worldwide. Inking or skin discoloration is characterized as discolored brown-and-black spots. In most cases, it 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.

Abrasion injury is one of the major precursors of inking (Crisosto et al., 1993). The other inking precursor 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. 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. 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). Foliar nutrient, fungicide, miticide, and insecticide preharvest sprays, depending on the preharvest application interval (PHI), may act as sources of contamination for inking development.

Last year our proposal objectives were to:

- Optimize Fe, Al, and Cu chemical analysis.
- Identify new sources of heavy metal contamination as a precursor for inking development.
- Screen new commercial pre and postharvest chemicals (foliar nutrients, additives, miticides, fungicides and insecticides) used in the tree fruit industry for potential inking precursors such as Fe, Cu and Al.
- Determine the minimum Fe, Al, and Cu ion concentration necessary to trigger the metallo-anthocyanin reaction and formation of dark areas.
- Evaluate where inking damage is being triggered (packinghouse versus field).
- Test other potential causes of inking development in the California industry.

***Optimizing Fe, Al, and Cu chemical analysis***

In the past 2 years, the same pesticide samples were analyzed for Al, Fe, and Cu at three different laboratories in California including the UC ANR Laboratory (University of California, Davis). Unfortunately, huge differences and inconsistent results were delivered by these laboratories (Table 1). Following this experience, we attempted to develop an improved testing protocol and focused on working with personnel at the UC ANR lab, located at UC Davis. The quantitative determination of Fe, Cu, Al and a variety of other elements is greatly affected by the digestion methods. The current analysis includes nitric acid/hydrogen peroxide microwave digestion as determined by atomic absorption spectrometry (AAS). This method has detection limits ranging from 0.1 mg $Kg^{-1}$ to 0.01% and is generally reproducible within 8% for all analyses. It is described in detail by Sah and Miller (1992), and also by Meyer and Keliher (1992). Improvement of the protocol by the UC ANR Laboratory (University of California, Davis) included a modification in the digestion step by adding 0.1 ml hydrofluoric acid in addition to the current nitric acid/hydrogen peroxide standard digestion. This modification increased the analytical accuracy and consistency of this method for Fe, Al, and Cu concentration measurements. This improved protocol performance was tested by using the same pesticide samples, sample dilution series, and adulterated samples. The use of duplicated samples as an internal control is recommended as a routine mode of operation during these heavy metal analyses.

***Screening new commercial chemicals (additives, foliar nutrients, fungicides, miticides, and insecticides) used in the tree fruit industry for potential inking precursors such as Fe, Cu and Al***

Different sources of chemicals (additives, pesticides and foliar nutrients) used in commercial peach production were collected and samples were prepared for Fe, Cu and Al determination. Following label recommended concentrations, preharvest applied chemicals were calculated based on 100 gallons per acre. Postharvest fungicides Scholar® (fludioxonil) and Mentor® (propiconazole)] were used based on their label recommendations. Total Fe, Cu, and Al concentrations were not detected in additive solutions such as Omnis Supreme, No Foam® B (surfactant), and Latron B-1956® (modified phthalic glycerol alkyd resin and butyl alcohol) (Table 2) while Fe and Al concentrations were detected in some foliar nutrients (Table 3) used commercially in our industry. Among the foliar nutrients analyzed last season Agri-Trend 20-20-20 (nitrogen, phosphoric acid, potash) and MicroPlex nutrients contained high concentrations of heavy metals. MicroPlex was richer in Fe (21 ppm) and Cu (8.0 ppm) while Agri-Trend 20-20-20 had 2.8 ppm Fe and 1.1 ppm Cu. The other foliar nutrients tested this season (Cal Ocho (calcium), Vigor Cal® (foliar calcium), and Goemar® (seaweed foliar fertilizer)] did not have any detected Fe, Cu and Al (Table 3).

***Preharvest Pesticides*** Total Fe and Al concentrations varied among miticide, insecticide and fungicide preharvest sprays that are frequently applied as foliar spraying (Tables 4-6). Copper concentrations were below detectable level (<0.2 mg per liter) in all of the tested pesticides. Thus, these low Cu concentrations should not be affecting inking incidence. Fe and Al concentrations ranged from < 0.2 to 16.7 ppm and 270 ppm, respectively. Vendex® 50WP-FL Miticide (fenbutatin oxide), Acramite® -50WS (bifenazate), Omite® 300W Wettable Powder Miticide (propargite), Imidan® 70-W (phosmet), Delegate™ WG (spinetoram), and Elite® 45 WP (tebuconazole), had higher Fe concentrations than other tested chemicals (Tables 4-6). At the same time, Elite, Vendex, Acramite, Imidan, Indar™ 75WSP Fungicide (fenbuconazole), Omite, Pristine® (pyraclostrobin and boscalid), Altacor® (chlorantraniliprole), and Delegate had higher Al than other tested pesticides (Tables 4-6). Specifically, Acramite and Vendex concentrations using 100 gallons per acre had a higher Fe concentration than the proposed 10 ppm minimum necessary to trigger inking formation on abraded tissue. Several chemicals tested had high aluminum concentrations that may be triggering inking reaction, and biochemical studies to establish a minimum safe threshold are currently being carried out. Envidor® 2 SC (spirodiclofen), Onager® (hexythiazox), Intrepid® 2F (methoxyfenozide), Success® (spinosad), DiPel® DF (*Bacillus thuringiensis*), Lannate® SP (methomyl), Deliver® (*Bacillus thuringiensis*), Pyganic EC 5.0 (pyrethrins), and Orbit® (propiconazole) did not have any detectable Fe, Cu and Al. Our preliminary laboratory results suggest that even 4 ppm Fe and 8 ppm Al concentrations can cause anthocyanin and chlorogenic acid color changes from red-yellow to dark under controlled laboratory conditions.

***Postharvest Fungicides*** Scholar and Mentor were analyzed, and at three potential postharvest application rates had higher Fe and Al and lower Cu concentrations than other available chemicals. Scholar and Mentor had 2,400 ppm and 2,800 ppm Fe in the original product while Orbit, which has the same active ingredient as Mentor, had almost no detectable heavy metals. The concentration of Scholar exceeded our proposed 10 ppm (~12-15 ppm) threshold when prepared using 8 oz per 8 and 12 gallons of water-wax solution. Mentor prepared using 3 oz per 8 gallons of water-wax solution had 7.3 ppm Fe and 13.8 ppm Al. Theoretically, these application volumes (8, 12, and 25 gallons) should be covering approximately 200,000 pounds of fruit. In our previous work, we demonstrated that an efficient washing prior to a known inking precursor overcame the potential inking development by removing all exposed pigment from the fruit surface. Detailed experiments to test potential involvement of fungicides in inking and to design and assure their safe and effective postharvest use are under careful planning and execution.

***Evaluating where inking damage is being triggered during the postharvest operation.***

The effect of the handling process on inking development was studied using 'Sugar Giant' and 'Snow King' peaches. These inking cases were carefully investigated because no preharvest pesticides were sprayed during the last 30 days of fruit growth prior to harvest in these cases. In fact, these fruit samples were submitted to a full organochlorine screen (OC), organophosphate and organonitrogen screen (OP-ON), carbamate screen (CB), and pyrethroid general pesticide residue analysis screening using the current CDFA extraction method to assure that they were not exposed to known potential inking precursors. These analyses showed no pesticide residues present in these samples. However, this screening did not include foliar nutrient sprays or other potential sources of contamination (Fe, Cu, Al and others) that can be acting as inking precursors and so it cannot be concluded that contamination did not play a role in the problem.

Of the 12 domestic inking cases investigated this season, two of them showed inking without known preharvest spray contamination. For this reason, we followed up these cases with the sampling protocol described below and full pesticide residue analysis looking for potential inking precursors. 'Sugar Giant' and 'Snow King' peach fruit samples from the same grower-lot were collected after transportation (packinghouse arrival) and after packing. After sampling 10 totes per each sampling location, fruit were carefully tray packed and gently transported to the Kearney Agricultural Center (KAC). Upon arrival at KAC, fruit were stored at $33^{o}$F for 3 days before inking incidence evaluations. Inking incidence was defined as percentage of fruit showing well defined inked (light brown to black) areas larger than an aggregated area of 64 square millimeters.

Inking incidence of ‘Sugar Giant’ and ‘Snow King’ was low at arrival at the packinghouse (~4.8%) and increased to ~18.0% after packaging (Fig. 1). Our previous work demonstrated that most of the inking incidence was triggered prior to packinghouse arrival and this inking incidence did not increase after packaging. In this particular case in 2008, it appears as if inking incidence was triggered during the packaging process, indicating that abrasion and/or contamination (Fe, Al or others) was potentially playing a role. In our previous work we demonstrated that abrasion is virtually always occurring during harvesting and hauling so the limiting factor for inking formation is, in general, contamination. Fludioxinil (Scholar) residues during the packing process varied from 1-3.8 ppm while propiconazole (Mentor) residues ranged from 0.35-2.00 ppm. These values are very high compared with standard commercial residues of Scholar and Mentor. Conversely, this indicates that 1) some other mechanism may be involved in inking development, or that 2) contamination occurred during the packaging process.

***Effect of pH on inking formation***

Skin discoloration development is associated with abrasion injury occurring during fruit handling and transportation. Our previous anatomical study, using light microscopy and scanning electron microscopy, demonstrated that the physical injury associated with discolored skin spots was abrasion. The epidermal cells in the discolored spots were broken but those in non-discolored skin spots were intact, while the flesh tissue cells (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. Phenolic oxidation is likely the cause of the brown skin discoloration found on injured peach and nectarine fruit. Dark discoloration (black, blue, purple spots) probably emanates from non-oxidation reactions (metallo-pigment) involving anthocyanins, chloragenic acid, and other phenolics, which are abundant in the skin cells of peach and nectarine fruit. Other types of the non-oxidation reactions involve the transformation of the molecular structure of anthocyanins at high pHs.

The color of anthocyanins depends on the pH of the solution. These pigments exist mainly in the red-colored flavylium salt form at low pH (1.0). As pH rises from pH 4 to 5, the pigments gradually transform into colorless carbinol pseudo-bases. 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. Thus, it is well established that skin red and yellow pigments released from broken damaged cells do not turn dark when exposed to fruit physiological pH which is around 4.0. The pigments have to be exposed to pH higher than 7.0 to trigger pigment changes from bright yellow- red to dark (inking). Skin pH is around pH 4.0 and flesh tissue is a little bit lower than skin tissue (Table 8).

During commercial packing, fruit are exposed to pH higher than 4.0 at the washing and waxing steps. Normally, water pH during washing and hydrocooling varies from 7.2-7.9 (Table 8). Under these conditions, color will change immediately as micro environmental pH changes. The postharvest use of sodium hypochlorite (chlorine) and fruit coatings has the potential to increase pH (Table 9). In some operations, pH of the chlorine solution is reduced to ~6.5-7.0 (neutral) to maximize chlorine action (hypochlorous) and reduce the potential “off-smell” during the hydrocooling process. It has been observed that immediately after the chlorine wash, some abraded fruit areas may turn dark as a consequence of temporary pH tissue increases. However, these light-dark areas will later turn back to normal color as tissue pH will equilibrate to ~3.8-4.5. Another type of the non-oxidation reaction that can cause a permanent dark discoloration is the formation of metallo-pigment complexes at physiological pH (pH ~3.8-4.5). Anthocyanins such as cyanidin-3-glucoside can react with metallic ions to give derivatives of blue and other colors. The extent of the metallo-pigment formation also depends on the cultivar pigment composition and its affinity to contaminants.

As micro-environmental conditions of a high pH and metallic iron often exist during postharvest fruit handling, understanding the fruit’s response to different metallic ions and pHs is important to reduce the skin discoloration disorder. Several laboratory studies of the anthocyanins and chlorogenic acids’ response to exogenous pH and metallic ions are being carried out using fruit solution and skin disk systems.

## PRELIMINARY CONCLUSIONS

Results from last season confirmed that abrasion and metal contamination are precursors to inking formation. Therefore, abrasion and heavy metal contamination need to be avoided. Careful screening for Fe and Al of any preharvest and postharvest chemicals used in our tree fruit industry should be carried out prior to any commercial use to reduce potential inking incidence. However, it is well worth noting that other metals or chemical compounds that we have not identified may also be involved in inking formation.

Our last season results encourage further detailed research on answering new questions related to inking formation. These could include: 1) how is inking triggered during packing operations; 2) establishing minimum safe thresholds for Fe and Al concentrations; 3) are there additional potential precursors for inking such as air pollution or other unknowns; 4) are specific new cultivars more highly susceptible to inking; 5) is cultivar pigment composition or quality composition related to inking susceptibility; 6) is cultivar antioxidant capacity related to inking susceptibility; and 7) can we reverse or protect fruit from inking?

## 2009 Season Inking Reduction Tips

We suggest that:

1. Chemical manufacturing companies attempt to identify and remove from their products any potential sources of contaminants that may contribute to inking formation.
2. Chemical manufacturing companies attempt to develop safe preharvest spray intervals (PHI) for foliar nutrients, fungicides, miticides, and insecticides.
3. Producers understand the preharvest and postharvest chemicals commonly used in their tree fruit preharvest and postharvest operations and how they may affect inking incidence.
4. Reduce abrasion damage, handle fruit gently, avoid long hauling distances and keep harvest containers free of dirt.
5. Contamination of fruit can be reduced by keeping equipment clean, avoiding dust contamination of fruit, checking water quality for (Fe Al, Cu) contaminations, and avoiding foliar nutrients sprays containing Fe, Al, or Cu.
6. In orchards where inking is a problem, delay packaging for ~48 hours so you will be able to remove fruit with inking prior to placing fruit in the box.
7. Fine tune your postharvest fungicide application to assure that your residues are above the effective minimum recommended, but well below the maximum residue limit (MRL) or tolerance (Adaskaveg personal communication, 2008).

| **Postharvest Fungicide** | | **Stone Fruit Residues (ppm) for Domestic and International Markets** | |
|---|---|---|---|
| **Chemical Names** | **Trade Names** | **Usage Residue*** | **Tolerance (MRL)** |
| **Fludioxonil** | **Scholar** | 0.5-1 | 5 |
| **Fenhexamid** | **Judge**** | 1-3 | 10 |
| **Propiconazole** | **Mentor***** | 0.5-1 | 2*** |

* Based on application method. Improved coverage (e.g., high volume systems) allows lower residues.

** Formerly named Elevate (preharvest name).

*** Mentor 45WP was registered under an emergency registration (Section 18) for the 2006-2008 (pending for 2009) seasons and is in the IR-4 program for full Section 3 registration. International CODEX MRL is 1 ppm.

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Table 1. Iron concentrations measured by three laboratories in different new chemicals used in the tree fruit industry.

| Sample # | Lab A | Lab B | DANR Lab (UC-Davis) |
|---|---|---|---|
| | ppm Fe total | ppm Fe total | ppm Fe total |
| 1 | <0.01 | 1.35 | <0.2 |
| 2 | <0.01 | 2.32 | <0.2 |
| 3 | <0.01 | 1.20 | <0.2 |
| 4 | <0.01 | 3.23 | <0.2 |
| 5 | <0.01 | 0.56 | <0.2 |
| 6 | 3.0 | 1.34 | 2.8 |
| 7 | <0.01 | <0.10 | 0.4 |
| 8 | 6.0 | 1.62 | 10.2 |
| 9 | 4.0 | 6.14 | 5.0 |
| 10 | <0.01 | 1.45 | 5.8 |
| 11 | <0.01 | 2.81 | 11.5 |
| 12 | <0.01 | 4.68 | 16.3 |
| 13 | 4.0 | 2.00 | 2.4 |
| 14 | 6.0 | 4.57 | 5.1 |
| 15 | <0.01 | 0.59 | <0.2 |

Table 2. Specific heavy metal concentrations in selected additive solutions prepared at concentrations according to their labels (100 gallons per acre rate).

| Additive Names | Total (mg per liter) | | |
|---|---|---|---|
| | Iron (Fe) | Copper (Co) | Aluminum (Al) |
| **Omnis Supreme** | <0.2 | <0.2 | <0.5 |
| **No Foam B** | <0.2 | <0.2 | <0.5 |
| **Latron B** | <0.2 | <0.2 | <0.5 |

Table 3. Specific heavy metal concentrations in selected foliar nutrient solutions prepared at concentrations according to their labels (100 gallons per acre rate).

| **Foliar Nutrient Names** | **Total (mg per liter)** | | |
|---|---|---|---|
| | **Iron (Fe)** | **Copper (Co)** | **Aluminum (Al)** |
| **Cal Ocho** | 0.2 | <0.2 | <0.5 |
| **Vigor-Cal** | <0.2 | <0.2 | <0.5 |
| **Agri-Trend 20-20-20** | 2.8 | 1.1 | <0.5 |
| **MicroPlex** | 21.0 | 8.0 | <0.5 |
| **Goemar** | <0.2 | <0.2 | <0.5 |

Table 4. Specific heavy metal concentrations in selected preharvest fungicides solutions prepared at concentrations according to their labels (100 gallons per acre rate).

| **Fungicides Names** | **Total (mg per liter)** | | |
|---|---|---|---|
| | **Iron (Fe)** | **Copper (Co)** | **Aluminum (Al)** |
| **Elite** | 2.8 | <0.2 | 52.5 |
| **Orbit** | <0.2 | <0.2 | <0.5 |
| **Indar 75** | 0.3 | <0.2 | 4.6 |
| **Pristine** | 0.3 | <0.2 | 13.6 |

Table 5. Specific heavy metal concentrations in selected miticide solutions prepared at concentrations according to their labels (100 gallons per acre rate).

| **Miticide Names** | **Total (mg per liter)** | | |
|---|---|---|---|
| | **Iron (Fe)** | **Copper (Co)** | **Aluminum (Al)** |
| **Envidor** | <0.2 | <0.2 | <0.5 |
| **Onager** | <0.2 | <0.2 | <0.5 |
| **Vendex** | 10.2 | <0.2 | 183 |
| **Acramite** | 12.4 | <0.2 | 198 |
| **Omite** | 4.2 | <0.2 | 114 |

Table 6. Specific heavy metal concentrations in selected insecticide solutions prepared at concentrations according to their labels (100 gallons per acre rate).

| **Insecticide Names** | **Total (mg per liter)** | | |
|---|---|---|---|
| | **Iron (Fe)** | **Copper (Co)** | **Aluminum (Al)** |
| **Imidan** | 1.1 | <0.2 | 27.0 |
| **Intrepid** | <0.2 | <0.2 | <0.5 |
| **Success** | <0.2 | <0.2 | <0.5 |
| **DiPel DF** | 0.2 | <0.2 | <0.5 |
| **Lannate** | <0.2 | <0.2 | <0.5 |
| **Deliver** | 0.4 | <0.2 | <0.5 |
| **Delegate** | 2.0 | <0.2 | 29.2 |
| **Pyganic** | <0.2 | <0.2 | <0.5 |
| **Altacor** | 0.5 | <0.2 | 5.1 |

Table 7. Specific heavy metal concentrations in selected postharvest fungicides solutions prepared at concentrations according to their labels (100 gallons per acre rate).

| **Fungicide Names** | **8 oz of Scholar applied per 200,000 pounds of fruit** | **Total (mg per liter)** | | |
|---|---|---|---|---|
| | | **Iron (Fe)** | **Copper (Co)** | **Aluminum (Al)** |
| Scholar 25 | 25 gallons | 5.2 | <0.2 | 2.3 |
| Scholar 12 | 12 gallons | 12.1 | <0.2 | 20.7 |
| Scholar 8 | 8 gallons | 15.7 | <0.2 | 25.5 |

| **Fungicide Names** | **3 oz Mentor applied per 200,000 pounds of fruit** | **Total (mg per liter)** | | |
|---|---|---|---|---|
| | | **Iron (Fe)** | **Copper (Co)** | **Aluminum (Al)** |
| Mentor - 25 | 25 gallons | 2.5 | <0.2 | 4.6 |
| Mentor - 12 | 12 gallons | 4.8 | <0.2 | 8.6 |
| Mentor - 8 | 8 gallons | 7.3 | <0.2 | 13.8 |

Table 8. Change of skin pH on peach exposed to hydrocooler water.

| | pH |
|---|---|
| Sound fruit | 4.3 |
| Rinsed fruit | 4.1 |
| Hydrocooled fruit | 4.2 |
| 3 hours after hydrocooling | 4.3 |
| 15 hours after hydrocooling | 4.4 |
| Hydrocooled and rinsed | 4.3 |
| | |
| Hydrocooling water | 7.9 |
| Water | 7.8 |
| Distilled water | 7.5 |

Table 9. Change in pH of hydrocooler water with different concentrations of chlorine.

| Chlorine solution | pH |
|---|---|
| 0 ppm | 7.7 |
| 100 ppm | 8.3 |
| 200 ppm | 8.5 |
| 400 ppm | 8.7 |
| 800 ppm | 9.0 |
| Skin-Waxed Peach | 5.8 |
| Skin-Waxed Low Acid Nectarine | 4.8 |

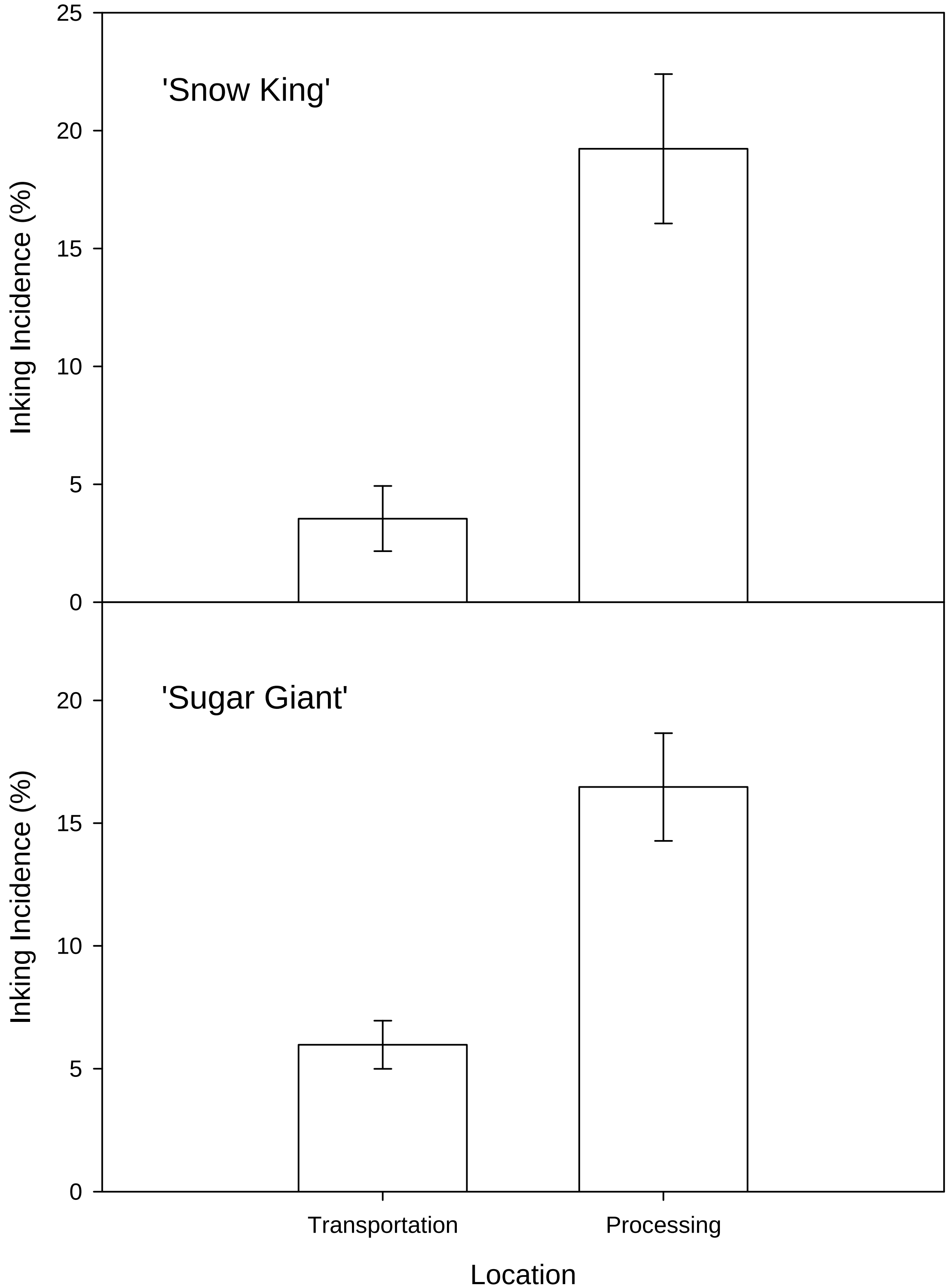


Fig. 1. Postharvest inking development on ‘Snow King’ and ‘Sugar Giant’ peaches sampled after transportation (left bar) or processing (right bar).

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