Sink activity in Washington navel orange fruit borne on leafy and leafless inflorescences

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**SUMMARY**

Washington navel orange (WNO) (*Citrus sinensis* L. Osbeck) fruit borne on leafy (LY) inflorescences (with leaves) were confirmed to be stronger sinks based on their faster-growth rate during the 9 weeks after petal fall than fruit borne on leafless (LS) inflorescences (without leaves). During this period, the activities of enzymes involved in phloem unloading and sucrose synthesis, including vacuole soluble acid invertase (VINV), sucrose synthase (SuSy), and sucrose phosphate synthase (SPS), were quantified in whole fruit and individual fruit tissues, exocarp (flavedo), mesocarp (albedo), vascular bundles, and endocarp (juice sacs), to identify the determinants of citrus fruit sink strength during Stage I of fruit development. Fruit set on LY and LS inflorescences exhibited no differences in VINV activity during the 9-week period. In contrast, SuSy activity was significantly greater in fruit developing on LY inflorescences, whereas SPS activity was significantly greater in fruit from LS inflorescences. For individual tissues isolated from fruit collected 7 and 9 weeks after petal fall, LY inflorescences had greater SuSy activity in vascular bundles (weeks 7 and 9), flavedo (weeks 7 and 9) and juice sacs (week 9) than fruit of LS inflorescences, whereas fruit from LS inflorescences had greater VINV activity in vascular bundles (week 9), flavedo (week 7) and juice sacs (weeks 7 and 9). The results provide strong evidence that SuSy activity is the determinant of greater sink strength during early development of WNO fruit borne on LY inflorescences.

**Index terms:** *Citrus sinensis*, acid invertase, sucrose synthase, sucrose phosphate synthase.

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**Atividade de dreno em frutos de laranja Washington navel desenvolvidos em inflorescências com folhas e sem folhas**

**RESUMO**

Frutos de laranja Washington navel (WNO) (*Citrus sinensis* L. Osbeck) desenvolvidos em inflorescências contendo folhas (LY) foram confirmados como sendo drenos mais fortes, com base na sua maior taxa de crescimento durante o período compreendido até nove semanas após a queda das pétalas, em comparação com frutos desenvolvidos em inflorescências sem folhas (LS). Durante este período, as atividades das enzimas envolvidas na descarga do floema e na síntese de sacarose, incluindo a inverteza de ácidos solúveis do vacúolo (VINV), sacarose sintetase (SuSy) e a fosfato de sacarose sintetase (SPS), foram quantificadas em frutos inteiros e tecidos individuais de frutos: exocárpico (flavedo), mesocárpico (albedo), feixes vasculares e endocárpico (sacos de suco), para
Sink activity in Washington navel orange fruit...

INTRODUCTION

Sweet orange (Citrus sinensis L. Osbeck) trees produce two general types of inflorescences: leafy (LY), bearing flowers and leaves, and leafless (LS), bearing only flowers. Based on a number of physiological criteria analyzed in fruit developing on LY and LS inflorescences of Valencia, Shamouti and Washington navel orange (WNO) trees, young developing fruit of LY inflorescences have been characterized as stronger sinks than fruit borne on LS inflorescences. Fruit retention (% fruit set) is greater for LY versus LS inflorescences (Emmer & Bravdo, 1983; Hofman, 1989; Lovatt et al., 1992; Moss et al., 1972). On average, fruit of the same age produced by LY inflorescences are larger than fruit on LS inflorescences, with significant differences in transverse diameter measurable just 1 week after petal fall (Hofman, 1989; Lovatt et al., 1992). By this time, fruit borne on LY inflorescences are metabolically different from fruit on LS inflorescences (Lovatt et al., 1992). For LY inflorescences, fruit growth rate is significantly greater as early as 2 weeks after petal fall. Thus, major differences in fruit set and fruit size potential of LY versus LS inflorescences are evident in Stage I of fruit development, which is dominated by cell division (Bain, 1958). In addition, the faster-growing fruit of LY inflorescences tend to remain faster growing, to persist on the tree longer, and to contribute more fruit to harvest (Lovatt et al., 1992; Zucconi et al., 1978). The greater yield and fruit size potential of LY inflorescences has been attributed (i) to photosynthate supplied by the leaves adjacent to the flowers and young developing fruit (Moss et al., 1972) and (ii) to the greater concentrations of gibberellins in the fruit (Hofman, 1989). However, despite evidence of greater sink strength in fruit developing on LY versus LS inflorescences, the activities of enzymes known to regulate sink strength have not been investigated.

Sucrose, the major form of sugar translocated in Citrus, is loaded into the sieve tube cells of the phloem in source tissues and unloaded in sink tissues (Katz et al., 2007; Koch, 2004). In sink tissue adjacent to the phloem, sucrose catabolism facilitates phloem unloading (Katz et al., 2007; Koch, 2004; Wind et al., 2010). In the apoplast, sucrose is hydrolyzed to glucose and fructose by cell wall insoluble acid invertase (CWINV; β-fructofuranosidase; sucrose → glucose + fructose; EC 3.2.1.26) (Koch, 2004; Wind et al., 2010; Sturm & Tang, 1999). Within sink cells, this reaction is carried out by invertases located in the cytosol (CINV) (soluble, neutral to alkaline invertase) and vacuole (VINV) (soluble, acid invertase) (Sturm & Tang, 1999). In addition, sucrose is cleaved by the cytosolic glycosyltransferase sucrose synthase (SuSy; UDP-glucose: D-fructose 2-α-D-glucosyltransferase; EC 2.4.1.13) to UDP-glucose and fructose in the presence of UDP (Geigenberger & Stitt, 1993; Koch, 2004). Thus, under high sucrose concentrations, sink strength is determined by the combined activities of the sucrose cleaving enzymes. However, in many sink tissues, sucrose is resynthesized and utilized in the synthesis and storage of starch. Under low sucrose or high hexose sugar concentrations, SuSy now synthesizes sucrose (Geigenberger & Stitt, 1993). Also in sink tissues, especially those that are not photosynthetic or do not store starch, sucrose phosphate synthase (SPS; UDP glucose: D-fructose-6-phosphate-2-glucosyltransferase; EC 2.4.1.14) contributes to sucrose synthesis in the reaction of UDP-glucose + fructose-6-phosphate → sucrose-6-phosphate + UDP, which in the presence of an abundance of sucrose phosphate phosphatase (SPP) (sucrose-6-phosphate → sucrose + Pi) renders the reaction irreversible and results in sucrose. Both SuSy and SPS have been documented to regulate sink strength (Komatsu et al., 1999; Sturm & Tang, 1999; Wind et al., 2010). The relative activity of these enzymes...
Ntoukakis et al. affects the ratio of sucrose to hexose sugars, which in turn influences cell metabolism, gene expression, and plant growth and development (Koch, 2004; Sturm & Tang, 1999; Wind et al., 2010). In general, high hexose sugar concentrations relative to sucrose favor cell division; whereas high sucrose supports cell differentiation, maturation and biomass accumulation (Koch, 2004; Sturm & Tang, 1999).

The role of these enzymes during Stage I of citrus fruit development is of interest since fruit growth rate during this stage of development influences both final fruit set and final fruit size (Erner & Bravdo, 1983; Hofman, 1989; Lovatt et al., 1992; Moss et al., 1972; Zucconi et al., 1978). This stage of citrus fruit development, which is dominated by cell division (Bain, 1958), is likely associated with high cellular concentrations of hexose sugars maintained by CWINV and/or VINV activity (Koch, 2004). Indeed, VINV was the most active enzyme in extracts from all tissues of Stage I fruit of grapefruit (Citrus paradisi L.) (Lowell et al., 1989). In later stages of fruit development, SuSy activity was greater in extracts of grapefruit transport tissues (vascular bundles), whereas SPS was the more active enzyme in juice sacs. Similar information is lacking for sweet orange.

Fruit of the WNO were used to investigate the roles of VINV, SuSy and SPS in controlling sink strength during the first nine weeks of fruit development following petal fall (Stage I) (Bain, 1958). The WNO provides an excellent system for investigating the contribution of these enzymes in regulating sink activity. The anatomy of the citrus fruit (a hesperidium) makes it feasible to separate the flavedo, albedo, juice sacs, and vascular bundles, which are located between the albedo and segment wall outside the juice sacs (Lowell et al., 1989). Quantification of the relative activities of enzymes regulating sink strength during Stage I of fruit development in fruit borne on LY versus LS inflorescences will contribute to our understanding of the determinants of sink activity in WNO fruit and might provide information essential for increasing fruit growth rate during this critical period to increase sweet orange yield and fruit size.

**MATERIALS AND METHODS**

**Plant material and chemicals used**

Commercially producing 20-year-old WNO scions on Carrizo citrange (C. sinensis × Poncirus trifoliata L. Raf) rootstock located at the University of California, Riverside, were the source of Stage I fruit. At 70% anthesis, 200 LY and LS inflorescences at the same developmental stage were tagged. Five fruit were collected from each inflorescence type at weeks 1, 3, 5, 7 and 9 after petal fall. Individual tissues could only be isolated from fruit collected in weeks 7 and 9. For these samples, fruit were cut in half, with half left intact and the other half dissected into flavedo, albedo, vascular bundles and juice sacs. Fruit halves or tissues collected on the same date from the same inflorescence type were pooled. All samples were pulverized in liquid nitrogen and stored at –80 °C. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise noted.

**Carbohydrate analysis**

Pulverized fruit tissue (0.1 g) was extracted in 5 mL of boiling 80% ethanol for 1 min, incubated at 65 °C in a water bath for 30 min, and then centrifuged for 10 min at 3,000g. The supernatant (soluble sugars) was decanted and saved; the pellet was extracted two more times with 5 mL of 80% ethanol. Pooled supernatants (15 mL) were dried in a SpeedVac concentrator (ThermoFisher Scientific, Waltham, MA), suspended in 1 mL of deionized water and applied to tandem anion and cation resin (AG1-X8/formate and AG50W-X8/H+) columns (1 mL each) (Bio-Rad, Hercules, CA). Soluble neutral sugars were eluted with 15 mL deionized water and dried as described above. The sugars were dissolved in 200 μL of deionized water, filtered (0.45-μm pore size), and a 20-μL aliquot analyzed by HPLC using a heated (85 °C) Sugar-pak I (300 mm × 6.5 mm) column (Waters Corp., Milford, MA) eluted with calcium-ethylenediaminetetraacetic acid (Ca-EDTA) (50 mg L⁻¹) at a flow rate of 0.5 mL per min. Separated sugars were quantified by refractive index relative to known sugar standards (Beckman Instruments, Irvine, CA).

**Partial purification of enzymes from fruit tissue**

Pulverized fruit or individual fruit tissues (5 g) were homogenized with four 10-sec bursts at full speed with a Polytron tissue homogenizer (Brinkman, Westbury, NY) in 15 mL of buffer A (100 mM HEPES [pH 7.5], 2% polyvinylpolyalcohol, 4 mM EDTA, 2 mM ethylene glycol-bis(β-aminoethylether)-tetraacetic acid [EGTA],
50 mM Na-ascorbate, 5 mM thiourea, 5 mM benzamidine, 1 mM phenyl methyl sulfonyl fluoride, 1 mM dithiothreitol [DTT], 5 mM \( \varepsilon \)-amino-\( n \)-caproic acid, and phosphatase inhibitors [50 mM NaF, 10 mM Na\(_2\)MoO\(_4\), and 1 mM activated orthovanadate]). The homogenate was filtered through a 0.45-\( \mu \)m pore-size nylon membrane and centrifuged at 30,000g for 30 min. The supernatant was brought to 30% saturation with (NH\(_4\))\(_2\)SO\(_4\), stirred for 30 min, and then centrifuged at 30,000g for 30 min. The clarified supernatant was passed through two layers of Miracloth (MilliporeSigma, Darmstadt, Germany) and brought to 70% saturation with (NH\(_4\))\(_2\)SO\(_4\). Precipitated proteins were collected by centrifugation at 30,000g for 30 min, suspended in a minimum volume of buffer B (20 mm HEPES [pH 7.5], 2 mm DTT, and 10% glycerol), and centrifuged for 30 min at 30,000g. The clarified supernatant was desalted on a PD-10 column (Amersham Biosciences, Piscataway, NJ) and concentrated using an Amicon Ultra-4 10,000 MWCO (MilliporeSigma). This fraction was frozen in liquid nitrogen, stored at \(-80^\circ\)C and served as the source of partially purified enzymes.

### The activity of the sucrose metabolizing enzymes in fruit tissues

Acid invertase activity was quantified as sucrose cleavage by coupling glucose and fructose formed to NADH production using commercial hexokinase, glucose phosphate isomerase and glucose-6-phosphate dehydrogenase (Bergmeyer & Klotzsch, 1965). The reaction contained 100 mM acetate buffer (pH 4.5), 100 mM sucrose, 5 mM MgCl\(_2\), 20 mM NaF, 5 mM DTT, 1 mM ATP, 0.2 mM NAD, 5 mM EDTA, 1 unit of hexokinase, 3 units of glucose phosphate isomerase, 1 unit of glucose-6-phosphate dehydrogenase in 200 \( \mu \)L; NADH formed was measured at 340 nm.

Activity of SuSy was determined as sucrose production by coupling glucose and fructose formed to NADH production using commercial hexokinase, glucose phosphate isomerase and glucose-6-phosphate dehydrogenase (Bergmeyer & Klotzsch, 1965). The reaction contained 80 mM fructose, 10 mM MgCl\(_2\), 20 mM NaF, 5 mM DTT, 10% glycerol and 10 mM UDP-[\(^3\)H]glucose (0.2 MBq/mmole specific activity) in 20 mM HEPES (pH 7.5) in 200 \( \mu \)L (final volume) incubated at 30 °C for 20 min and terminated by heating at 100 °C for 1 min. After cooling, 0.5 g cation resin AGI-X8/formate was added to remove unincorporated UDP-[\(^3\)H]glucose. The resin was washed three times with 400 \( \mu \)L of distilled water, followed by centrifugation and collection of the supernatant after each centrifugation. An equal volume of Liquiscint (National Diagnostics, Sommerville, NJ) was added to the pooled supernatant, and [\(^3\)H]sucrose was quantified using a Beckman LS 500 TD scintillation counter (Beckman Instruments).

Activity of SPS was quantified as the combined production of sucrose and sucrose-6-phosphate (Huber et al., 1989). The reaction contained 10 mM UDP-glucose, 10 mM fructose-6-phosphate, 40 mM glucose-6-phosphate, 5 mM MgCl\(_2\), 5 mM DTT, 10% glycerol and phosphatase inhibitors (20 mM NaF, 5 mM Na\(_2\)MoO\(_4\), and 1 mM activated orthovanadate) in 20 mM HEPES (pH 7.5). The assay was run at 28 °C in 100 \( \mu \)L (final volume) for 20 min and terminated with an equal volume of 30% KOH and boiling for 10 min to break down any remaining fructose-6-phosphate, UDP-glucose, glucose-6-phosphate, sucrose-6-phosphate, and other reducing sugars to prevent reaction with anthrone, whereas sucrose was left intact (van Handel, 1968). After boiling, the denatured protein was pelleted by centrifugation and 150 \( \mu \)L of supernatant was mixed with 1 mL of 0.14% anthrone in 13.8 N H\(_2\)SO\(_4\) to detect sucrose. The solution was incubated at 40 °C for 20 min and optical density (OD) at 620 nm was determined. SPS was also assayed in the direction of sucrose synthesis in the presence of UDP-glucose and fructose-6-phosphate by coupling the production of UDP to pyruvate kinase and lactic acid dehydrogenase and measuring the depletion of NADH at 340 nm (Adam, 1965). The reaction contained 0.2 mM NADH, 1.63 mM 2-phosphoenolpyruvate, 5 mM MgCl\(_2\), 6.8 mM KCl, 10 units of lactic acid dehydrogenase, 10 units of pyruvate kinase, 10 mM UDP-glucose, 10 mM fructose-6-phosphate, 40 mM glucose-6-phosphate, 5 mM DTT, phosphatase inhibitors (20 mM NaF, 5 mM Na\(_2\)MoO\(_4\), 1 mM activated orthovanadate) and 10 \( \mu \)L of SPS in 20 mM HEPES (pH 7.5) and 10% glycerol in a final volume of 100 \( \mu \)L. Sucrose formed was detected with anthrone as described above.

Enzyme activities are reported as µmol product formed per min per mg protein, which was measured as OD at 595 nm with γ-globulin as the standard (Bradford, 1976).

### RESULTS

#### Fruit growth rates

One week after petal fall, the average transverse diameter of fruit borne on LY inflorescences was significantly greater than that of fruit borne on LS inflorescences (Table 1). The difference in fruit size persisted through
the last fruit collection date 9 weeks after petal fall. In addition, fruit growth rate per day was greater for fruit developing on LY inflorescences throughout the 9-week period (Figure 1a). For fruit from both inflorescence types, fruit growth rate increased steadily during the first 3 weeks after petal fall, remained constant between the third and fifth week, and then increased dramatically from week 5 through 9, i.e., from 0.3 mm per day to a high of 0.75 mm per day for fruit on LY inflorescences and from 0.2 to only 0.4 mm per day for fruit of LS inflorescences.

### Fruit carbohydrate concentrations

For both inflorescence types, as fruit increased in size, glucose, fructose and sucrose accumulated (data not shown). Glucose concentrations in fruit developing on

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**Table 1.** Transverse diameter (mm) of Washington navel orange fruit developing on leafy (LY) and leafless (LS) inflorescences

| Inflorescence type | Weeks after petal fall |
|-------------------|------------------------|
|                   | 1          | 3          | 5          | 7          | 9          |
| LY inflorescence  | 5.5 a     | 9.7 a     | 13.8 a     | 21.0 a     | 30.7 a     |
| LS inflorescence  | 4.4 b     | 7.1 b     | 10.2 b     | 16.6 b     | 22.0 b     |
| P-value           | 0.001     | 0.001     | 0.001      | 0.001      | 0.001      |

*Means within a vertical column followed by different letters are significantly different at the 5% level using Fisher’s Protected LSD test.*

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**Figure 1.** (a) Fruit growth rate (based on transverse diameter) and (b) activities of vacuole soluble acid invertase (VINV), (c) sucrose synthase (SuSy), and (d) sucrose phosphate synthase in partially purified extracts of intact fruit from leafy (LY) (••-) and leafless (LS) (-o-) inflorescences of Washington navel orange. Data are the means ± SE (n = 3).
LY and LS inflorescences increased in parallel during the 9-week period after petal fall. For LY inflorescences, fruit sucrose concentration increased 6-fold, from 15 to 90 mg per g fruit tissue from week 1 to week 9, whereas fruit on LS inflorescences exhibited a 12.5-fold increase in glucose (from 8 to 100 mg per g fruit tissue) during this period, resulting in a significantly greater glucose concentration in fruit from LS inflorescences by week 9. The accumulation of fructose was less dramatic from week 1 to week 9, increasing from 30 to 60.25 mg per g tissue for fruit from LY inflorescences versus an increase from 20 to 50 mg per g tissue for fruit from LS inflorescences. Fructose concentrations were significantly greater in fruit of LY inflorescences at weeks 1, 7 and 9 after petal fall. For both inflorescence types, fruit sucrose concentrations remained relatively constant at ~30 mg per g fruit tissue from week 1 to 9, with the exception of a 67% decrease in sucrose concentration from week 1 to week 3 and full recovery to 30 mg per g fruit tissue by week 5. The decrease in sucrose concentration was coincident with the period when fruit on both inflorescences types failed to show an increase in growth rate, i.e., weeks 3 to 5 after petal fall (Figure 1a).

During the first 3 weeks of fruit development after petal fall, the ratio of the concentrations of sucrose to hexose sugars (glucose + fructose) was significantly lower in fruit from LY inflorescences than those of LS inflorescences (Table 2). The greater hexose sugar concentration relative to sucrose in fruit from LY inflorescences is consistent with cell division as the predominant type of growth during Stage I of WNO fruit development. In contrast, the ratio of sucrose to hexose sugars was greater than one during the first week after petal fall in fruit from LS inflorescences (Table 2). The ratio dropped below one by week 3, but was still significantly greater than that of fruit from LY inflorescences. For weeks 5 through 9 after petal fall, fruit on LY and LS inflorescences had low ratios of sucrose to hexose sugar concentrations that were not significantly different.

### Enzyme activities

In partially purified extracts of intact WNO fruit, sucrose was metabolized by VINV (soluble, pH optimum 4.5; $K_m$ for sucrose of 4.975 mM), SuSy (pH optimum 7.5; $K_m$ for UDP-glucose of 0.957 mM, $K_m$ for D-fructose of 1.46 mM, and SPS (pH optimum 7.0-7.5; $K_m$ for UDP-glucose of 9.54 mM, $K_m$ for fructose-6-phosphate of 0.755 mM); the activity of CINV (soluble, pH optimum 6.5) was very low. There were no significant differences in VINV activity in fruit from LY versus LS inflorescences during the 9 weeks after petal fall (Figure 1b). For both, VINV activity was greater during the first 5 weeks after petal fall, decreasing 3-fold by week 9. At 1 week after petal fall, fruit from LY inflorescences had significantly lower SuSy activity (20%) than fruit from LS inflorescences (Figure 1c). By week 3, SuSy activity increased 60% in fruit from LY inflorescences, whereas it decreased 20% in fruit from LS inflorescences, making SuSy activity in fruit collected from LY inflorescences significantly greater than fruit from LS inflorescences. For fruit of LY and LS inflorescences, SuSy activity reached a minimum rate 5 weeks after petal fall, but increased 5-fold by week 7, with no differences in SuSy activity between fruit of LY and LS inflorescences at week 5 or 7. However, fruit from LY inflorescences sustained the high rate of SuSy activity attained in week 7 through week 9, whereas SuSy activity decreased 50% in fruit from LS inflorescences by week 9 to a rate significantly lower than that of fruit from LY inflorescences. In contrast, SPS activity, which was much lower than VINV or SuSy activity throughout the 9-week period, was always greater in fruit from LS inflorescences than fruit of LY inflorescences, except for the first week after petal fall (Figure 1d). The observed differences in the activity of SuSy versus SPS in fruit from LY and LS inflorescences resulted in LY inflorescences having a greater ratio of SuSy activity to SPS activity through the entire 9-week period, except during the first week after petal fall ($P = 0.001$) (Table 3).

### Table 2. Ratio of sucrose to hexose sugar (glucose + fructose) concentrations in Washington navel orange fruit from leafy (LY) and leafless (LS) inflorescences

| Inflorescence type | 1      | 3      | 5      | 7      | 9      |
|-------------------|--------|--------|--------|--------|--------|
| LY inflorescence  | 0.622 b | 0.195 b | 0.305 a | 0.227 a | 0.222 a |
| LS inflorescence  | 1.065 a | 0.305 a | 0.221 a | 0.198 a | 0.219 a |
| $P$-value         | 0.003  | 0.003  | 0.084  | 0.657  | 0.657  |

*aRatios within a vertical column followed by different letters are significantly different at the 5% level using Fisher’s Protected LSD test.
Individual tissues were isolated from WNO fruit collected 7 and 9 weeks after petal fall and the activities of VINV and SuSy were assayed in partially purified extracts of flavedo, vascular bundles and juice sacs. For the flavedo, fruit from LS inflorescences had greater VINV activity than fruit from LY inflorescences at week 7, but VINV activity was greater in LY inflorescences by week 9 (Figure 2a). In contrast, SuSy activity was consistently greater in the flavedo of fruit from LY inflorescences than fruit from LS inflorescences (Figure 2a). In juice sacs, fruit of LS inflorescences had consistently greater VINV activity than fruit from LY inflorescences (Figure 2b).

For juice sacs, SuSy activity was equally low in fruit from LY and LS inflorescences at week 7. However, by week 9, SuSy activity in juice sacs had increased more than 3-fold for fruit from LY inflorescences, but had only doubled for fruit of LS inflorescences, resulting in greater SuSy activity in juice sacs of fruit borne from LY inflorescences (Figure 2b). For vascular bundles, VINV activity was not significantly different for fruit from the two types of inflorescences at week 7 after petal fall, but at week 9, vascular bundles from fruit of LS inflorescences had significantly greater VINV activity than those from fruit on LY inflorescences (Figure 2c). In contrast, SuSy

Table 3. Ratio of sucrose synthase activity to sucrose phosphate synthase activity in Washington navel orange fruit from leafy (LF) and leafless (LS) inflorescences

| Inflorescence type | Weeks after petal fall |
|--------------------|------------------------|
|                    | 1          | 3          | 5          | 7          | 9          |
| LY inflorescence   | 54.1 b\(^c\) | 48.1 a    | 1.8 a    | 20.1 a    | 59.9 a    |
| LS inflorescence   | 69.1 a     | 20.6 b    | 0.8 b    | 10.4 b    | 9.3 b     |
| \(P\)-value        | 0.001      | 0.001     | 0.001    | 0.001     | 0.001     |

\(^c\)Ratios within a vertical column followed by different letters are significantly different at the 5% level using Fisher’s Protected LSD test.

Figure 2. Activities of vacuole soluble acid invertase (VINV) \(\square\) and sucrose synthase (SuSy) \(\square\) in partially purified extracts of (a) flavedo, (b) juice sacs, and (c) vascular bundles from fruit borne on leafy (LY) and leafless (LS) inflorescences of Washington navel orange. Data are the means ± SE (n = 3).
activity was significantly greater in vascular bundles from fruit of LY inflorescences than those from fruit of LS inflorescences at weeks 7 and 9 after petal fall (Figure 2c).

**DISCUSSION**

Fruit borne on LY inflorescences were confirmed to be stronger sinks based on their larger size and faster rate of growth during the 9-week period after petal fall compared to fruit on LS inflorescences, consistent with earlier reports (Hofman, 1989; Lovatt et al., 1992). Differences in the accumulation of sucrose, glucose and fructose during the first 3 weeks of fruit development resulted in fruit from LY inflorescences having a low ratio of sucrose to hexose sugars that was significantly lower than fruit of LS inflorescences. Since growth of WNO fruit during the 9 weeks after petal fall is dominated by cell division (Bain, 1958) and cell division is favored by high concentrations of hexose sugars relative to sucrose (Koch, 2004; Komatsu et al., 1999; Sturm & Tang, 1999), the low sucrose to hexose sugar ratio is likely a positive factor in the growth of fruit borne on LY inflorescences.

The lower ratio of sucrose to hexose sugar concentrations in fruit of LY inflorescences is consistent with fruit of LY inflorescences having greater combined activity of sucrose cleaving enzymes during early development. The activity of VINV during the first five weeks after petal was equally high in fruit from both LY and LS inflorescences. However, fruit from LY inflorescences also had high SuSy activity during first 3 weeks after petal fall. The interpretation that SuSy was functioning in sucrose cleavage is supported by the low ratio of sucrose to hexose sugars in fruit from LY inflorescences. During the first week after petal fall, when SuSy activity was greater in fruit of LS inflorescences the ratio of sucrose to hexose sugar was greater than one, suggesting that SuSy might have been synthesizing sucrose in fruit of LS inflorescences. This interpretation is supported by the fact that the decrease in SuSy activity paralleled the decrease in the ratio of sucrose to hexose sugar concentrations in fruit of LS inflorescences during the 9 weeks after petal fall. Sucrose was also synthesized by SPS; SPS activity was significantly greater in fruit from LS versus LY inflorescences from week 3 through week 9.

The contribution of SuSy activity to fruit from LY versus LS inflorescences was similarly reflected in individual fruit tissues at weeks 7 and 9 after petal fall. Fruit from LY inflorescences had greater SuSy activity in vascular bundles and flavedo on both dates and juice sacs at week 9 than fruit of LS inflorescences; fruit from LS inflorescences had greater VINV activity in vascular bundles at week 9, flavedo at week 7 and juice sacs on both dates. By week 9, SuSy activity in fruit from LY inflorescences was greater than VINV activity in fruit from both LY and LS inflorescences.

The results of this research suggest that the faster-growth rate and larger size of fruit borne on LY inflorescences is related to their greater sucrose cleaving activity during early development. The activity of VINV was equal in fruit from both types of inflorescences, but fruit borne on LY inflorescences had greater SuSy activity, which resulted in a lower ratio of sucrose to hexose sugars favoring cell division and growth. In fruit of LS inflorescences, VINV activity alone maintained sink strength. For fruit borne on LS inflorescences, synthesis of sucrose possibly by SuSy during the first 3 weeks after petal fall and by SPS throughout the 9 weeks after petal fall likely contributed to the greater ratio of sucrose to hexose sugars unfavorable to cell division during the critical first 3 weeks after petal fall. Taken together, the results provide strong evidence that SuSy activity is the key determinant of the greater sink strength of WNO fruit borne on LY inflorescences during early fruit development.

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