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Activities of Sucrose and Sorbitol Metabolizing Enzymes in Vegetative Sinks of Peach and Correlation with Sink Growtb Rate

Riccardo Lo Bianco and Mark Rieger
Department of Hortkulture, University of Georgia, Athens, GA 30602

She-Jean S. Sung
Institute of Tree Root Biology, Southem Research Station, U.S. Department of Agriculture Forest Sewice, 320 Green Street, Athens, GA 30602

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ABSTRACT. Terminal portions of 'Flordaguard' peach roots [*Prunus persica* (L.) Batsch] were divided into six segments and the activities of NAD+-dependent sorbito1 dehydrogenase (SDH), sorbito1 oxidase (SOX), sucrose synthase (SS), soluble acid invertase (Al), and soluble neutral invertase (NI) were measured in each segment 10, 15, and 20 days af'ter seed germination. The same type of experiment was conducted with terminal portions of 'Flordaguard' and 'Nemaguard' peach shoots except that one of the six segments consisted of the leaflets surrounding the apex. Independent of the age of individual roots, activities of SDH and AI were consistently highest in the meristematic portion and decreased with tissue maturation. In shoots, AI was the most active enzyme in the elongating portion subtending the apex, whereas SDH was primarily associated with meristematic tissues. A positive correlation between SDH and AI activities was found in various developmental sones of roots (r = 0.96) and shoots (r = 0.90). Sorbito1 and sucrose contents were low in roots regardless of distance from tip, while sucrose showed a decreasing trend with distance and sorbitol, fructose, and glucose increased with distance from the meristem in shoots. Activity of SDH in internodes, but not apices, correlated with shoot elongation mte of both cultivars, whereas activities of otber enzymes did not correlate with shoot elongation rate. We conclude that AI and SDH are tbe predominant enzymes of carbohydrate catabolism and the best indicators of sink growth and development in vegetative sinks of peach.

In peach (*Prunus persica*), as well as in many species of the Rosaceae, the polyol sorbito1 represents the major photosynthetic product and the main form of translocated carbon, although sucrose is also present and functions similarly (Bieleski, 1982). Little is known about sucrose and sorbito1 metabolism and their relative importance in carbon partitioning and carbon use efficiency of plants such as peach.

In plants that use only sucrose as the translocated form of carbon, Sung et al. (1989) found that sucrose cleavage enzyme activity in sink tissues was correlated with sink growth tate. In plants where sucrose is not the major form of translocated carbon, this same principle may apply, but with different enzymes. In peach, sorbito1 dehydrogenase (SDH) is found primarily in sink tissues and may be the main enzyme responsible for the oxidation of sorbito1 and eventual use of its carbon in growth (Lo Bianco et al., 1998, 1999; Loescher, 1987). Currently, it is unclear whether SDH activity is related to sink strength in Rosaceous tree fruit, although in apple [*Malus domestica* (L.) Borkh], SDH activity does not seem to be correlated with fruit relative growth rate (Yamaguchi et al., 1996), and SDH activity was not detected in 'Hakuto' (Moriguchi et al., 1990) and 'Encore' (Lo Bianco et al., 1999) peaches.

Our previous work indicated that sorbito1 and sucrose may play different roles in peach sinks, depending on the developmental stage of the sink (i.e., young versus mature fruit) and on the type of sink (i.e., reproductive versus vegetative organ) (Lo Bianco et al., 1999). In particular, sucrose represented the major carbon form used for fruit growth and soluble acid invertase (AI) was the enzyme of sucrose cleavage that best correlated with fruit growth rate. On the other hand, sorbito1 seemed to have a predominant role in vegetative growth, where SDH activity, but not sucrose cleavage enzyme activities, followed the same pattem as shoot growth rate. However, further experiments were needed to verify the latter.

Activities of invertase and other enzymes have been studied in successive stages of development in pea (*Pisum sativum* L.), broad bean (*Vicia faba* L.), and com radicles (*Zea mays* L.) (Hellebust and Forward, 1962; Robinson and Brown, 1952), supporting the idea that sucrose cleaving enzymes play specific roles in root apex growth. Studies on levels of transcripts for sucrose cleavage *enzymes* in carrot (*Daucus carota* L.) suggest that sucrose synthase (SS) regulates sucrose utilization in developing tap roots, while a soluble acid invertase located in the vacuoles controls sucrose storage and sugar composition (Sturm et al., 1995). Very few studies, however, have focused on the association between tissue development and carbohydrate metabolism of vegetative sinks in species that primarily use polyols as the form of translocated carbon. The 'transition from sink to source stage of developing leaves has been studied and associated with carbohydrate biosynthesis and biodegradation in apple (Loescher et al., 1982) and peach (Merlo and Passera, 1991). Therefore, the objectives of this work were to characterize the activities of sorbito1 and sucrose metabolizing enzymes in the developmental zones of the root and shoot apex of peach, and relate them to the stage of growth and tissue maturation. We then tested the hypothesis that sorbito1 and/or sucrose cleaving enzyme activities were associated to sink growth and to the developmental stage of tissues within the sink in vegetative organs of peach.

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Materials and Methods

PLANT MATERIALS AND EXPERIMENTAL PLAN. Stratified (moist-

prechilled) seeds of 'Flordaguard' peach were sown in trays of a composted pine bark medium in a greenhouse on 9 Dec. 1997. Thirty to thirty-four temiinal portions of roots were collected 10, 15, and 20 d after seed germination. At 10 and 15 d after germination, radicles were sampled. However, 20 d after germination the radicle was no longer distinguishable from lateral roots, so it is likely that lateral roots were sampled. Each root tip was divided into six segments, a 3-mm-long segment that consisted of mostly meristematic tissue and five 5-mm-long segments that consisted of elongating or mature tissue. Twenty to thirty terminal portions of shoots from 3-month-old 'Nemaguard peach seedlings and 1-year-old 'Flordaguard' peach rooted cuttings were used for localization of enzymes within shoot tips. Plants were grown hydroponically (Rieger and Scalabrelli, 1990) in a greenhouse in Athens, Ga. (35 °N latitude and 85 °W longitude), yielding approximately 70% integrated daily solar radiation transmission, natural photoperiod, and temperatures ranging from 22 to 35 "C. Each terminal portion was divided into six segments, the leaflets surroundingthe meristem, a 3-mm-long segment including the meristem, and four subsequent 5-mm-long segments consisting of mostly elongating or mature internode tissue. Samples were transported quickly from the greenhouse to the laboratory in aluminum foil on ice and then rinsed in distilled water, blotdried. One half of the samples was assayed immediately for SDH since the enzyme is not stable in frozen tissues (Lo Bianco et al., 1998), whereas the other half was stored at -20 °C for subsequent determination of SS, AI, soluble neutral invertase (NI), and sorbito1 oxidase (SOX) activities (no significant loss of activity occurred as compared to fresh samples).

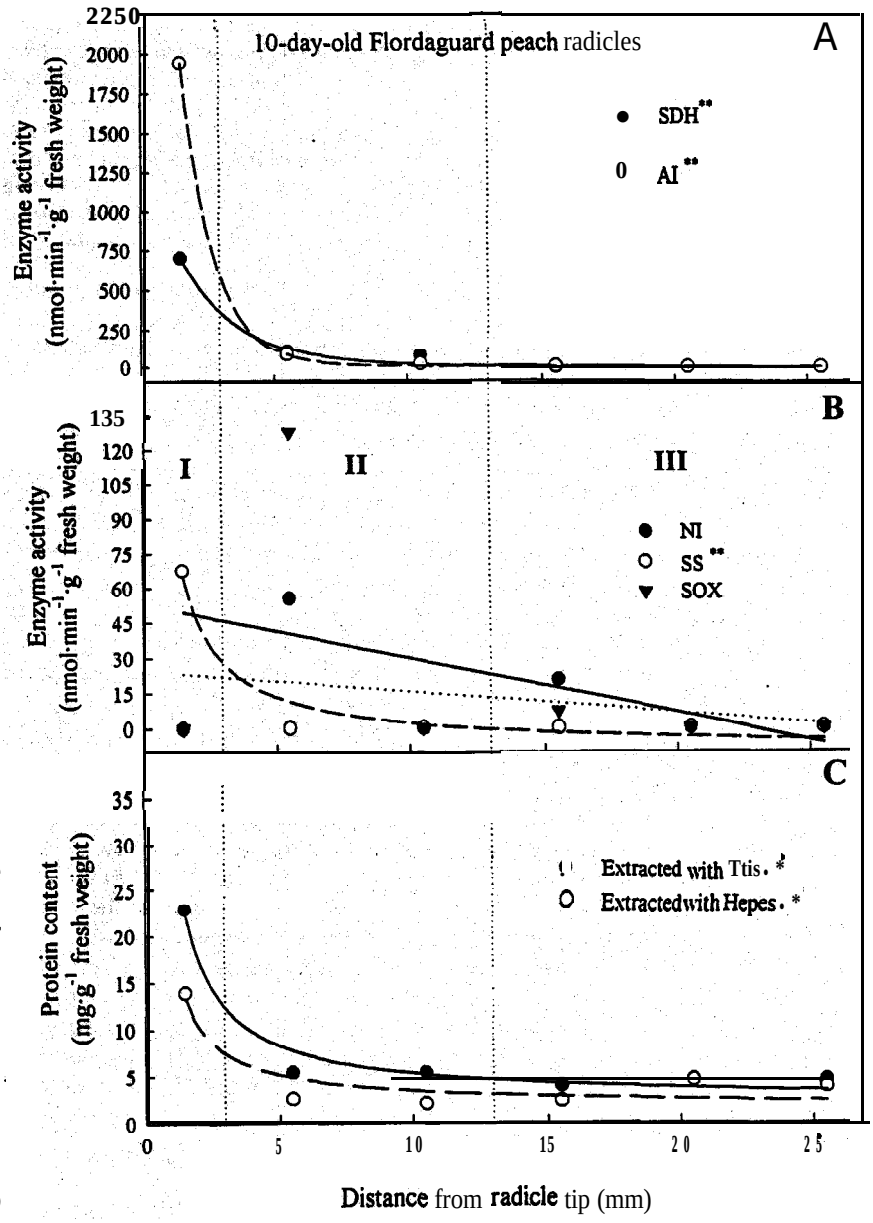
Two to three root tips of 'Flordaguard' seedlings were collected 10, 15, and 20 d after seed germination and submerged immediately in formaldehyde–acetic acid-ethanol (FAA, 70% ethanol). After fixation in FAA, roots were stained with 0.01% safranin, embedded in parafin wax, and sectioned in thin, free hand segments at known distances from the apex. Sections were examined under a Zeiss SV8 (Carl Zeiss, Thomwood, N.Y.) light microscope at 100×, and a distinguishable stele was used as a transition point between meristematic and elongating portions, while the presenceofmaturemetaxylemelementsindicatedroot maturity. Shoot tips were not examined microscopically since meristematic, elongating, and mature zones were easily located by thenaked eye.

Growth analysis. Shoots of 'Flordaguard' seedlings and 'Nemaguard' rooted cuttings were labeled and their terminal two intemodes were measured in length daily for at least 3 d. A shoot relative growth rate (RGR) in millimeters per d per millimeter was obtained according to the following formula: RGR = $(\ln L_2 - \ln L_1)/(T_2 - T_1)$ where L is length in millimeters and T is time in days.

Eachshoottiporintemoderepresentedasample and, in each experiment, six samples from shoots growing at relatively different rates were harvested to be assayed for enzyme acuvity.

Enzyme extraction and assays. SDH was extracted and assayed followmg the protocol of Lo Bianco et al. (1998). Tissues were homogenized in 0.1 M Tris buffer (pH 9 at 25 "C) containing 8% (v/v) glycerol, with 2-mercaptoethanol (20 mM) added immediately before each extraction since it was unstable in stored buffer. Tween 20 (0. 1%, v/v) and polyvinylpolypyrrolidone (PVPP, 1%, w/v) were added during grinding. SS, SOX, and the soluble fraction of AI and NI were extracted using 0.2 M Hepes/NaOH buffer (pH 7.5 at 25 "C) containing 10 mM dithiothreitol (DTT), 3 mM Mg-acetate, and 6% (v/v) glycerol; 0.1% (v/v) Tween 20 and 1% (w/v) PVPP were addedduring grinding. In al1 cases, the tissue was ground in buffer

Fig. 1. Activity of (A) SDH and AI, (B) NI, SS, and SOX, and (C) protein content in various segments of 'Flordaguard' peach radicles 10 d after seed germination. Data points represent midpoints of various segments. Regression equations are $SDH = 13477.4^{-0.44 \cdot distance}$, $R^2 = 0.991$; $AI = 6470.37^{-0.80 \cdot distance}$, $R^2 = 0.999$; $SS = 11.14 \cdot distance^{-1} + 9.04$, $R^2 = 0.955$; Tris protein $= 30.04 \cdot distance^{-1} + 2.34$, $R^2 = 0.970$; Hepes protein $= 17.26 \cdot distance^{-1} + 1.76$, $R^2 = 0.865$. 'Significance of regression at $P \leq 0.05$, "significance of regression at $P \leq 0.01$. Absence of asterisk indicates that regression was nonsignificant. Vertical dotted lines indicate transition from meristematic (I) to elongating (II), to mature (III) tissue.



and sand using a precooled (2 to 4 "C) mortar and pestle. The homogenate was centrifuged at 3000 g_n for 15 min and the supematant was assayed directly without desalting (no significant difference in enzyme activity was obtained when extracts were desalted),

SDH was assayed using 0.1 mL of crude extract, 0.1 M Tris buffer (pH 9.5 at 25 "C), 1 mM NAD^+, and 300 mM sorbito1 in 1 mL final volume (Lo Bianco et al., 1998). SS, AI, and NI were assayed as described in Xu et al. (1989). Briefly, SS was assayed by measuring the continuous change in optical density at 340 nm at 25 °C on a Spectronic 21-D spectrophotometer (Milton Roy, Rochester, N.Y.) using 100 mM sucrose, 0.5 mM UDP, and 1 mM PPi as substrates and phosphoglucomutase (1 U), *Leuconostoc* glucose-6-phosphate dehydrogenase (1 U), and endogenous UDP-glucopyrophosphorylase as coupling enzymes. UDP-glucopyrophosphorylase activity was measured in al1 extracts before conducting the SS assay. Values of UDP-glucopyrophosphorylase activity were always at least 100 times greater (≈1100 $nmol \cdot min^{-1} \cdot mg^{-1}$ protein or 20,000 $nmol \cdot min^{-1} \cdot g^{-1}$ fresh weight) than those of SS activity in the same tissues, and thus did not limit the apparent SS activity. AI and NI were assayed with 25 mM sucrose at pH 5.0 and 100 mM sucrose at pH 7.0, respectively, whereas SOX was assayed with 400 mM sorbitol at pH 4.0. Aftera 15- to 20-min incubation at 25 °C, the reaction was stopped by boiling for 10 min. The AI and SOX reaction mixtures were neutralized before boiling. The glucose formed was then measured using hexokinase (1 U) and *Leuconostoc* glucose-6-phosphate dehydrogenase (1 U) in presence of ATP and NAD.

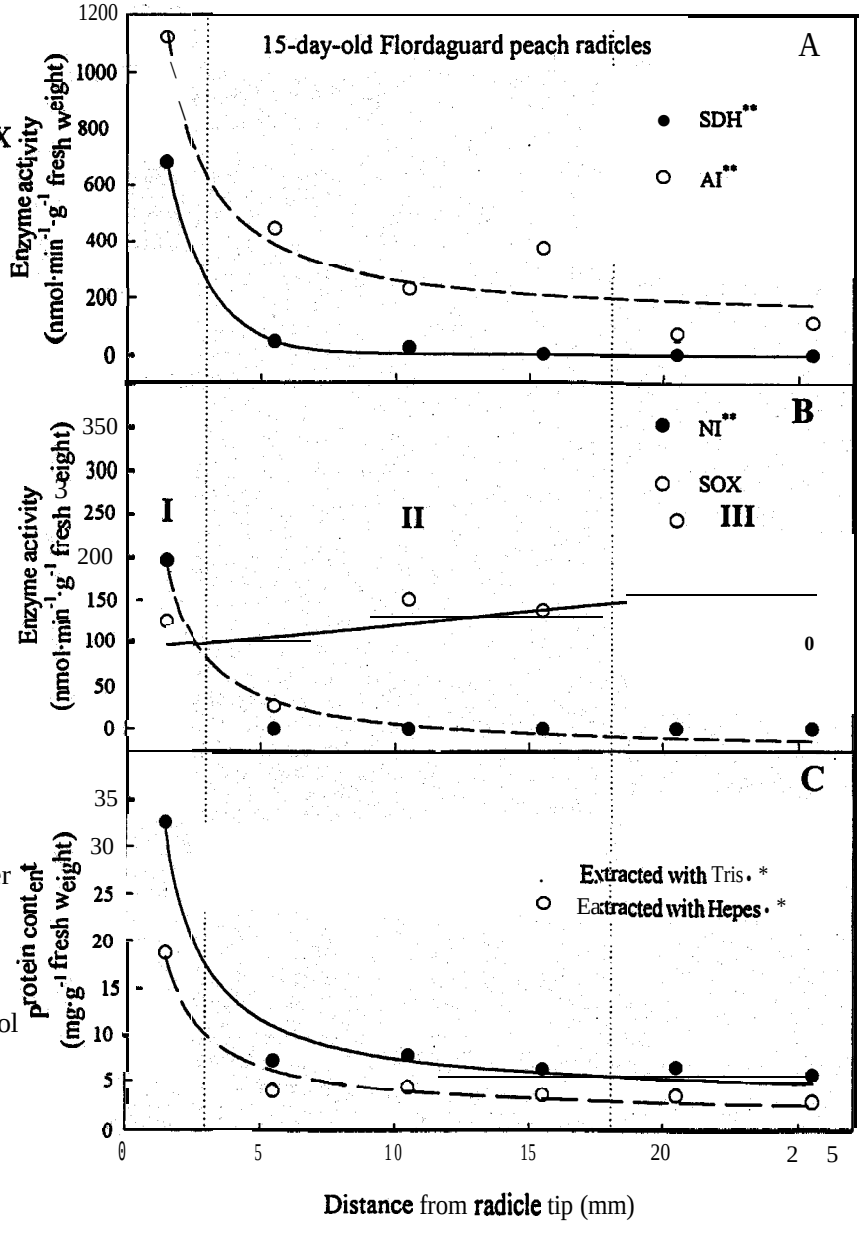
Protein content was determined by the method of Bradford (1976). Enzyme specific activity was expressed as nanomoles of NADH produced per minute per gram of fresh weight.

SORBITOLANDSUGARCONTENT. Soluble carbohydrates, including sorbitol, were measured following the procedure of Rieger and Marra (1994). Shoot tips and root tips were collected from the same 'Flordaguard' peach seedlings growing in hydroponics as used for enzyme assays. Several hundred root tips were collected from 24 plants and cut into the segments described above. The segments at each developmental zone were pooled together to accumulate ≈50 mg of dry weight, yielding one extraction per segment type. Three to 5 shoot tips were collected from each of the 24 'Flordaguard' plants, pooled together, and divided into leaflets, meristem, and intemode segments as described above. Enough tissue was available for 3 separate extractions (50 mg dry weight per extraction) for shoot tips. Sugar content was not determined in 'Nemaguard' shoots due to lack of sufficient amount of tissue for extraction.

Freezedried tissue was fmely ground and 50 mg were' extracted in 5 mL 80% (v/v) methanol containing 0.44 $mg \cdot mL^{-1}$ phenyl-β-D-glucopyranose as an intemal standard. After homogenization, samples were centrifuged for 3 min at 3900 g_n and the supematant was stored at 4 °C. One hundred micro liter samples were dried in GC vials at 40 °C and derivitized according to the method of Chapman and Horvat (1989). Briefly, dried samples were rediluted in 0.025 mL of hydroxylamine-HCl(25 $mg \cdot mL^{-1}$ in pyridine) at 75 °C for 30 min, and subsequently derivitized with 0.07 mL N, O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) plus 1% trimethylchlorosylane (TMCS) at 75 °C for 20 min. Soluble carbohydrates were quantified using a Hewlett Packard HP 5940 gas chromatograph (Avondale, Pa.), using a DB5 column (30 m length, 0.3 mm inner diameter, 0.25 mm film thickness).

CORRELATIONOFCROWTHRATEANDENZYMEACTMTY. Terminal portions of actively growing shoots were collected from the

Fig. 2. Activity of (A) SDH and AI, (B) NI and SOX, and (C) protein content in various segmenta of 'Flordaguard' peach radicles 15 d after seed germination. Data points represent midpoints of various segments. Regression equations are SDH = $1838.77^{-0.66 \cdot distance}$, $R^2 = 0.999$; $AI = 1529.72 \cdot distance^{-1} + 112.58$, $R^2 = 0.932$; NI = $321.75 \cdot distance^{-1} - 26.17$, $R^2 = 0.955$; Tris protein = $42.83 \cdot distance^{-1} + 3.18$, $R^2 = 0.969$; Hepes protein = $25.06 \cdot distance^{-1} + 1.64$, $R^2 = 0.970$. 'Significance of regression at $P \leq 0.05$, "significance of regression at $P \leq 0.01$. Absence of asterisk indicates that regression was nonsignificant. Vertical dotted lines indicate transition from meristematic (I) to elongating (II), to mature (III) tissue.



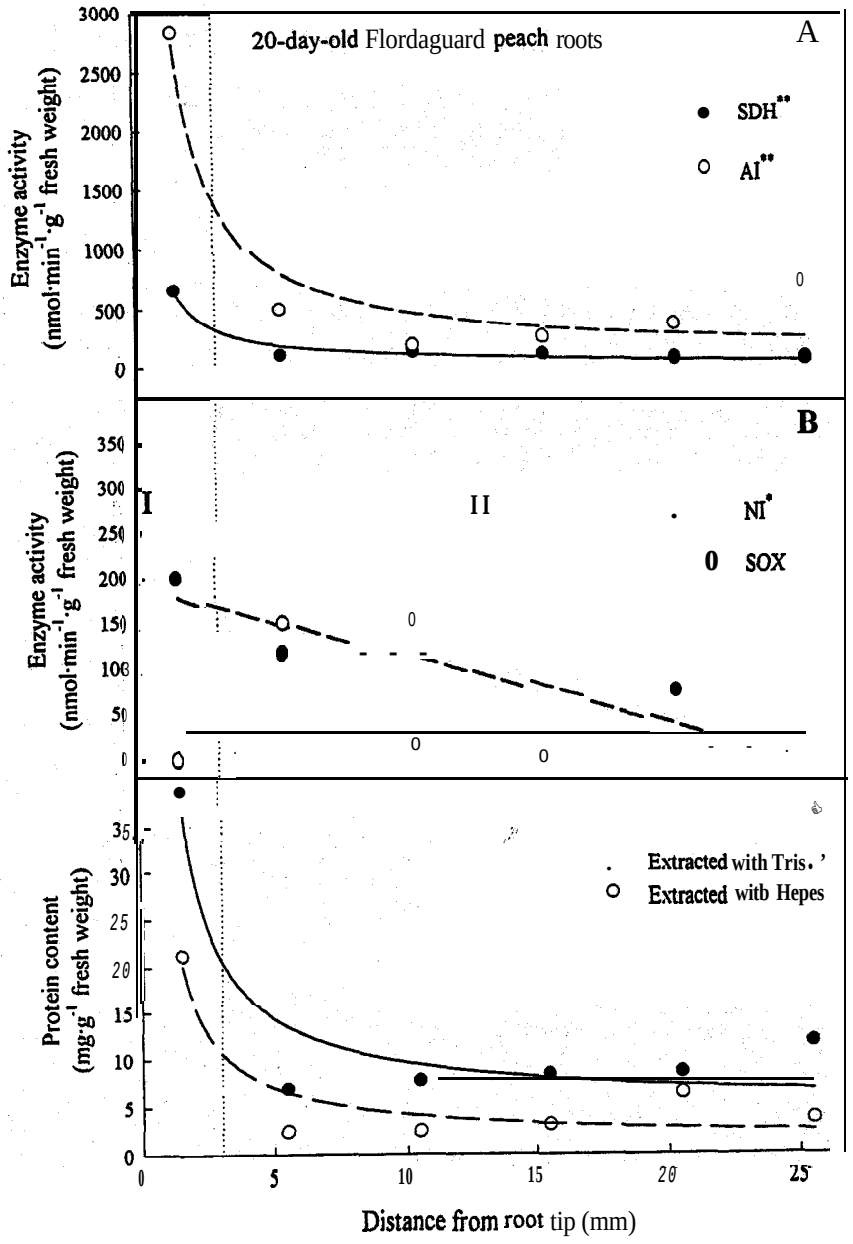


Fig. 3. Activity of (A) SDH and AI, (B) NI and SOX, and (C) protein content in various segments of 'Flordaguard' peach roots 20 d after seed germination. Data points represent midpoints of various segments. Regression equations are SDH = 926.02 · distance^{-1} + 31.39, R^2 = 0.972; AI = 3981.18. distance^{-1} + 81.13, R^2 = 0.919; NI = –7.55 · distance + 189.25, R^2 = 0.708; Tris protein = 47.29 · distance^{-1} + 4.94, R^2 = 0.896; Hepes protein = 28.17 · distance^{-1} + 1.39, R^2 = 0.868. *Significance of regression at $P \leq 0.05$, **significance of regression at $P \leq 0.01$. Absence of asterisk indicates that regression was nonsignificant. Vertical dotted lines indicate transition from meristematic (I) to elongating (II) tissue.

same plants indicated above. Shoot tips or shoot internodes were used to determine enzyme activities in the growth correlation study. Shoot tips consisted of the apical meristem and all the nonexpanded leaves that were considered carbon-importing organs. Leaf length varied at ≈1 to 3 cm, and shoot tip fresh weight ranged from 30 to 70 mg. Internodes consisted of the elongating portion of the shoots between the apical meristem and the first or second visible node and weighed ≈20 to 50 mg each. In each experiment, samples were harvested all at once (in the morning) to reduce variation due to fluctuations in the daily metabolism of plants. Samples were transported quickly from the greenhouse to the laboratory in aluminum foil on ice and then rinsed in distilled water, blot-dried, and half immediately assayed for SDH, the other half stored at -20 °C for subsequent determination of SS, AI, NI, and SOX activities.

Relative growth rates of shoots were plotted versus activities of al1 enzymes, and linear regression was used to determine significance of these relationships. A correlation between root growth rate and enzyme activity was not possible due to difficulties in measurement of root elongation rate.

Statistical analysis. Linearandnonlinear regressions were used to analyze data trends. In particular, Sigma Plot 4.01 (Chicago, Ill.) was used to fit lines (y = ax + n) and inverse (y = a . x-r + n), exponential decay (y = a^{-bx}), and exponential rise (y = a · (1^{-bx})) curves to data according to goodness of fit. SAS procedures (SAS Institute, Cary, N.C.) were used to determine simple correlation coefficients between enzyme activities and sugar contents in 'Plordaguard' roots and shoots.

Results

Association between developmental stage and enzyme activity

Root tips. Microscopic observation of 'Plordaguard' root sections confirmed that the terminal 3-mm segment of all roots always consisted of mostly meristematic tissue, and that in 10- and 15-d-old radicles, mature tissues were present 13 to 18 mm from the tip. However, in 20-d-old lateral roots, no fully mature tissue was present within the 28 mm examined.

In radicles collected 10 d after seed germination, activity of all enzymes was confined within the first 18 mm from the tip (Fig. 1A and B). SOX and NI activities were barely detectable in the elongating segments behind the meristem and ranged from 0 to 127 nmol·min^{-1}·g^{-1} fresh weight and from 0 to 55 nmol·min^{-1}·g^{-1} fresh weight, respectively. In 15-d-old radicles, only SDH and NI activities were confined within the first 13 mm, whereas AI and SOX activities were present all along the 28-mm segment (Fig. 2A aud B). SS activity was detected (68 nmol·min^{-1}·g^{-1} fresh weight) only in meristems of 10-d-old radicles (Fig. 1B). In 20-d-old roots, all 5-mm root segments behind the 3-mm tip segment were still elongating and exhibited enzyme activities (Fig. 3A and B).

Regardless of root age, both AI and SDH were several times more active in the meristematic zone, where the protein content was highest, than in the elongating zone, following an exponential or inverse pattern (Figs. 1, 2, and 3). However, moderate AI activity was still present in relatively mature portions of 15-d-old radicles (Fig. 2A). In all cases, SOX activity did not follow any significant trend with distance from the tip. In 15-d-old radicles, NI activity exhibited a significant decreasing trend with distance from the tip (Fig. 2B).

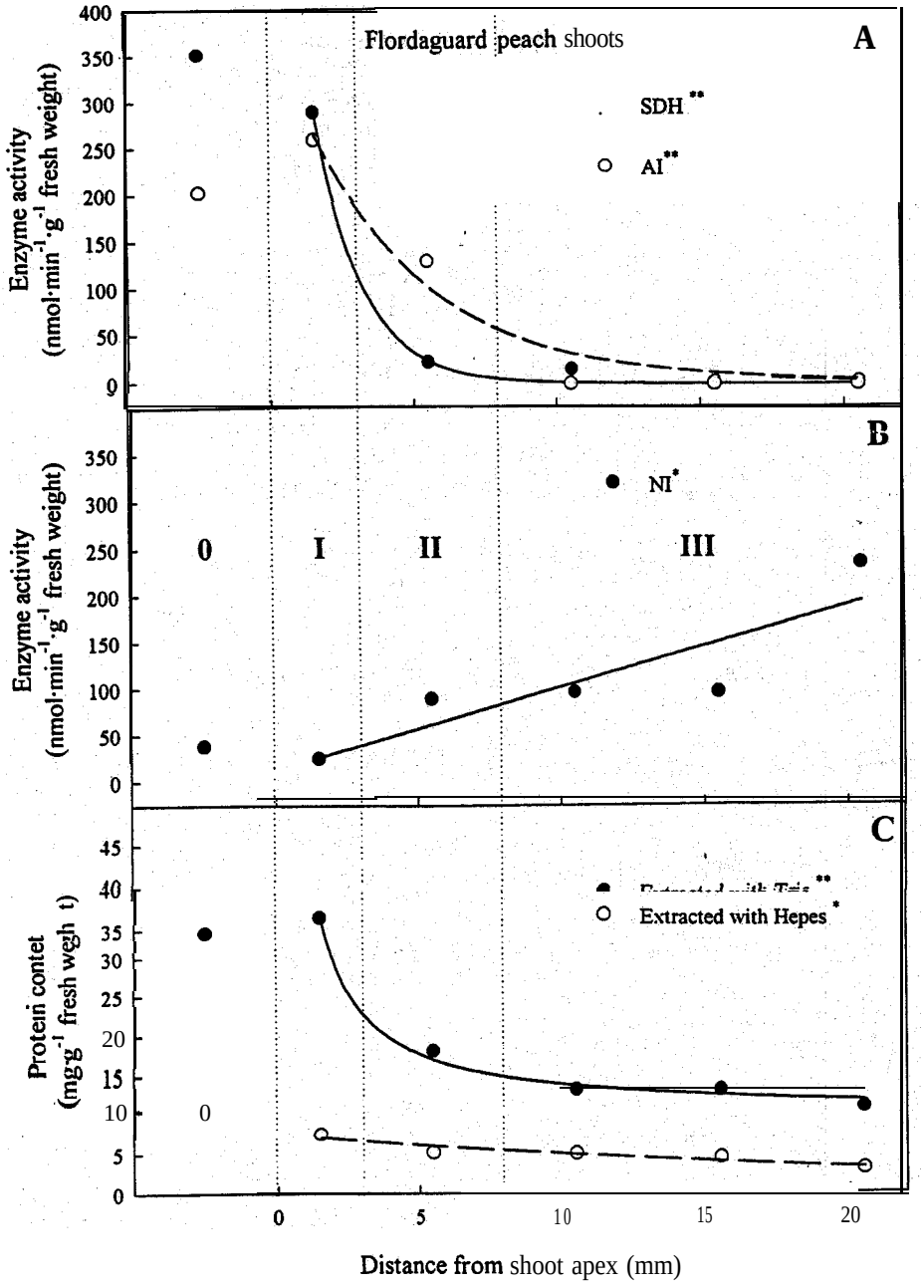


Fig. 4. Activity of **(A)** SDH and AI, **(B)** NI, and (C) proteincontent in young leaves and various segments of shoot terminal portions of 'Flordaguard' peach. Data points represent midpoints of various segments. Regression equations are SDH = $719.68^{-0.61 \cdot \text{distance}}$, $R^2 = 0.997$; AI = $376.17^{-0.23 \cdot \text{distance}}$, $R^2 = 0.965$; $NI = 8.80 \cdot \text{distance}^{-1} + 13.34$; $R^2 = 0.768$; Tris protein = 37.21 . $\text{distance}^{-1} + 10.19$, $R^2 = 0.993$; Hepes protein = $7.58^{-0.04 \cdot \text{distance}}$, $R^2 = 0.886$. 'Significance of regression at $P \leq 0.05$, **significance of regression at $P \leq 0.01$. Absence of asterisk indicates that regression was nonsignificant. Vertical dotted lines indicate transition from leaflets (0) to meristematic (I), to elongating (II), to mature (III) tissue.

SHOOT TIPS. In shoots of both 'Flordaguard' and 'Nemaguard', the apical 3 mm consisted of mostly meristematic tissue. However, the elongating tissue was concentrated in the first 10 mm behind the meristem in 'Flordaguard' shoots (Fig. 4), whereas it extended along al1 the 20 mm below the apex in 'Nemaguard' (Fig. 5). SDH was 10 to 15 times more active in meristematic portions of both 'Flordaguard' and 'Nemaguard' shoots, where protein content was highest, than in elongating segments below the apex and followed an exponential decrease pattem (Figs. 4A and C and 5A and C). SS activity was detected in shoot tips of 'Nemaguard', but not 'Flordaguard', and decreased exponentially with distance from the meristem (Fig. 5B). AI was very active in the meristematic portions of shoots, but also active in the internodes below the meristem, particularly in 'Nemaguard', where the enzyme activity decreased linearly from tip to base (Figs. 4A and 5A). NI activity decreased exponentially with distance from the meristem in 'Nemaguard' shoots (Fig. 5B), whereas it increased linearly in 'Flordaguard' shoots (Fig. 4B). No SOX activity was detected in shoots of either 'Flordaguard' or 'Nemaguard' peach.

In shoots, the amount of protein extracted witb Tris buffer was three to four times higher than that extracted with Hepes buffer.

SUGAR CONTENT IN ROOTS AND SHOOTS. In 20-d-old roots, sorbito1 and sucrose were consistently low along all 28 mm of tissue examined (Fig. 6A). No significant trend of sugars with distance from the root tip was observed. However, sucrose was negatively correlated ($P = 0.05$) with AI activity.

In 'Flordaguard' shoots, sorbitol, glucose, and fructose increased with distance from the apex, following an exponential, an inverse, and alinear trend, respectively (Fig. 6B). Sucrose, on the other hand, was the only sugar that exhibited an exponential decrease with distance from the apex. SDH activity was inversely related to sorbito1 ($P = 0.005$) and glucose ($P = 0.03$ 1), but positively correlated to sucrose ($P = 0.029$). Similar correlations were observed between Al activity and sugars, as AI activity was always positively correlated with SDH activity ($P = 0.036$), even in roots ($P = 0.002$).

Enzyme activities and sugar contents in the leaflets surrounding the apex were reported separately and not included in the overall trends along the shoots because of their different behavior in terms of growth. However, in general, enzyme activities in the leaflets were comparable to those in the meristematic portion of the stem (Figs. 4 and 5).

Correlation of growth rate and enzyme activity

Regressions of SDH activity versus relative growth rate were significantly positive when internodes of 'Flordaguard' and 'Nemaguard' shoots were sampled for the assays (Fig. 7A and B). On the other hand, no significant correlation was found between SDH activity and RGR when shoot tips (meristem and surrounding leaflets) were sampled for the assays (data not shown). Also, no significant correlation was found between SS, AI, NI activity, and RGR when either shoot tips or intemodes from both cultivars were sampled for the assays (Fig. 7).

Discussion

Vegetative sinks such as shoot or root apices, unlike reproduc-

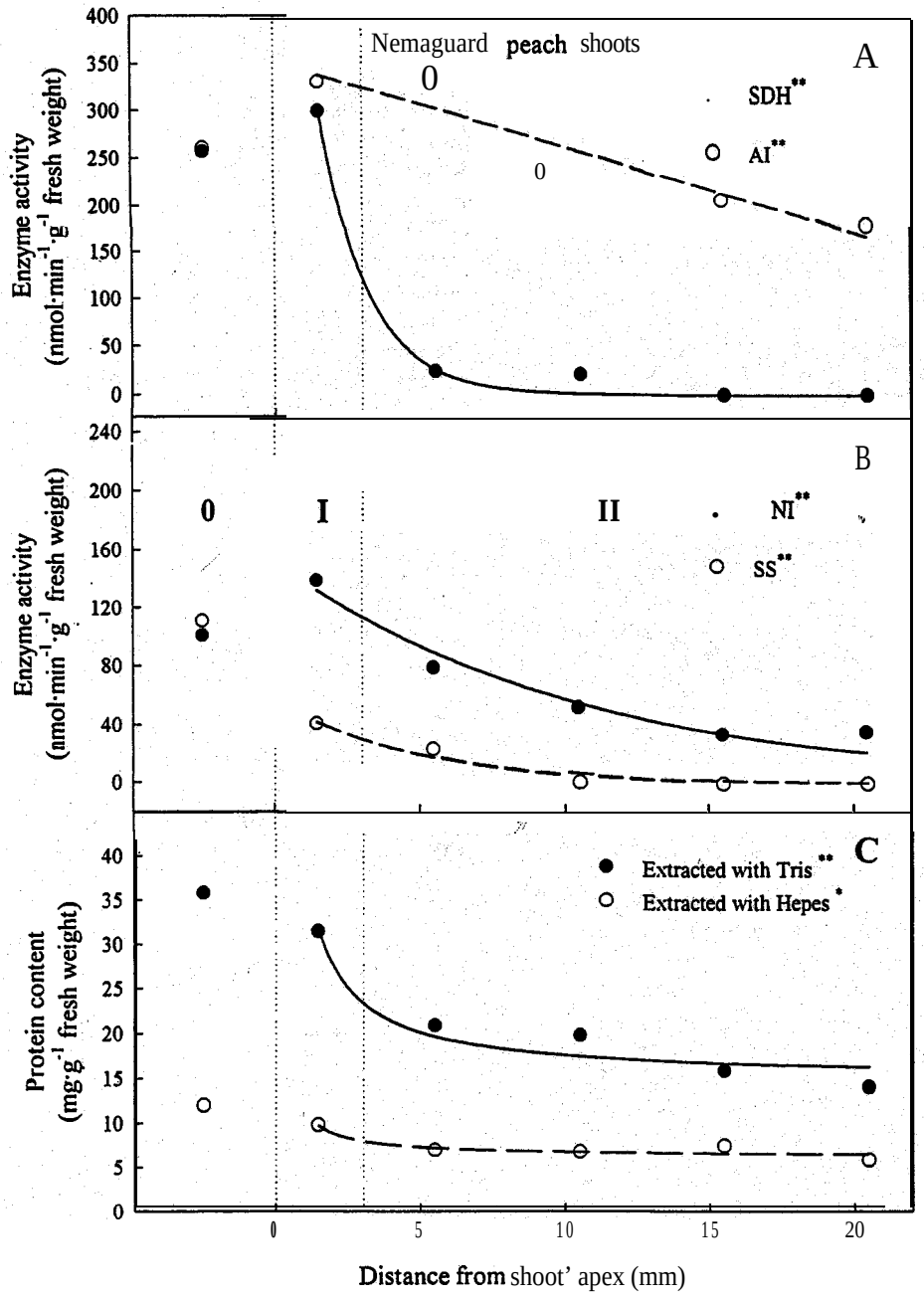


Fig. 5. Activity of (A) SDH and AI, (B) NI and SS, and (C) protein content in young leaves and various segments of shoot terminal portions of 'Nemaguard' peach. Data points represent midpoints of various segments. Regression equations are SDH = $745.35^{-0.61 \cdot \text{distance}}$, $R^2 = 0.994$; SS = $58.50^{-0.22 \cdot \text{distance}}$, $R^2 = 0.947$; AI = $-8.93 \cdot \text{distance} + 351.59$, $R^2 = 0.925$; NI = $152.34^{-0.10 \cdot \text{distance}}$, $R^2 = 0.952$; Tris protein = $25.19 \cdot \text{distance}^{-1} + 15.11$, $R^2 = 0.932$; Hepes protein = $5.20 \cdot \text{distance}^{-1} + 6.30$, $R^2 = 0.855$. 'Significance of regression at $P \leq 0.05$, "significance of regression at $P \leq 0.01$. Absence of asterisk indicates that regression was nonsignificant. Vertical dotted lines indicate transition from leaflets (0) to meristematic (I), to elongating (II) tissue.

tive or storage sinks, present a spatial separation of successive stages of development. The apex generally consists of a meristematic zone of rapidly diviclmg cells located at the tip, subtended by an elongating zone where various tissues are already distinguishable, but not yet mature, and ends with fully mature tissues where longitudinal growth has ceased (Esau, 1977).

In roots of 'Flordaguard' peach seedlings, the spatial separation of successive stages of development gradually changed with root age and type. These changes in leve1 of tissue maturity revealed by microscopic observations were followed mainly by changes in SDH and AI activity. Regardless of root age and type, both AI and SDH were always most active in the meristematic portion of the root, where cells divide rapidly, and diminished in the elongating portion, finally disappearing in the mature zone (Figs. 1, 2, and 3). These results agree with those of Hellebust and Forward (1962), where AI activity on a fresh weight basis was highest in the first 3 mm of the radicle (meristematic portion). The decrease in SDH and AI activity exhibited along the root from the meristem to the mature tissue is an indication that these enzymes are associated with growth in terms of ce11 division and expansion, and not with metabolic processes in mature root tissues. The same type of behavior is exhibited by invertases during seed development (Weber et'al., 1997). In particular, invertase activity seems to be important during the initial stages of seed growth in com, *Sorghum*, and broad bean (Weber et al., 1997).

Similar results were obtained in shoots as in roots. However, SDH was most active exclusively in the meristematic portions of the shoot, while AI was relatively active also in the internodes subtending the apex (Figs. 4 and 5). This is consistent with reports of high AI activity in elongating cells of pea radicles (Robinson and Brown, 1952), and elongating pods of snap bean (*Phaseolus vulgaris* L.) fruit (Sung et al., 1994).

Differences between 'Flordaguard' and 'Nemaguard' shoots in the association of enzyme activity and developmental stage may be due to differences between cultivars, or perhaps between seedlings and rooted cuttings. In shoots from seedlings, developing tissues (meristematic and elongating portions) were clearly concentrated toward the tip (first 13 mm), whereas, in 'Nemaguard' shoots, elongation occurred well past the zone 20 mm behind the tip. A number of differences between seedlings and rooted cuttings in growth and leafmorphology have been reported for peach (Rieger, 1992).

In shoot tissues, the higher amount of protein extracted with Tris buffer could be due to easier extractabiity of some proteins at higher pH. However, since the difference in protein contents extracted with the two buffers was much lower in the roots, the high amount of protein extracted with Tris buffer in the shoots may be attributed mostly to chloroplast proteins present in green tissues rather than the enzymes of interest.

Results of correlations between enzyme activities and sugar contents are difficult to interpret. The expected negative correlation between enzyme activities and substrates (sorbito1 and sucrose) may be strongly affected by the import rate of the substrates into the sink or by enzyme induction due to substrate increase. On the other hand, the expected positive correlation

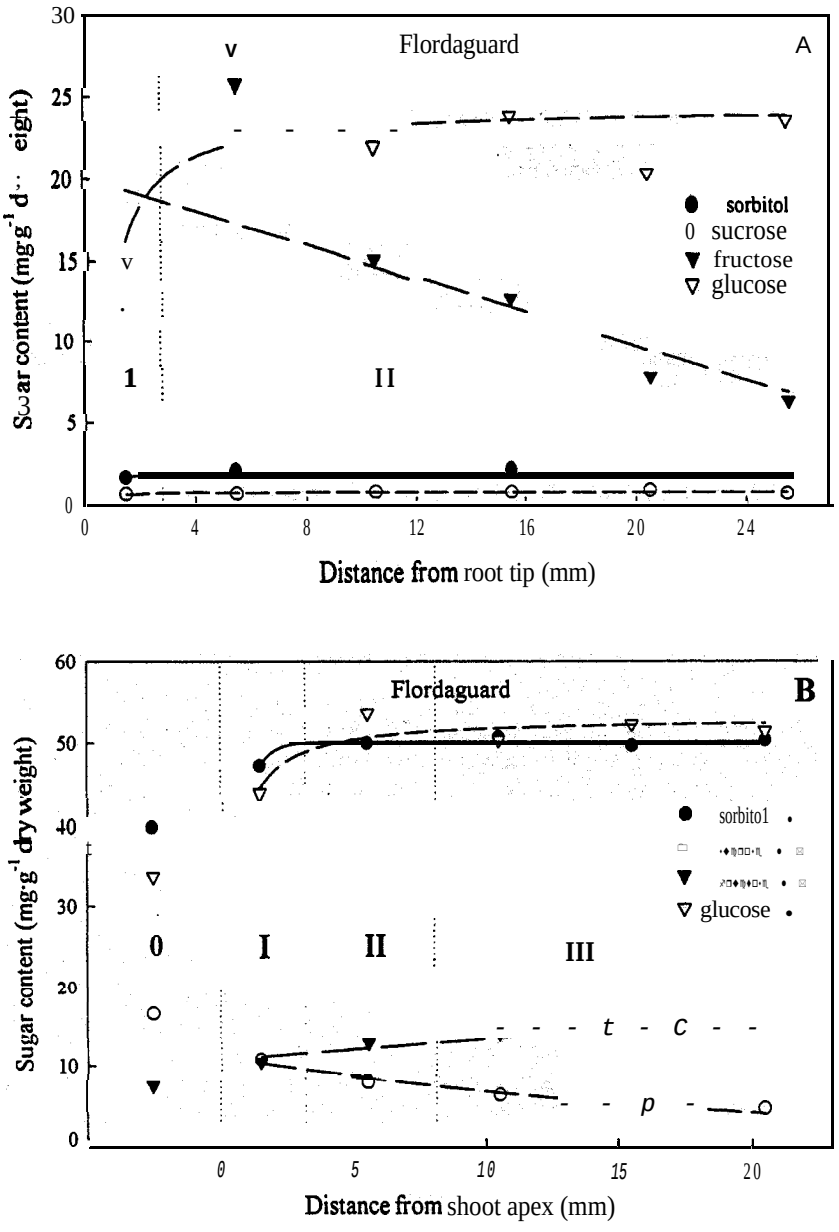


Fig. 6. Content of sorbito1 and sugars in various segments of (A) 'Flordaguard' shoot terminal portions and (B) 20-d-old roots. Data points represent midpoints of various segments. Regression equations in the shoots are sorbito1 = $50.23 \cdot (1-1.90 \cdot \text{distance})$, $R^2 = 0.909$; sucrose = $11.10^{-0.05 \cdot \text{distance}}$, $R^2 = 0.961$; fructose = 0.25 . distance + 10.81, $R^2 = 0.926$; glucose =-12.85 . distance-' + 53.08, $R^2 = 0.796$. 'Significance of regression at $P \leq 0.05$, "significance of regression at $P \leq 0.01$. Absence of asterisk indicates that regression was nonsignif'icant. Vertical dotted lines indicate transition from leaflets (0) to meristematic (I), to elongating (II), to mature (III) tissue.

between enzyme activities and products (reducing sugars) may not be seen because of the rapid phosphorylation of fructose and glucose and their subsequent glycolysis.

By definition, sinks are organs that import and use assimilates in respiration, growth, and storage. The rate of shoot and root elongation alone, however, should give a good indication of sink strength since these actively growing sinks do not store much carbon (Lo Bianco et al., 1999) and their growth rate is positively correlated with respiration (Amthor, 1994). Results obtained in the present work support the hypothesis of a positive correlation between enzyme activity and sink growth only for SDH (Fig. 7). Lack of statistically significant correlation between SDH and shoot growth when leaflets and meristems were included in the assay was at first surprising since SDH was most active in these organs. However, in our experiments we measured shoot growth in terms of elongation and intemodes contribute more directly to shoot elongation than meristems. Also, shoot tips consisted of meristematic apices and the surrounding young leaves, which may follow a different temporal pattem of growth during their development. In other words, leaflets and intemodes may actas two separate sinks supported by a common meristematic zone acting as a source of cells, and these separate sinks may not be synchronized in their growth. Therefore, it is reasonable that the rate of shoot elongation correlates better with enzyme activities in the internodes than in the tips.

In conclusion, our results show a predominant importance of SDH for sorbito1 metabolism and Al for sucrose metabolism in vegetative sinks of peach. Also, SDH seems to be more important in the meristematic portions of the sink, where sorbitol is preferentially used, whereas AI has an equally important role in cell elongation. A separate mechanism for sucrose and sorbito1 unloading could explain the preferential use of sorbito1 in meristematic tissues (Moing et al., 1992). Moreover, sucrose metabolism appears to be more complex since various enzymes may be involved in different ce11 functions, such as growth, storage, or maintenance metabolism. Lack of association between SS and growth of vegetative peach sinks observed in the present study, for example, suggests an implication of the enzyme in either starch accumulation, as in tomato (*Lycopersicon esculentum* Mill.) (Wang et al., 1993) and peach fruit (Lo Bianco et al., 1999), or maintenance metabolism. However, starch accumulation data is needed to support this idea.

Furthermore, Al and SDH can be considered adaptive enzymes in peach roots and shoots since they both show some association with sink growth and development, whereas NI and SOX resemble maintenance enzymes more closely (Black et al., 1987). Fmally, results obtained in this study confirm the predominance of SDH and sorbito1 in the vegetative growth of peach sinks already seen in a previous study (Lo Bianco et al., 1999) and partly clarify the relative importance of sucrose and sorbito1 along zones of tissue differentiation during sink development.

Literature Cited

Amthor, J.S. 1994. Respiration and carbon assimiiate use, p. 221-250. In: K.J. Boote, T.R. Sinciair, and J.M. Bennett (eds.). Physioiogy and determination of crop yieid. Amer. Soc. Agron. Madison, Wis.

Bieleski, R.L. 1982. Sugar alcohols, p. 158–192. In: F.A. Loewus and W. Tanner (eds.). Plant carbohydrates 1. Intracellular carbohydrates. Encyciopedia Plant Physioi. New Ser. vol. 13A. Springer-Veriag, Berlin.

Biack, C.C., L. Mustardy, S.S. Sung, P.P. Kormanik, D.P. Xu, and N. Paz. 1987. Reguiation and roles for alternative pathways of hexose metabolism in plants. Physioi. Plant. 69:387–394.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principie of protein-dye

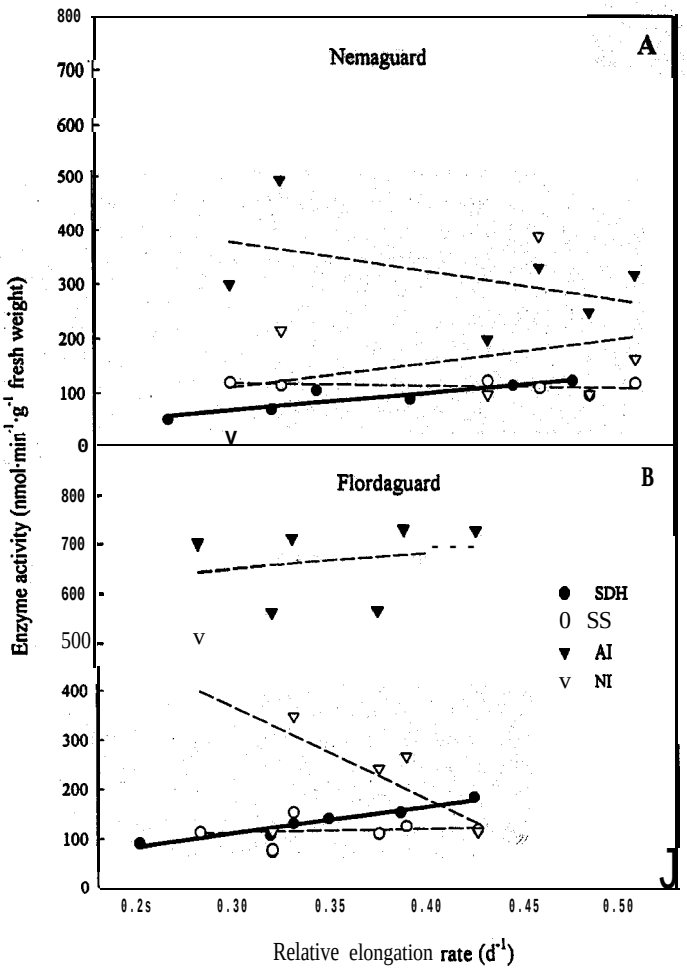


Fig. 7. Linear regression between the activity of each enzyme and the relative elongation rate of (A) 'Nemaguard' and (B) 'Flordaguard' shoots when internodes were used for the assays. A solid line indicates significance of the slope ($P \leq 0.05$).

binding. Anal. Biochem. 72:248–254.

Chapman, G.W. and R.J. Horvat. 1989. Determination of nonvolatile acids and sugars from fruits and sweet potato extract by capillary GLC and GLC/MS. J. Agr. Food Chem. 37:947–950.

Esau, K. 1977. Anatomy of seed plants. 2nd ed. Wiley, New York.

Hellebust, J.A. and D.F. Forward. 1962. The invertase of the com radicle and its activity in successive stages of growth. Can. J. Bot. 40:113–126.

Lo Bianco, R., M. Rieger, aud S.S. Sung. 1998. A simple, rapid extraction and assay procedure for NAD+-dependent sorbito1 dehydrogenase (SDH) in peach. J. Amer. Soc. Hort. Sci. 123:1065–1068.

Lo Bianco, R., M. Rieger, and S.S. Sung. 1999. Carbohydrate metabolism of vegetative and reproductive sinks in the late-maturing peach cultivar 'Encore'. Tree Physiol. 19:103–109.

Loescher, W.H. 1987. Physiology and metabolism of sugar alcohols in higher plants. Physiol. Plant. 70:553–557.

Loescher, W.H., G.C. Marlow, and R.A. Kennedy. 1982. Sorbito1 metabolism and sink-source interconversions in developing apple leaves. Plant Physiol. 70:335–339.

Merlo, L. and C. Passera. 1991. Changes in carbohydrate and enzyme levels during development of leaves of *Prunus persica*, a sorbito1 synthesizing species. Physiol. Plant. 83:621–626.

Moing, A., F. Carbonne, M.H. Rashad, and J.P. Gaudillère. 1992. Carbon fluxes in mature peach leaves. Plant Physiol. 100: 1878-1884.

Moriguchi, T., T. Sanada, and S. Yamaki. 1990. Seasonal fluctuations of some enzymes related to sucrose and sorbito1 metabolism in peach fruit. J. Amer. Soc. Hort. Sci. 115:278–281.

Rieger, M. 1992. Growth, gas exchange, water uptake, and drought response of seedling- and cutting-propagated peach and citrus rootstocks. J. Amer. Soc. Hort. Sci. 117:834–840.

Rieger, M. and F. Marra. 1994. Responses of young peach trees to root confmement. J. Amer. Soc. Hort. Sci, 119:223–228.

Rieger, M. and G. Scalabrelli. 1990. Paclobutrazol, root growth, hydraulic conductivity, and nutrient uptake of 'Nemaguard' peach. HortScience 25:95–98.

Robinson, E. and R. Brown. 1952. The development of the enzyme complement in growing root cells. J. Expt. Bot. 3:356–374.

Sturm, A., V. Sebková, K. Lorenz, M. Hardegger, S. Lienhard, and C. Unger. 1995. Development and organ-specific expression of the genes for sucrose synthase and three isozymes of acid b-fructofuranosidase in carrot. Planta 195:601–610.

Sung, S.S., W.J. Sheih, D.R. Geiger, and C.C. Black. 1994. Growth, sucrose synthase, and invertase activities of developing *Phaseolus vulgaris* L. fruits. Plant Ce11 Environ. 17:419–426.

Sung, S.S., D.P. Xu, and C.C. Black. 1989. Identification of actively filling sucrose sinks. Plant Physiol. 89: ll 17-1121.

Wang, F., A. Sanz, M.L. Brenner, and A. Smith. 1993. Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol. 101:321–327.

Weber, H., L. Borisjuk, and U. Wobus. 1997. Sugar import and metabolism during seed development. Tr. Plant Sci. 2:169–174.

Xu, D.P., SS. Sung, and C.C. Black. 1989. Sucrose metabolism in lima bean seeds. Plant Physiol. 89:1106-l 116.

Y amaguchi, H., Y. Kanayama, J. Soejima, and S. Yamaki. 1996. Changes in the amounts of the NAD-dependent sorbito1 dehydrogenase and its involvement in the development of apple fruit. J. Amer. Soc. Hort. Sci. 121:848–852.