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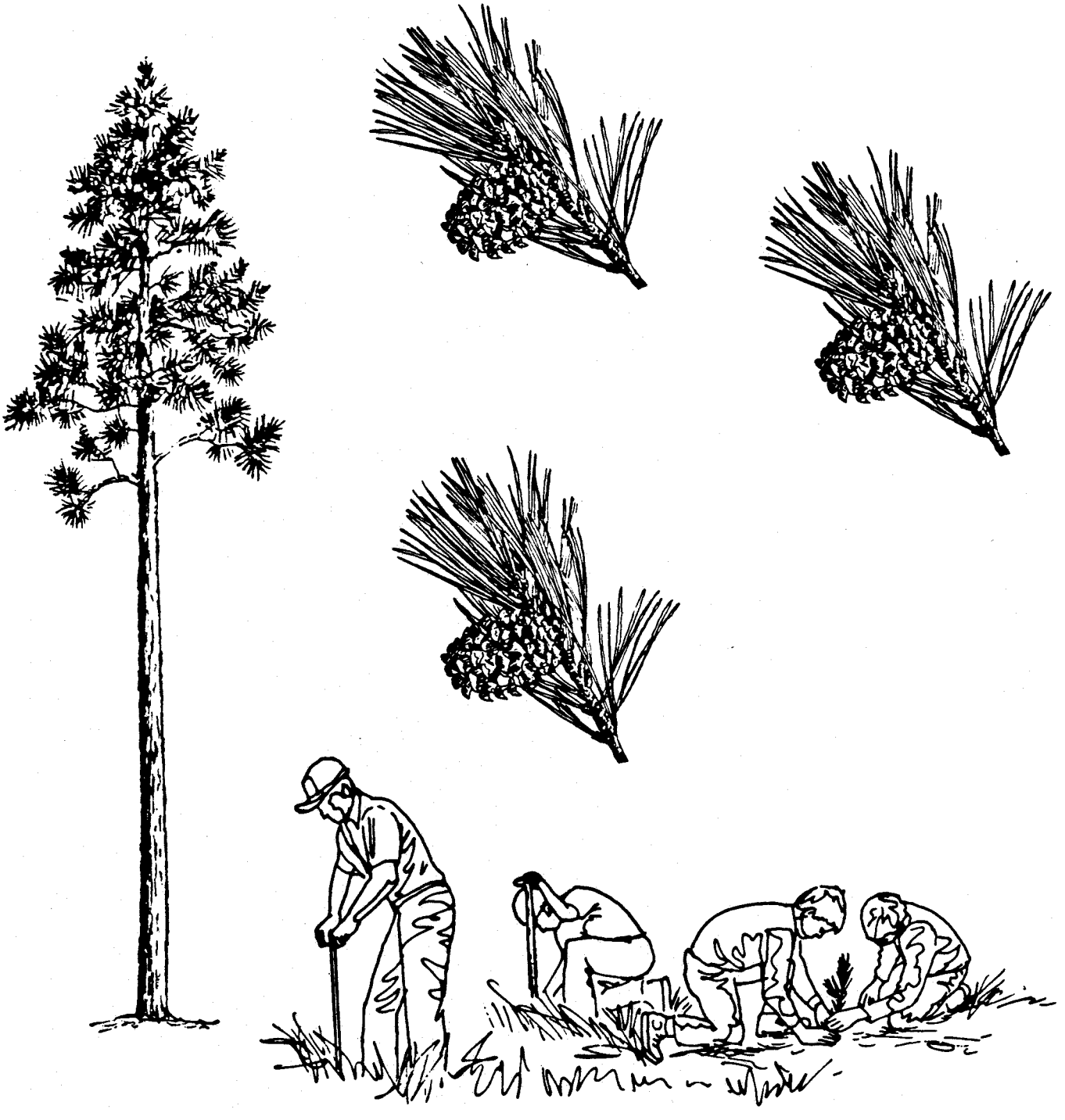
Sung, Kormanik and Black



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Vascular cambial sucrose metabolism and growth in loblolly pine (*Pinus taeda* L.) in relation to transplanting stress

SHI-JEAN S. SUNG,[1] PAUL P. KORMANIK[1] and CLANTON C. BLACK[2]

[1] Institute of Tree Root Biology, Forestry Sciences Laboratory, Southeastern Forest Experiment Station. USDA Forest Services. 320 Green Street. Athens, Georgia 30602, USA

[2] Department of Biochemistry, Life Science Building, University of Georgia, Athens, Georgia 30602, USA

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Summary

Sucrose synthase (SS) was the dominant enzyme of sucrose metabolism in both stem and root vascular cambial zone tissues of nursery-grown loblolly pine (*Pinus taeda* L.) seedlings. Acid invertase (AI) and neutral invertase (NI) activities were generally less than 10% of the SS activity in both tissues. In both cambial tissues, seasonal patterns of enzyme activity were observed for SS but not for AI or NI. The seasonal patterns of SS activity in stem and root cambia paralleled the periodic growth of stems and roots. Stems had high SS activity and growth during summer and early fall. Roots had substantial SS activity and growth during summer and fall, but SS activity and growth were even higher in winter.

When seedlings were transplanted, about eight months elapsed before stem and root cambia resumed rates of growth and sucrose metabolism similar to those in control nontransplanted seedlings. Two months after transplanting, root SS was at its lowest, whereas AI activity in transplants was 50% higher than in control nontransplanted seedlings. In stems, SS activity decreased in response to transplanting, whereas AI and NI activities did not change appreciably.

In loblolly pine tissues, SS was specific for uridylates, whereas the nucleotide triphosphatedependent phosphofructokinase (NTP-PFK) had similar activity with either UTP or ATP. Except in winter, the NTP-PFK was less active than the pyrophosphate-dependent phosphofmctokinase (PPi-PFK) during all seasons. The PPi-dependent PFK activity in nontransplanted seedlings followed similar seasonal and spatial patterns to those of SS activity. In actively growing tissues, such as stem cambial tissues in summer and root cambial tissues in winter, the measured total PFK to SS ratio ranged between 1.5/1 and 3/1. In contrast, in less actively growing tissues or transplanted seedlings, a greater decrease occurred in SS than in PFK activity, hence the ratio rose to as high as 12/1.

It was concluded that: (1) SS was the dominant enzyme for sucrose metabolism in root and stem cambial tissues of loblolly pine seedlings; (2) both SS and PPi-PFK in the cambial tissues can be used as biochemical indicators of growth sink strength in stems and roots; and (3) both enzymes can be used as indicators of seedling stress caused by events such as transplanting and winter freezing.

Keywords: invertase, phosphofructokinase, sucrose synthase, transplanting stress, winter freezing.

Introduction

In the southern United States about one billion loblolly pine seedlings are lifted from nursery beds and transplanted on natural forest sites each year (Johnson et al. 1982). Successful artificial regeneration of loblolly pine forests depends on seedlings surviving the transplanting process, which imposes severe stresses on them (Weaver et al. 198 1, Johnson et al. 1982). Because bareroot transplanting is not a natural occurrence in the life history of forest trees, it is unlikely that trees have specific

mechanisms to cope with the associated stresses. According to Barghoom (1964), the development of a vascular cambium, which is one of the most important structural evolutionary improvements to occur in the plant kingdom, enabled plants to cope with terrestrial-type stresses. The vascular cambial zone tissues are the tissues most indicative of radial tree growth (Zimmermann and Brown 197 1). It is in this region that active cell division, cell growth, secondary cell wall deposition, and the storage of starch and lipids occur. Despite the importance of the vascular cambium, there have been few studies of the metabolic processes occurring in tree cambial tissues (Ford 198 1, Berlyn and Battey 1985, Higuchi 1985, Kimmerer and Stringer 1988, Savidge 1989, Whetten and Sederoff 1992).

Metabolic processes in cambial tissues depend on imported sucrose, which is the major form of translocated carbon in trees (Shiroya et al. 1962, Zimmermann and Brown 1971). The role of sucrose metabolism during plant growth and development has been extensively studied in various annual crops (Claussen et al. 1986, Hubbard et al. 1989, Sung et al. 1989*a*, Xu et al. 1989*a*, Ross and Davies 1992, Sun et al. 1992). Less information is available on trees, although there has been some recent work on seasonal root cambial sucrose metabolism in deciduous trees (Sung et al. 1989b) and in germinating pinyon pine (*Pinus edulis* Engelm.) organs (Murphy et al. 1992). Here we have deten-nined which enzymes are involved in sucrose metabolism in the vascular cambium of loblolly pine (*Pinus taeda* L.) seedlings. We tested the hypothesis that sucrose metabolism in the vascular cambium of loblolly pine has seasonal and spatial patterns within the tree that are closely associated with growth. We also tested the hypothesis that transplanted seedlings adjust their cambial sucrose metabolism both spatially within the plant and at specific seasons in order to survive the stress of bareroot transplanting and to resume growth.

Materials and methods

Plant materials

Loblolly pine (*Pinus taeda* L.) seeds of mixed seed lots were stratified at 4 °C for 60 days and sown in beds (18.3 x 1.2 x 1.2 m) at the Whitehall Nursery in Athens, Georgia, in April 1989. Nursery cultural practices were as described by Kormanik et al. (1990, 1992). In mid-January 1990, 3120 of the 12,480 one-year-old nursery-grown seedlings were lifted by hand and immediately transplanted to nearby nursery beds of similar fertility. Unlifted seedlings remained in the beds and were thinned from 284 seedlings m^{-2} to the same density, 71 seedlings m^{-2}, as the transplanted seedlings; the nontransplanted seedlings served as controls. All seedlings received regular watering and cultural care. Sampling was made twice a month from January 1990 until May 199 1. In the 1990 study, there were two replicates for each sampling. Results reported are the average of the two replications. Variations in enzyme activities between two replications were less than 15% at all times.

In 1991, the 1990 study was repeated to verify the earlier results and to relate seedling growth to enzyme activities. In the 1991 study, loblolly pine seeds were

sown in April 1990 and seedlings were transplanted in February 1991. All cultural practices were similar to those of the 1990 study. Individual nontransplanted and transplanted seedlings were tagged in June 199 1. Height and root collar diameter were measured. Some of these seedlings were then sampled for enzyme analyses and for establishing the relationship between growth and enzyme activities. For each treatment, a minimum of 200 seedlings were measured for growth monthly.

Tissue preparation for enzyme extraction

Taproot and stem vascular tissues from the cambial zone were obtained by peeling the bark and scraping off the inner (xylem-side) cambial tissue with a razor blade (Sung et al. 19896). To ensure that tissues from control and transplanted seedlings were comparable, tissue was only taken from the portion of stem formed during the first year. In preliminary tests, cambial tissue from the xylem side was more enzymatically active than cambial tissue from the bark side in taproots and in stems; therefore, this cambial zone tissue was used for subsequent enzyme extractions. At each sampling date, 10 to 40 pine seedlings were used to obtain 3 to 4 g of taproot and stem cambial tissues. To ensure comparability of results, all tissues were harvested and extracted between 1000 and 1200 h.

Enzyme extraction procedures

Cambium tissues were quickly placed in liquid N_2 and powdered with a pestle and mortar. Extraction buffer was added to powdered tissues at a 5/1 ratio (v/w) along with 1% (w/v) insoluble polyvinylpynolidone (PVP), 1% (w/v) Dowex-1 chloride form, 0.1 mM phenylmethylsulfonyl fluoride, and sand. The extraction buffer was similar to that used by Sung et al. (19896) with 200 mM Hepes/NaOH (pH 7.8), 3 mM Mg acetate, 5 mM DTT, 10% (v/v) glycerol, and 1% (w/v) soluble PVP-40. The homogenate was passed through one layer of Miracloth and centrifuged at 34,000 g for 20 min at 4 °C. The supematant protein was concentrated with 70% ammonium sulfate precipitation, the pellet was resuspended in 25 mM Hepes/NaOH (pH 7.5) buffer containing 3 mM Mg acetate, 5 mM DTT, and 15% (v/v) glycerol and then desalted on a Sephadex G-25 column equilibrated with the same buffer. Recovery from the ammonium sulfate concentration step was between 90 and 100% for all enzymes tested. Freshly harvested potato (***Solanum tuberosum*** L.) tubers were homogenized alone and with loblolly pine tissues in a l/l ratio (w/w) to ensure that there was no loss of enzyme activities during the extraction and assay procedures (data not shown).

Enzyme assays

Sucrose synthase (SS), acid invertase (AI), neutral invertase (NI), PPi-PFK, and ATP-PFK were assayed in the same soluble extracts as described previously (Xu et al. 1989*a*) with minor modifications. In the SS reaction, UDP-glucose and fructose are formed. The UDP-glucose was measured utilizing the abundant, endogenous UDP-glucopyrophosphorylase in the extracts. The assay mixture for SS contained 100 mM sucrose, 0.5 mM UDP, and 1 mM PPi (Xu et al. ***1989a).*** Changes in $OD_{340\ nm}$

at 25 °C were monitored continuously with a Beckman DU-7 spectrophotometer for SS, PPi-PFK, and ATP-PFK. In all of the enzyme assays, activities were proportional to the amount of each extract and time. Acid invertase and neutral invertase were assayed with 25 and 100 mM sucrose, respectively. Reactions usually were incubated at 25 °C for 15 min and then boiled for 7 min to stop the reaction. All AI incubation mixtures were neutralized with 2 M NaOH before boiling. Under the standard assay conditions, invertase activities were linear for up to 60 min and proportional to the amount of extract used. The protein concentration of each extract was determined with Bradford reagents using bovine serum albumin as the standard. Enzyme specific activities were expressed both on a fresh weight basis (g) and on a protein basis (mg).

Results and discussion

In stem tissues, protein concentration and SS activity were 4 to 15 times greater in xylem-side than in phloem-side cambial tissue or xylem tissue (Table 1). Pyrophosphate-dependent PFK activity was 6 to 24 times greater in xylem-side cambial tissue than in phloem-side cambial tissue. Fivefold more NTP-PFK activity was found in xylem-side cambial tissue than in phloem-side cambial or xylem tissue. Similar data sets were obtained with loblolly pine taproot tissues (data not shown). In all cases, xylem-side root cambial tissues contained more protein and higher enzyme activities than phloem-side root cambial tissues.

The nucleotide specificity of SS was tested by assaying fructose formed (Sun et al. 1992). In contrast to a recent report of the presence of an ADP-specific SS in extracts of sycamore (*Acer pseudoplutanus* L.) cell cultures and spinach (*Spinach oleracea* L.) leaves (Pozueta-Romero et al. 1991), SS in loblolly pine stem and root cambial tissues was specific for UDP (data not shown). For loblolly pine PFK, UTP was as efficient in phosphorylating Fru 6-P as ATP (data not shown). Hence we designated loblolly pine PFK as an NTP-PFK, though we routinely used ATP. In contrast to reports of a 20-fold stimulation of PPi-PFK by Fru 2,6-P_2 in certain plant tissues (Kombrink et al. 1984), stimulation of PPi-PFK activities by Fru 2,6-P_2 was less than 50% in both pine root and stem extracts (data not shown). Low stimulation of PPi-PFK activities by Fru 2,6-P_2 has also been reported in pineapple (*Ananas comosus* (L.) Merr.) leaves (Carnal and Black 1983). In the present studies, PPi-PFK activities were assayed routinely with 2 μM Fru 2,6-P_2.

Seasonal sucrose metabolism in root cambial tissues of nontransplanted loblolly pine seedlings

Seasonal enzyme activity patterns of SS, AI and NI, the three enzymes that catalyze sucrose breakdown in taproot and stem cambial tissues, were assessed over a 17-month period beginning January 1990. Seasonal activities of SS, AI, and NI in root cambial tissues of nontransplanted control seedlings are given in Figure 1. Because seedling growth in the nursery was not synchronized and the seedlings were from mixed seed lots, the arrows marking times of bud set and bud break, which

Table 1. Extractable proteins and enzyme activities in various loblolly pine stem tissues with control seedlings.

Stem tissue[1]	SS		PPi-PFK		ATP-PFK		Protein
	(nmol g_{fw}^{-1} min^{-1})	(nmol mg^{-1} protein min^{-1})	(nmol g_{fw}^{-1} min^{-1})	(nmol mg^{-1} protein min^{-1})	(nmol g_{fw}^{-1} min^{-1})	(nmol mg^{-1} protein min^{-1})	(mg g_{fw}^{-1})
Phloem-side cambia	ND[2]	ND	49	53	95	102	0.93
Xylem-side cambia	513	120	1292	302	578	135	4.28
Xylem	30	29	202	198	88	86	1.02

[1] Stem bark was peeled and the phloem-side and xylem-side cambial tissues were obtained by scraping cambium off the inner side of the bark and the xylem side, respectively. Xylem tissues, identified by microscopic examination, were isolated by rescraping the xylem-side cambium.

[2] ND = Not detectable.

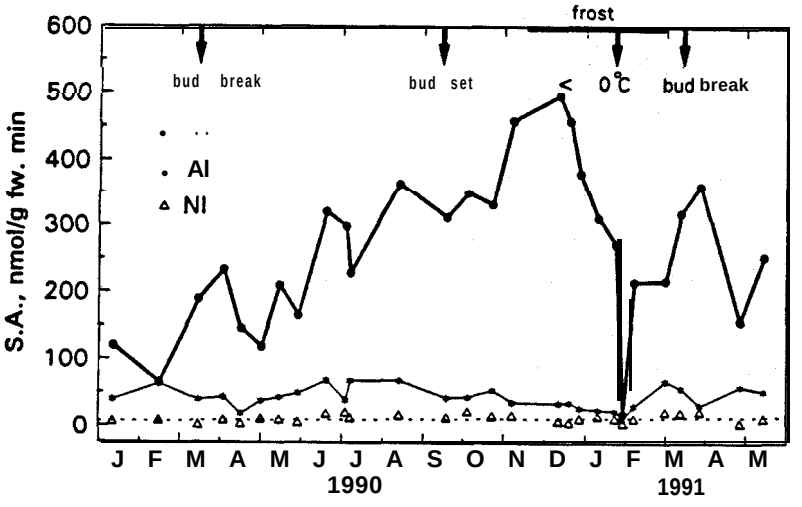

Figure 1. Seasonal activity patterns of sucrose metabolism enzymes in control loblolly pine seedling taproot cambial tissues. In all figures approximate times for bud break and bud set of the loblolly pine seedlings are marked with arrows. Freezing temperatures occurred on January 22 to 26, 1991. The first and last frosts were on November 18, 1990 and February 27. 199 1, respectively.

occurred over a 2- to 3-week period, are approximate. The first frost was on November 18, 1990, the last frost on February 27, 1991, and freezing temperatures occurred from January 22 to 25, 1991.

Sucrose synthase activity was the dominant sucrose metabolism activity in root cambial tissues. It was an order of magnitude greater than those of the invertases (cf. Sung et al. 1989*a*, 1989*b*, Xu et al. 1989*a*). Although root AI activity fluctuated throughout the year, no specific seasonal patterns were detectable (Figure 1). Neutral invertase was the least active sucrose breakdown enzyme in roots and its activity remained almost constant throughout the year (Figure 1). As in. sweetgum (*Styraciflua liquidambar* L.) and pecan (*Carya illinoiensis* (Wangenh.) K. Koch) root cambial tissues, neither AI nor NI played dominant roles in sucrolysis (Sung et al. *19896).*

Root SS activity exhibited a seasonal pattern with up to 50-fold differences over a year (Figure 1). Increases in root SS activity from March to December were interspersed with periods of decreased SS activity (Figure 1). In both years, one period of low root SS activity coincided with new shoot elongation during April and May. Root SS was most active in November and early December and declined with decreasing winter temperatures to a minimum during the coldest periods in late January and early February.

The seasonal patterns for root SS activity (Figure 1) paralleled the reported seasonal patterns for radial root growth in loblolly pine seedlings (DeWald and Feret 1988, Kuhns and Gjerstad 1991). The steady increases in root SS activity (Figure 1) and root collar diameter (Table 2) during summer and early fall were followed by more rapid increases in both during winter. Field-grown loblolly pine seedlings exhibit net photosynthesis (measured at 20 or 25 °C) during winter (McGregor and Kramer 1963, Drew and Ledig 198 1). Also seedlings labeled with ^{14}C allocated most

Table 2. Growth and sucrose synthase specific activity of control and transplanted loblolly pine seedlings.[1]

Date	Control seedlings				Transplanted seedlings			
	RCD (mm)	Height (cm)	Stem SS (nmol $g_{fw}^{-1} min^{-1}$)	Root SS (nmol $g_{fw}^{-1} min^{-1}$)	RCD (mm)	Height (cm)	Stem SS (nmol $g_{fw}^{-1} min^{-1}$)	Root SS (nmol $g_{fw}^{-1} min^{-1}$)
June 4, 1991	7.8[2,3]	65			5.8	51		
June 24.1991	8.9	78	435[3,4]	308	6.3	62	269	29
August 13.1991	IO.3	87	322	217	6.8	69	255	98
September 13,199 I	12.4	94	364	253	8.0	79	426	340
October 22, I99 I	13.9	96	901	469	9.6	81	873	435
November 27, 199 I	14.4	96	596	528	9.6	82	519	701
December 18.1991	14.3	96	39	478	9.5	82	74	302

[1] Average seedling root collar diameter (RCD) and height were 3.77 mm and 3 I cm, respectively, at transplanting in February 1991.

[2] Each value is the average of 200 seedlings. The same seedlings were measured for growth at each sampling date.

[3] Each value is the average of two replications.

[4] Enzyme data were from composite samples of seedlings similar to those that were measured for growth.

of the radioactivity to roots in October through January when root growth was rapid (Kuhns and Gjerstad 1991). Because sucrose is the starting point of sucrolysis in higher plants (Sung et al. 1988, Xu et al. 19896, Sun et al. 1992) and SS is the dominant sucrose cleavage enzyme activity in the root cambium of loblolly pine (Figure 1), the close relationship between root SS activity and increases in root biomass could be expected. The rapid (less than 2 weeks) resumption of root SS activity after a 4-day period of freezing temperatures (Figure 1) indicated that sucrose metabolism in loblolly pine roots was only quiescent during periods of severe winter cold. This pattern of activity contrasts with the very low root cambial SS activity throughout the late fall and winter in dormant, deciduous hardwoods, such as sweetgum and pecan (Sung et al. 19896).

Seasonal sucrose metabolism in root cambial tissues of transplanted loblolly pine seedlings

Transplanted seedlings often encounter severe seasonal water stress as a result of loss of and damage to fine roots, poor hydraulic continuity between soil and root, and poor water absorption by suberized roots (Burdett et al. 1984, Sands 1984, Grossnickle 1988, Johnsen et al. 1988). Thus the availability of carbohydrates for new root development in transplanted seedlings is of paramount importance for establishing soil and watercontacts (Johnsen et al. 1988). The carbon sources for new root growth vary with tree species. For example, new root growth of Sitka spruce (*Picea sitchensis* (Bong.) Car-r.) seedlings depends on root starch reserves, whereas Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings depend on current photosynthates (Philipson 1988).

The delayed growth and high mortality of loblolly pine seedlings during the first year after transplanting on natural forest regeneration sites can be understood in terms of sucrose metabolism. No strong seasonal patterns or obvious effects of transplanting were apparent for root AI or NI activity (Figure 2). Within two months after transplanting, however, root SS activity of transplants (Figure 2) was lower than that of control seedlings (Figure 1). By late March, root SS activity in transplants had increased to 50% of that in control seedlings, but it decreased again during the period of shoot elongation and remained low until July. Thereafter, root SS activity in transplants gradually increased, but it remained lower than in nontransplanted controls until November (Figure 2 versus Figure 1). One year after transplanting, the activities of the three sucrose-cleaving enzymes in root cambial tissues were similar in transplanted and nontransplanted seedlings except when the shoot tip was actively elongating.

Seasonal sucrose metabolism in stem cambial tissues of nontransplanted loblolly pine seedlings

Sucrose synthase was also the dominant sucrose metabolizing enzyme in the stem cambium of control seedlings (Figure 3), but the seasonal patterns for stem and root SS activities differed. Sucrose synthase activity was very low in the stem cambium from December until February. Sometimes it was almost impossible to peel the stem

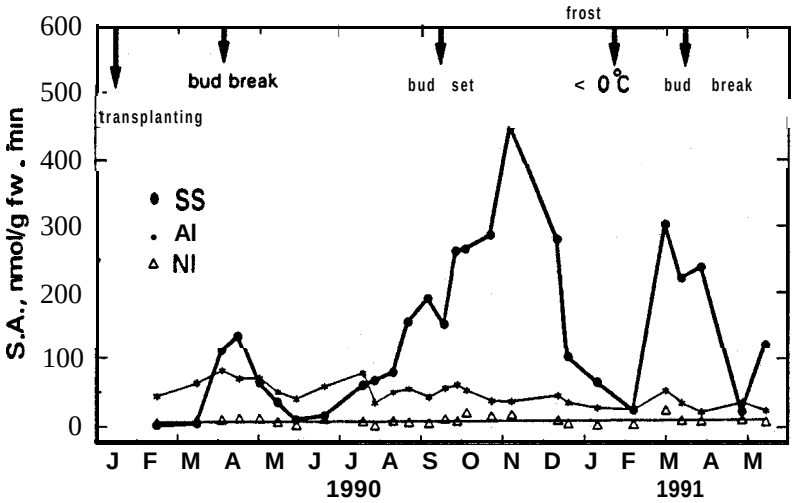

Figure 2. Seasonal activity patterns of sucrose metabolism enzymes in transplanted loblolly pine seedling taproot cambial tissues. Seeds were sown in April 1989 and seedlings were lifted during the second week of January 1990 and immediately transplanted.

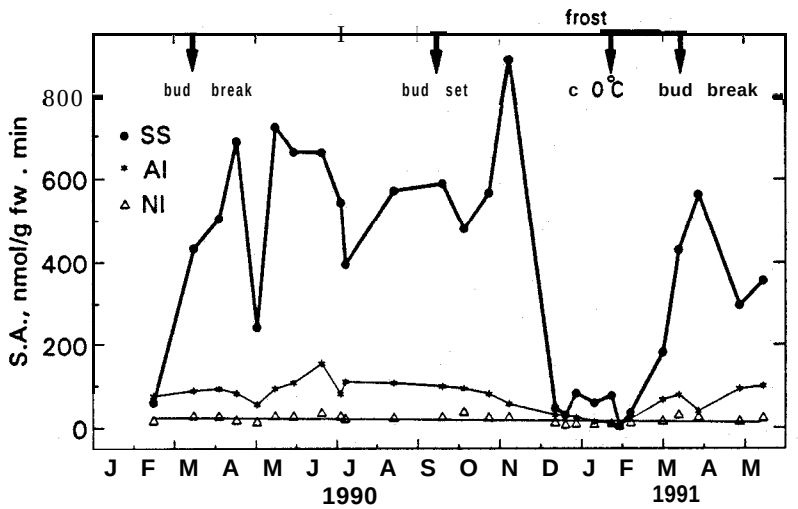

Figure 3. Seasonal activity patterns for sucrose metabolism enzymes in control loblolly pine seedling stem cambial tissues. Stem segments of 1989 growth from the seedlings sampled for root enzyme activities in Figure 1 were used.

bark from seedlings sampled in January. It is commonly acknowledged that the ease with which the stem bark peels is positively correlated with active stem growth (Zimmermann and Brown 197 1, Kimmerer and Stringer 1988). No increases in loblolly pine seedling stem diameter and weight were observed after late November (DeWald and Feret 1988, Kuhns and Gjerstad 1991, Table 2). In a study on 30-year-old loblolly pine trees in Louisiana, tree stem cambial growth, based on the number of cells formed, stopped during the first week of December and did not resume until the last week of March (Blanche et al. 1992).

In both years, stem SS activity decreased in April and May (Figure 3) when

competition for sucrose amongst the new shoot, the previous-year stem and roots was most severe. Sucrose synthase was active in stems throughout the summer and peaked in early fall after bud set (Figure 3) when stem growth was most active (Kuhns and Gjerstad 1991, Blanche et al. 1992, Table 2). Similar results have been reported with several other actively growing plant tissues (Claussen et al. 1986, Sung et al. 1989*a*, 19896, Xu et al. 1989*a*, Ross and Davies 1992, Sun et al. 1992).

Our results with vascular cambial tissues differ from those obtained with cotyledons, hypocotyls, and radicles of germinating pinyon pine seedlings (Murphy et al. 1992). During a period of 14 days after radicle emergence, SS was more active than AI in cotyledons, whereas AI was 3 to 5 times more active than SS in hypocotyls. There were positive correlations between AI activities and fresh weights of hypocotyls and cotyledons, but there were no correlations between SS activity and organ fresh weight (Murphy et al. 1992). Because AI is associated with elongating tissues (Sung et al. 1988, and references cited therein), correlations between AI activity and extension growth in young germinating seedlings are expected. In the present study, however, results were obtained from cambial tissues that contribute mainly to the radial growth of the organ. Hence, both studies indicated that the sucrose metabolizing enzymes, SS and AI, can play vital roles in pine seedling growth, but perhaps at different stages of plant development.

Seasonal sucrose metabolism in stem cambial tissues of transplanted loblolly pine seedlings

Stem cambial tissues could not be obtained from transplanted seedlings harvested in February 1990 because the barks would not peel. Two months after transplanting, stem SS activity was only half as much in transplants as in controls (Figure 4). When stems were competing for sucrose with elongating new shoots, stem SS activity, which had increased in early April, decreased again. Stem SS activity in transplants

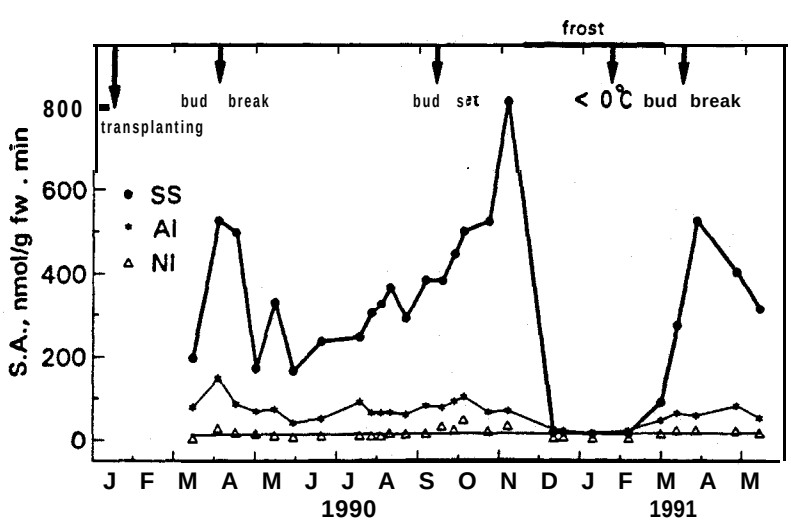

Figure 4. Seasonal activity patterns for sucrose metabolism enzymes in transplanted loblolly pine seedling stem cambial tissues. Stems segments of 1989 growth from the seedlings sampled for root enzyme activities in Figure 2 were used. Sampling began in March 1990.

increased slightly from July through September and finally equalled that in non-transplanted seedlings in October (Figure 4 versus Figure 3). Transplanting did not result in any specific changes in the seasonal patterns of AI or NI activity in stems (Figure 4).

Seasonal and spatial patterns of sucrose *synthase* activities in loblolly pine seedlings

Figure 5 shows the calculated differences between stem and root SS activities over a 17-month period. Generally the stem was a stronger sucrose sink than the root from March until October. From late November until February, the root was the major growth sink. The temporal (seasonal) and spatial (stem versus root) aspects of sucrose metabolism in loblolly pine seedlings (Figure 5) were in good agreement with reports on the periodic growth patterns of loblolly pine stems and roots (DeWald and Feret 1988, Kuhns and Gjerstad 1991, and Table 2). We conclude that SS activity in the vascular cambium can be used as a biochemical indicator of sucrose metabolism and of growth sink strength in loblolly pine seedlings,

Transplanting did not change the seasonal or spatial patterns of sucrose metabolism. Between bud break and bud set, stems were stronger sucrose sinks than roots in both transplanted and control seedlings. In the transplanted seedlings, stems resumed growth before roots.

Seasonal phosphofructokinase activities in cambial tissues of loblolly pine seedlings

In root (Figure 6) and stem (Figure 7) cambial tissues, PPi-PFK activities increased steadily from bud break until bud set, and then decreased with decreasing temperature. Seventeen- and 15-fold decreases in PPi-PFK activities were found in stems in November and in roots during the coldest period, respectively. Stem cambial PPi-PFK was twice as active as root cambial PPi-PFK throughout most of the year except during winter (Figures 6 and 7). The seasonal and spatial patterns of PPi-PFK activity in nontransplanted seedling root (Figure 6) and stem cambial tissues (Figure 7) were

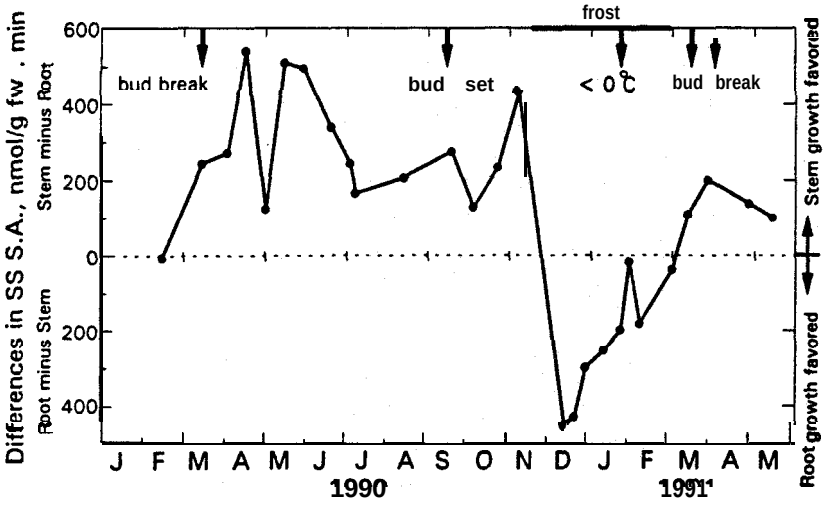

Figure 5. Differences between root and stem cambial sucrose synthase (SS) specific activities of control loblolly pine seedling throughout a season. Data were calculated from Figures 1 and 3.

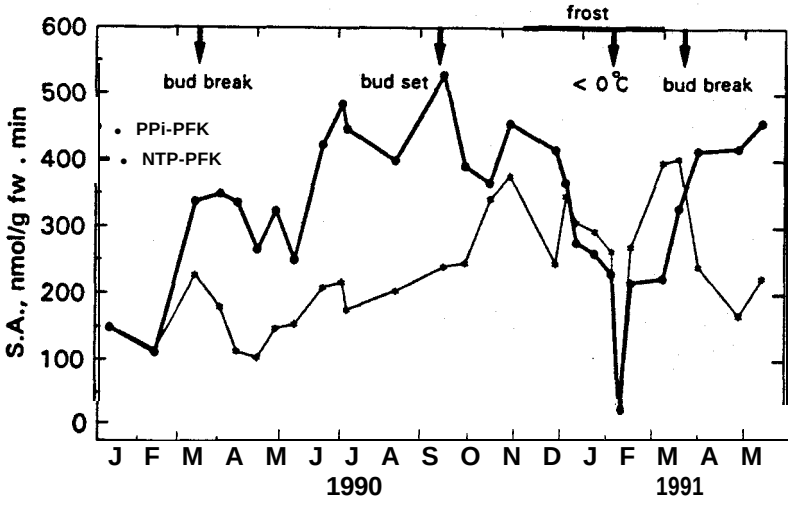

Figure 6. Seasonal activity patterns for Fru 6-P phosphorylating enzymes in control loblolly pine seedling taproot cambial tissues. The same extracts used for Figure 1 were assayed.

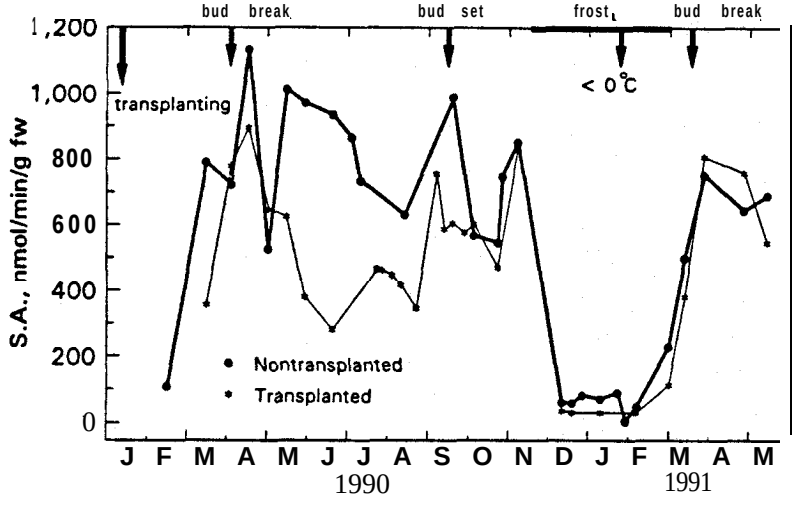

Figure 7. Seasonal activity patterns for pyrophosphate-dependent phosphofntctokinase in control and transplanted loblolly pine seedling stem cambial tissues. The same extracts used for Figures 3 and 4 were assayed.

similar to those of SS activity (Figures 1 and 3). There was a linear correlation between SS and PPi-PFK activities (r = 0.8), suggesting that both enzymes were responding to a common signal, possibly sucrose or another common metabolite, for example, in glycolysis.

In many plant species, ATP-PFK has a low specific activity in the range of 1 to 60 nmol mg^{-1} protein min^{-1} (Carnal and Black 1983), and does not respond markedly to development or environmental changes (Sung et al. 1989*b*, Xu et al. 1989*a*, Sung et al. 1990). We observed root NTP-PFK activities as high as 200 nmol mg^{-1} protein min^{-1} and ranging from 30 to 140% of the PPi-PFK activities (Figure 6). However, changes in root NTP-PFK maximal activities throughout the year were less than

3-fold except during the coldest period (Figure 6). Stem NTP-PFK activities in the dormant season were higher than stem PPi-PFK activities, but no clear developmental pattern was observed (data not shown).

Theoretically, the sum for PPi-PFK and NTP-PFK activities represents the potential of a plant tissue to phosphorylate Fru 6-P; whereas SS activity represents the potential of a tissue to cleave one molecule of sucrose into two molecules of hexose for entry into intermediary metabolism. Hence, in loblolly pine tissues actively growing on sucrose, a theoretical total PFK/SS ratio of 2/1 or less might be expected. In roots, which grow almost year around in the southern U.S., PFK/SS ratios were near 2/1 except during the period of bud break and stem elongation (calculated from Figures 1 and 6). From March through November, PFK/SS ratios in stem cambial tissue were generally close to 2/1. In winter when no stem growth occurred, greater decreases in SS than in PFK activities resulted in an increase in the ratios. When similar calculations were made for sweetgum and pecan tap roots (data not shown), from the study of Sung et al. (1989*b*), the PFK/SS ratios of roots were greater than 3/1 after leaf abscission, but the ratios were near 2/1 during active summer root growth.

Transplanting resulted in decreased PPi-PFK activities in stem (Figure 7) and root (data not shown) cambial tissues. These decreases occurred in similar patterns seasonally and spatially to those of SS activity except that the decreases were smaller for PPi-PFK than for SS. Transplanting had no effect on either stem or root cambial NTP-PFK activity. Stems and roots of transplants had PFK/SS ratios greater than 3/1 for three and six months after transplanting, respectively. Although a PFK/SS ratio of 2/1 is theoretical, it was correlated with the seasonal growth periods in control and transplanted loblolly pine seedlings (Figures l-4, 6 and 7) as well as in two deciduous tree seedlings (Sung et al. 19896).

Sucrose synthase as an indicator of growth sink strength in roots and stems of control and transplanted *loblolly* pine seedlings

In the 199 1 study, the most active root collar diameter growth occurred between late June and late October, whereas stem height growth decreased from June until late October (Table 2). There was a positive correlation between seasonal activities of growth and SS in the control seedlings in 1990 (Figures l-4) and 1991 (Table 2). A 14-fold decrease in stem SS activity occurred after root collar diameter growth stopped in November, whereas root SS activity was nearly constant from October to December. Stems were the major sucrose sinks during summer and early fall and roots were the major sinks in winter.

The data in Tables 2 and 3 also provide evidence to support the use of SS as a biochemical indicator of sucrose sink strength in tissues subjected to transplanting stress. From June to September, the increase in root collar diameter of transplants was only half as much as that of controls. Shoot elongation was delayed in the spring after transplanting and transplanting stress lasted through August in the 1991 study. Transplants also had lower root and stem SS activities than control plants. Similarly

Table 3. Relationship between seedling growth and sucrolytic enzyme activities in transplanted and control loblolly pine seedling stem and root cambial tissues.

Tissue	Increase in RCD[1] (mm per 3 weeks)	Specific activity[2] (nmol g_{fw}^{-1} min^{-1})					Ratio[3] PFK/SS
		SS	AI	NI	PPi-PFK	NTP-PFK	
Transplanted seedlings							
Stem	0.35[4]	268	52	16	511	312	3.1
Root		28	82	4	105	73	6.1
Control seedlings							
Stem	1.67	518	99	16	918	452	2.6
Root		326	99	15	580	295	2.7

[1] Root collar diameter (RCD) measurements were made on June 4 and 25, 1991 on control seedlings and on seedlings transplanted in February 1991.
[2] Only seedlings with less than 20% variation in RCD increase rate were combined for enzyme extraction and assays.
[3] Ratio between total PFK (PPi and NTP dependent) and SS activities.
[4] Root collar diameter (RCD) measurements were made on June 4 and 25, 1991 on control seedlings and on seedlings transplanted in February 1991.
[5] Each value is the average of two replications.

PPi-PFK activities were lower in transplants than in control plants. The ratios of total PFK/SS activities for transplants were 6/1 and 3/1 in roots and stems, respectively.

Conclusions

Three sucrose cleavage enzymes were present in loblolly pine stem and root cambial tissues, but in all tissues SS was the dominant enzyme throughout the year. Strong seasonal and spatial patterns of SS activity paralleled the periodic growth pattern of loblolly pine seedlings. There was a close relationship between SS and PPi-PFK activities throughout the year, and these enzymes rapidly and significantly changed their activities in response to both seasonal environment changes and the stress of transplanting. Transplanting stress caused decreases in root and stem SS activities but did not greatly alter the basic seasonal and spatial patterns. Actively growing tissues had total PFK/SS activity ratios near 2/1, whereas nonactively growing tissues had ratios as high as 12/1. The major enzyme activity that changed was SS.

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