A. Biochemical Assessment of the Value of Top Clipping Nursery-Grown Loblolly Pine Seedlings[1]

Shi-Jean S. Sung, Paul P. Kormanik, and Clanton C. Black[2]

ABSTRACT

Seasonal sucrose metabolism (sucrolysis) was studied in taproot cambial tissues of nursery-grown loblolly pine seedlings to assess the value of top clipping. In sucrose-importing taproots of nonclipped seedlings, sucrose synthase (SS) was the dominant enzyme for sucrose cleavage, and its activity exhibited a distinct seasonal activity. Both root SS activity and growth were most active during fall. Sucrose synthase activity decreased to the lowest level in mid-January and resumed after that. Neither root acid invertase (AI) nor neutral invertase (NI) changed activity appreciably through the seasons. Both August and September top clipping treatments decreased seedling top weight by 20 percent to 45 percent whereas total root weight was slightly decreased by August clipping only. Top clipping did not change the basic seasonal pattern of sucrolytic pathway in taproot cambial tissues. However, 2 to 3 months after top clipping, losses of root SS activity in clipped seedlings were observed. The largest decreases in SS activity occurred from November through early January followed by another decrease during active shoot elongation. Generally, August clipping decreased more root SS activity than September clipping. Neither root Al nor NI activity was affected by the clipping treatment. It was concluded that: (1) sucrose synthase was the dominant sucrolytic enzyme in cambial tissues of pine seedling taproots; (2) sucrose synthase activity can be used as an indicator for the physiological status of tissues; and (3) top clipping, especially in August, imposes stress on nursery seedlings based on biochemical analysis and growth measurements.

Keywords: Sucrolysis, sucrose metabolism, siicrose synthase.

INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is the most commonly planted conifer throughout the United States, with 1 billion seedlings produced in the Southern United States in peak years (Johnson and others 1982). Since World War II, great progress has been made in the genetic improvement of seed source and in the nursery technology to produce this large number of seedlings. A general rule was then suggested to eliminate culling if the percentage of small, damaged, or diseased seedlings was less than 10 percent in any seedlot (May 1985b). Consequently, about 95 percent of each nursery crop was commonly reported to be "plantable seedlings" (May 1985a). However, many of these "plantable seedlings" either did not survive transplanting stress or grew poorly in the field (Weaver and others 1981, Johnson and others 1982). Nonetheless, these practices continued because the seedlings appeared fairly uniform and were readily used in artificial forest regeneration work.

Uniformity of the planting stock was achieved principally by heavy fertilization, irrigation, and by top clipping the faster growing seedlings several times during the growing season (Dierauf 1976, Mexal and Fisher 1984). But we think these pro&d&es are flawed. For example, the more vigorous the growth of an individual seedling, the more often it was clipped. Indeed, many seedlings that were in fact poor competitors were repeatedly released from do&petition by these cultural practices. A close examination of these released seedlings revealed that about 20 to 30 percent of the crop still shared many morphological traits with those considered culls in Wakeley's original seedling grading system (1954). These traits include succulent stems, poor development of secondary needles, and general absence of terminal buds.

With a better understanding of loblolly seedling biology and Sucrose metabolism (sucrolysis) (Kormanik and others 1990, 1991; Sung and others 1993), Kormanik and others (1992) developed a new nursery cultural technology both for growing

[1] Paper presented at Research and Applications of Chemical Sciences in Forestry; 1994 February l-2; Starkville, MS.

[2] USDA Forest Service, Southeastern Forest Experiment Station, Institute of Tree Root Biology, Forestry Sciences Laboratory, Athens, GA 30602; USDA Forest Service, Southeastern Forest Experiment Station, Athens, GA 30602; Department of Biochemistry, University of Georgia, Athens, GA 30602, respectively.

and selecting loblolly pine seedlings. The technology requires as little as one-third to one-half of the top dressing nitrogen and water of those traditionally prescribed for nurseries. This technology does not employ top clipping to meet uniform target height for planting because 70 to 80 percent of the crop are plantable seedlings of uniform size.

Sucrose is the major form of translocated carbohydrate in plants (Shiroya and others 1962, Zimmermaxm and Brown 1971), and it initiates metabolism in recipient cells leading to growth and storage (Hubbard and others 1989; Sung and others 1989a, 1989b; Xu and others 1989). Of three enzymes cleaving sucrose, sucrose synthase (SS) is the dominant activity in actively growing aud storing tissues such as loblolly pine seedling stems (Sung and others 1993) or sweetgum seedling roots (Sung and others 1989a). Sucrose synthase also has been reported to be an indicator for sucrose sink strength in growing potato tubers (Sung and others 1989b) and for the physiological status of loblolly pine tissues under cold or transplanting. stress (Sung and others 1993).

It is clear that top clipping is another form of stress artificially imposed on nursery seedlings. When an actively growing loblolly pine seedling is top clipped, it loses almost half of the photosynthetically active secondary needles (Mexal and Fisher 1984). A few weeks later, numerous new shoots are initiated from cut needle fascicles (Dierauf 1976, Mexal and Fisher 1984). This observation suggests tbat reduced net photosynthate production and incnased demand for sucrose by new shoot growth will drastically change sucrose metabolism and hence change the source-sink dynamics between seedling tops and roots. 'This study was a part of the nursery cultural technology trials by Kormanik and others (1992). Objectives of this study were to determine how top clipping pine seedling influenced sucrolysis and to make a reassessment of the putative value of top clipping by following the seasonal patterns of seedling sucrolysis and growth.

METHODS

Loblolly pine seeds of mixed seedlots were stratified at 4 °C for 60 days and sown in mid-April, 1988, in 18.3- by 1.2- by 1.2-m beds at the Whitehall Experimental Nursery in Athens, GA Nursery cultural practices were as described by Kormanik and others (1990) with similar levels of nitrogen fertilizer and irrigation commonly used in most commercial nurseries. One-third of the seedlings were top clipped to 30 cm in height in mid-August, and the second one-third of seedlings were top clipped to 37 cm in mid-September. One-third of the seedlings were never clipped and served as controls. Beginning in August, 100 seedlings from each treatment were carefully harvested, and growth measurements such as top and root dry weight and root collar diameter (RCD) were made at 2-week intervals.

Biweekly sampling for enzyme analysis also began in August, and there were two replicates for each sampling. Results reported in this paper are the average of the two replications. Variations in enzyme activities between two replications were less than 15 percent at all times. Procedures for tissue sampling and biochemical analysis were tbe same as those described by Sung and others (1993). Root vascular cambial tissues were obtained by peeling the bark from taproots and scraping off the inner (xylem-side) cambial tissue with a razor blade. At each sampling date, 10 to 40 pine seedlings were used to obtain a composite sample of 2.5 g fresh weight root cambial tissues. Enzyme extraction and assay procedures for sucrolytic enzymes, namely sucrose synthase (SS), acid invertase (Al), and neutral invertase (NI), followed those by Sung and others (1993). The protein content of each extract was determined with Bradford reagent (Bradford 1976) using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Seasonal growth measurements for all treatments are presented in figure 1. From August througb October, when top growth was most active (fig. 1A), periodicity of root growth was observed (fig. 1B). In November, between the two *most* active periods of root growth, there was a period of slow growth. Both seedling top and root weights decreased in December and January (figs. 1A and 1B). This was also reported by DeWald and Feret (1988). It was suggested that this winter weight loss was due to a maintenance type respiration (Kuhns and Gjerstad 1991).

In addition to removal of part of the seedling top at clipping, August clipping treatment decreased the rate of top weight accumulation during the following active growing months (fig. 1A). September clipping treatment did not change top weight accumulation rate, which had almost plateaued at clipping (fig. 1A). Similarly, Dierauf (1976) reported that top clipping decreased stem diameter but not root system size. In this study, there were no significant differences in seedling root weight (fig. 1B) or root collar diameter (RCD) (data not shown) between nonclipped and September clipped seedlings. Up to 20 percent decreases in root weight (fig. 1B) and RCD (data not shown) were found in August clipped seedlings. There were also losses in both top and root weights during winter in clipped seedlings as in nonclipped controls. However, it is not known why the winter weight losses by clipped seedlings were not so obvious as those by the nonclipped controls.

One of the justifications for top clipping is to grow uniform size seedlings (Dierauf 1976, May 1985a, Mexal and Fisher 1984). Indeed, in this study, the standard deviations for nonclipped seedling top and root weights were greater than those of clipped seedlings (figs. 1A, 1B). Another reason to top clip is to improve seedling top:root ratio to 1.5:1 to 3:1 (May

1985a). In this study, top:root ratio was decreased from 6: 1 in the nonclipped seedlings to 4.5: 1 in the clipped seedlings (fig. 1C). This improvement of top:root ratio, however, was achieved because of decreased top weight rather than increased root biomass as reported by Mexal and Fisher (1984). These oversized seedlings, with a RCD of 5.0 to 5.5 mm when stem growth was finished for the year, were the results of heavy nitrogen fertilization and irrigation as used by most nurseries. It became clear why nurserymen top clipped the same seedlings several times in the year when weather condition was most suitable for growth. In this study, one-time top clipping in September did not affect much of the seedling top and root growth, whereas August clipping had small negative effects on top'growth.

A pine seedling can be looked at in a classical manner as composed of sucrose source and sucrose sink tissues or organs. The photosynthetic needles that produce and export photosynthate in the form of sucrose are sources. Tissues such as roots that import sucrose for growth are sinks. The seasonal patterns for sucrolytio enzyme activity in nonclipped taproot cambial tissues are shown in figure 2. Similar to the fmdings by Sung and others (1993), SS was the dominant sucrose-cleaving enzyme activity that adapted to seasonal and developmental changes. There was a three-fold difference in SS activity between the lowest and the highest levels with the former occurring in the coldest month and the latter in November (fig. 2). A close relationship between the periodicity growth of root weight and taproot SS activity was observed throughout the study (fig. 1B vs. fig. 2). Neither invertases played a significant sucrolytio role in pine roots, and the invertase activity did not vary either seasonally or developmentally (fig. 2). It is quite clear from the results presented in figure 2 that SS can be used as a sucrose sink strength indicator with loblolly pine root.

In this study, 3 to 4 weeks after August clipping, new pine shoot tips emerged from cut fascicles. It took the September clipped seedlings 4 to 6 weeks to develop new shoots. Most of these new shoots, especially on the September clipped seedlings, did not form terminal buds in the nursery bed. Top clipping not only decreases seedling sucrose source size by removing photosynthetic needles, but also increases sucrose sink size by initiating growth of new shoot tips. This contrasting response to top clipping provided a chance to assess sucrolysis pathways in seedlings under a nonenvironmental, human-made stress.

The effects of August clipping on seedling sucrolysis are presented in figure 3. Seasonal sucrolysis pathway in August clipped taproot cambial tissues (fig. 3) was similar to that of the nonclipped controls (fig. 2) with SS being the dominant and adaptive activity. Sucrose synthase activity was 20 to 60 percent less in the August clipped seedling roots than that of nonclipped seedlings (fig. 2 vs. fig. 3). The largest SS activity losses occurred in November, December, and March. Neither AI nor NI activity was noticeably affected by clipping (fig. 3). This clipping study establishes that loblolly pine seedling taproot SS activity changes can be mediated by either seasonal or human-made stress as suggested earlier by Sung and others (1993).

All three sucrose cleaving enzyme activities in September clipped seedling taproots (data not shown) did not differ in the seasonal patterns from those of the nonclipped and August clipped seedlings. There is a similarly close relationship between root growth and SS activity for September clipped seedlings to that of nonclipped seedlings. Furthermore, SS loss by September clipping was less when compared with that by August clipping.

Both August and September clipping affected SS activity more than root weight. For example,average seasonal losses in taproot SS activity and total root weight were 35 percent and 5 percent for the August clipped seedlings and 26 percent and 0.5 percent for the September clipped seedlings. This suggests that the physiological status of pine seedlings at lifting is more,critical for seedling's surviving transplanting stress than total root weight. Dierauf (1976) reported a higher field survival percentage from September clipped seedlings than from August clipped seedlings although both types of seedlings had similar top:root ratio. A general term like "physiological response" was used by Dierauf to reason this discrepancy. Results of this study, however, suggested that SS activity can be used as the qualitative and quantitative indicator for seedlmq physiological status.

CONCLUSIONS

Sucrose synthase was the dominant sucrose metabolizing activity in taproot cambial tissues during active root growth in fall and early winter with both invertase activities constant through development. The negative effects of top clipping, especially the August clipping, on loblolly pine seedlings were at least two-fold: losses of taproot cambial tissue SS activity through the seasons and decreases in top weight accumulation rate. These results support earlier conclusions about the detrimental effects of top clipping on loblolly pine seedlings.

LITERATURE CITATIONS

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry. 72; 248-254.

DeWald, L.E.; Feret, P.P. 1988. Changes in loblolly pine seedling root growth potential, dry weight, and

dormancy during cold storage. Forest Science. 34: 41-54.
Dierauf, T.A. 1976. Top clipping in the nursery bed In: Proceedings of the Southeastern Area nurserymen's conference; 1976 August; Charleston, SC. 37-43.
Hubbard, N.L.; Huber, SC.; Pharr, D.M. 1989. Sucrose phosphate synthase andacid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. Plant Physiology. 91: 1,527-1,534.
Johnson, J.D.; Bramlett, D.L.; Burns, RM. [and others] 1982. Pine seedling production in the South: a problem analysis. Forestry Wildlife Science Publ. 6-82. Blacksburg, VA: Virginia Polytechnic Institute and State University. 33 p.
Kormanik, PP.; Ruehle, J.L.; Muse, H.D. 1990. Frequency distribution and heritability of first-order lateral roots in loblolly pine seedlings. Forest Science. 36: 802-814.
Kormanik, PP.; Muse, H.D.; Sung, S.J. 1991. Impact of nursery management practices on heritability estimates and frequency distributions of first-order lateral roots of loblolly pine. In: Proceedings of the 21st southern forest tree improvement conference; 1991 June; Knoxville, TN: 248-257.
Kormanik, P.P.; Sung, S.S.; Kormanik, T.L. 1992. Controlling loblolly pine seedling growth through carbon metabolism regulation rather than mechanical procedures.-In: Proceedings of the Southern Forest Nursery Association conference; 1992 July; Pine Mountain, GA: 6- 11.
Kuhns, M.R.; Gjerstad, D.H. 1991. Distribution of 14C-labeled photosynthate in loblolly pine (*Pinus taeda*) seedlings as affected by season and time after exposure. Tree Physiology. 8: 259-271.
May, J.T. 1985a. Seedling growth and development. In: Southern pine nursery handbook Atlanta. GA: U.S. Department of Agriculture, Forest Service, Southern Region Cooperative Forestry: I-16. Chapter 7.
May, J.T. 1985b. Seedling quality, grading, culling and counting. In: Southern pine nursery handbook. Atlanta, GA: U.S. Department of Agriculture, Forest Service, Southern Region Cooperative Forestry: l-10. Chapter 9.
Mexal, J.G.; Fisher, J.T. 1984. Pruning loblolly pine seedlings. In: Proceedings of the southern nursery conference; 1984 June; Alexandria, LA; 75-83.
Shiroya, T.; Lister, G.R.; Slankis, V. [and others]. 1962. Translocation of the products of photosynthesis to roots of pine seedlings. Canadian Journal of Botany. 40: 1,125-1,135.
Sung, S.S.; Kormanik, P.P.; Xu, D.-P.; Black, C.C. 1989a. Sucrose metabolic pathways in sweetgum and pecan seedlings. Tree Physiology. 5: 39-52.
Sung, S.S.; Xu, D.-P.; Black, C.C. 1989b. Identification of actively filling sucrose sinks. Plant Physiology. 89: 1,1 17-1,121.
Sung, S.S.; Kormanik, P.P.; Black, C.C. 1993. Vascular cambial sucrose metabolism and growth in loblolly pine (*Pinus taeda* L.) in relation to transplanting stress. Tree Physiology. 12: 243-258.
Wakeley, P.C. 1954. Planting the southern pines. Monogr. 18. Washington, DC: U.S. Department of Agriculture. 233 p.
Weaver, C.H.; Izlar, B.; Xydias, K.; Broerman, F.S. 198 1. Trends in southern forest industry pine plantation survival 1960-78. In: Forest regeneration: Proceedings of the American Society of Agriculture Engineering symposium on engineering systems for forest regeneration. ASAE Publ. 10-8. St. Joseph, MI: American Society of Agriculture Engineers: 53-55.
Xu, D.-P.; Sung, S.S.; Black, C.C. 1989. Sucrose metabolism in lima bean seeds. Plant Physiology. 89: 1,106-1,116.
Zimmermann, M.H.; Brown, C.L. 1971. Trees, structure and function. Berlin: Springer-Verlag. 336 p.

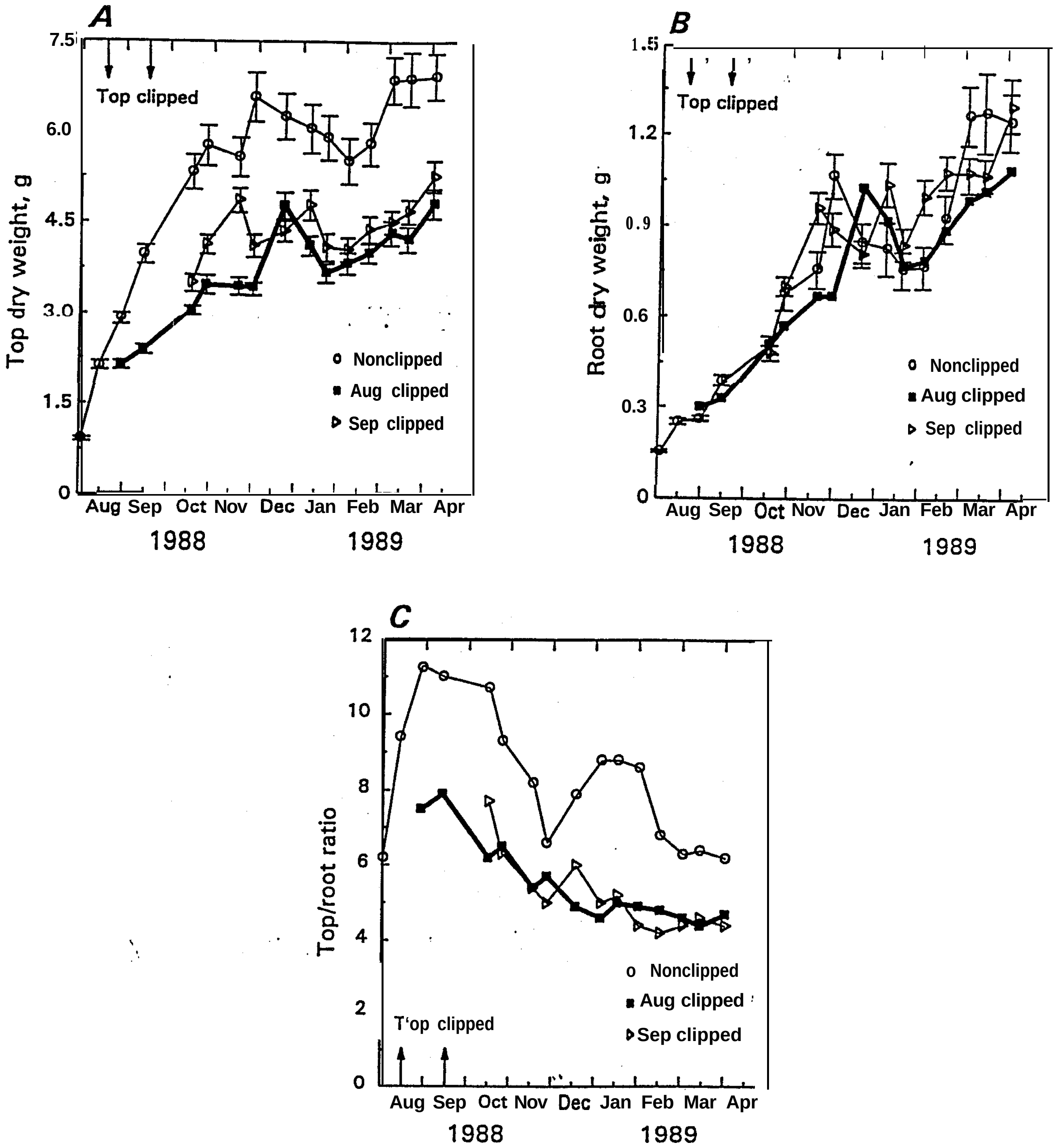

Figure *1. Growth measurements of loblolly pine seedlings. Seeds were sown in mid-April. Data are means of 100 seedlings. Bar represents ± 1 SE. (A) Top dry weight. (B) Total root dry weight. Standard error (SE) for August clipping treatment were similar to those of September clipping and were not shown here. (C) Ratio between seedling top and total root dry weight.*

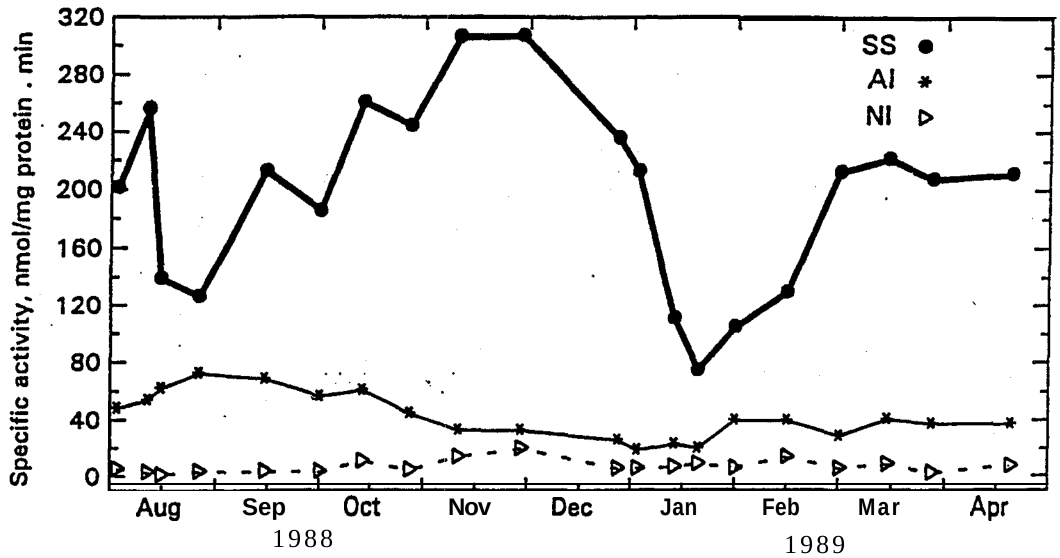

Figure 2. *Seasonal specific* activity *patterns of three sucrose metabolizing enzymes, sucrose syhtnase (SS), acid invertase, (AI), and neutral invertase (NI), in taproots of nonclipped loblolly pine seedlings. Soluble extracts from taproot cambial tissues were used in all assays. Each value is the average of two replications.*

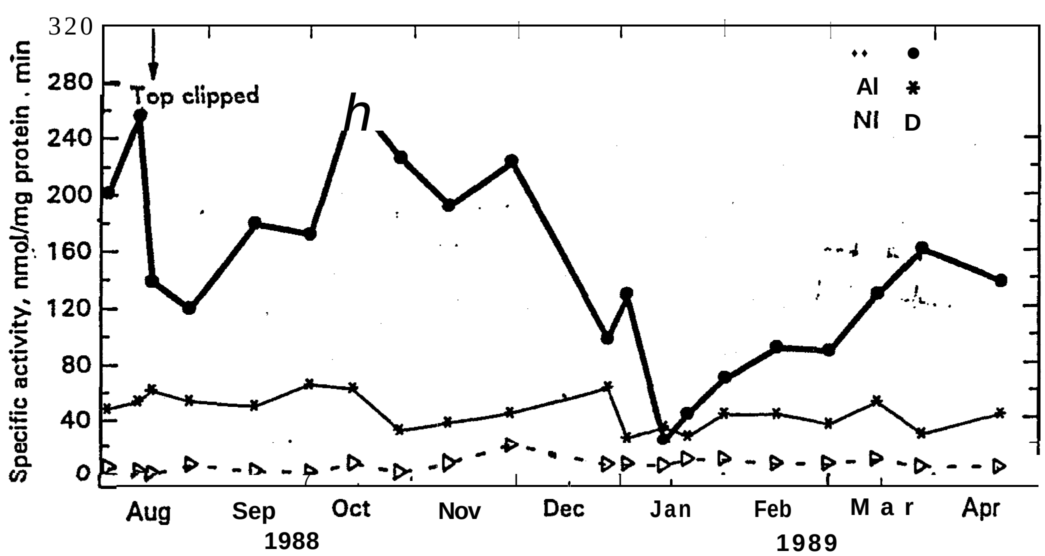

Figure 3. *Seasonal specificty activity patterns of three sucrose metabolizing enzymes in taproots of August clipped loblolly pine seedlings. Soluble extracts from taproot cambial tissues were used in all assays. Each value is the average of two replications.*