SEASONAL SUCROSE METABOLISM IN LONGLEAF PINE TREE STEM CAMBIAL TISSUES

Shi-Jean S. Sung, William J. Otrosina, and Stanley J. Zarnoch[1]

Abstract—This study was a part of a long-term study on factors contributing to the decline of a 40+-year-old longleaf pine stand where prescribed burning has occurred. Burn treatments were implemented between January and March 1997. From April 2002 through February 2003, stem cambial tissues were sampled periodically from healthy longleaf pine trees preselected from each treatment in early April 2002. Sucrose synthase activity increased from April through June but decreased 63 percent from June to July. After several rainstorms in September, the September and October sucrose synthase activity was as high as that of June. The lowest sucrose synthase activity occurred in February. The seasonal pattern of pyrophosphate-dependent phosphofructokinase was similar to that of sucrose synthase. Other measured enzymes in the sucrose metabolic pathway did not show clear seasonal patterns. No treatment effects on these enzyme activities were observed. The seasonal physiological status of longleaf pine trees, as evidenced by their stem cambial sucrose synthase activity, might be useful in determining the optimal time for prescribed burning in longleaf pine stand management.

INTRODUCTION

Longleaf pine (*Pinus palustris* Mill.) is both tolerant of and sensitive to fire, whether prescribed or wild, depending on fire frequency, intensity, and time of occurrence. March burning had more adverse effects on height growth of longleaf pine saplings (approximately 5 to 6 years old) than did burning in May or July (Haywood 2002). Three years after prescribed burning in a 40-year-old longleaf pine stand where temperatures reached approximately 54 to 66 ˚C at duff-soil interface, mortality and number of crown-symptomatic trees had increased after burn treatment (Otrosina and others 2002). Further, increased crown symptom severity was correlated with decreased sucrose synthase activity in longleaf pine stem cambial tissues (Otrosina and others 2002).

Sucrose synthase has been used as a sink strength and physiological status indicator in annual plants (Sung and others 1989b, Xu and others 1989), transplanted loblolly pine (*P. taeda* L.) seedlings (Sung and others 1993b), and loblolly pine trees (Sung and others 1996). The overall objective of this study was to establish the seasonal growth pattern of longleaf pine trees inferred by their cambial tissue sucrose synthase activity. This information will aid ongoing efforts to determine optimal windows for prescribed burning in stands where fire is reintroduced after being long absent.

MATERIALS AND METHODS

The original study was initiated in 1996 at the Savannah River Site (New Ellenton, SC) (Otrosina and others 2002). A 40-year-old planted longleaf pine stand was subjected to four burning treatments in a randomized complete block design. Each 2.0-ha treatment plot was replicated four times with unburned control, cool, medium, and hot burn intensities randomly assigned. Four 0.0079-ha subplots were located in each plot, starting with one at the plot center and three others located 30 m from plot center at 120-degree intervals from due N. Burning took place between January and March 1997. The low- and medium-intensity burns were head fires, whereas the hot burn was a backing fire tending to move more slowly across the landscape. Temperatures at the duff-soil interface registered potentially lethal levels (approximately 54 to 66 ˚C) in the hot-burn plots only. The decomposed organic layer depth in the cool- and medium-burn treatment plots was similar to that of the unburned controls. The hot-burn plots had about one-half the decomposed organic matter of the controls (Otrosina and others 2002).

Periodical sampling of stem cambial tissues for seasonal sucrose metabolic enzyme activities began in mid-April 2002 and continued through February 2003. Due to the summer drought, August sampling was not conducted. Three subplots were randomly selected in each treatment and block. Within each subplot, trees with healthy-appearing crowns were preselected for sampling in early April. A total of 48 trees were sampled each time. After a section of 8- by 15-cm stem bark was peeled; xylem-side cambial tissues were scraped into a weight boat, transferred to plastic bags, and stored immediately in liquid nitrogen. Samples were stored at -80 ˚C until processing. The protein extraction protocol and enzyme assays followed methods described by Sung and others (1993b). Enzymes assayed included sucrose synthase, pyrophosphate-dependent phosphofructokinase, ATP-dependent phosphofructokinase, fructokinase, glucokinase, UDP-glucopyrophosphorylase, phosphoglucomutase, and phosphoglucose isomerase. All enzyme activities were expressed on protein basis. This study was analyzed as a split-plot design with replication by means of four blocks. The whole plot factor was burn treatment (control, cool, medium, hot), while the split-plot factor was the seven sampling months. Thus, blocks were a random factor, while burn treatments and months were fixed factors. The three trees (one from each of three subplots) were considered a random sample of nonsymptomatic trees from each block, burn, and month combination. The random variable for analysis was the mean enzyme

[1] Research Plant Physiologist and Supervisory Plant Pathologist, Institute of Tree Root Biology, USDA Forest Service, Southern Research Station, Athens, GA 30602; and Mathematical Statistician, USDA Forest Service, Southern Research Station, Asheville, NC, respectively.

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activity of the three subplot trees. PROC GLM was used for the analysis of variance, and pairwise comparisons were performed using Tukey's test (SAS Institute 1989). All statistical analyses were performed at the 0.05-significance level.

RESULTS AND DISCUSSION

Mean breast height diameter from all sampled trees was 31.7 cm. No differences in breast height diameter existed among treatments or months of sampling. No burn-treatment effects were observed on any of the eight enzymes assayed over time. To our best knowledge, this is the first report on seasonal sucrose metabolism in longleaf pine trees.

Sucrose synthase activity increased from April to June and decreased significantly (63 percent) in July (fig. 1). The Southeastern United States was in the fifth year of drought in 2002. In fact, August sampling was not conducted due to severe drought conditions. After several rainstorms in early September, both September and October sucrose synthase activity resumed to levels recorded in June (fig. 1). The lowest sucrose synthase activity was found in February 2003 samples, but it was not significantly different from that of July samples. The observed seasonal sucrose synthase pattern in longleaf pine stem was increased activity from early winter through early summer, decreased activity in summer drought, and resumed activity after several rainstorms in early fall. This pattern is similar to that of loblolly pine trees planted at the Savannah River Site (Sung and others 1996). However, loblolly pine did not show significant decreases in sucrose synthase activity from June to August. Loblolly pine seedlings grown in nursery beds for 2 years showed some decreases in stem sucrose synthase activity from June to July in the second year (Sung and others 1993b). It is possible that sucrose synthase activity reduction in longleaf pine stems during summer was caused by either high temperature or drought. Sucrose synthase activity is closely associated with active growth and/or storage in annuals and trees (Otrosina and others 1996; Sung and others 1989a, 1989b, 1993a, 1993b, 1996; Xu and others 1989). In longleaf pine stems, high sucrose synthase activity, thus active growth, began in mid-April and continued through late October (except in July and August). Because we observed low sucrose synthase activity in February, we recommend that burn treatment should be restricted in winter months (December through February) when stem cambial activity is low. Episodic growth patterns of the above- and belowground parts of loblolly pine seedlings and trees have been reported (Sung and others 1993b, 1996). However, during January and February, sucrose synthase activity in both stems and roots of loblolly pine was the lowest. Effects of fire on longleaf pine roots and associated mycorrhizae were also reported in the original burn study and were attributed to delayed mortality in the stand (Otrosina and others 2002). Seasonal sucrose metabolic enzyme patterns in longleaf pine taproots and lateral roots have not been established.

The seasonal pattern of pyrophosphate-dependent phosphofructokinase, but not ATP-dependent phosphofructokinase, was similar to that of sucrose synthase (fig. 1). This was also the case with loblolly pine (Sung and others 1993b, 1996). Like loblolly pine and Jeffrey pine (*P. jeffreyi* Grev. and Balf.) (Otrosina and others 1996), longleaf pine also had similar ATP-dependent phosphofructokinase activity to that of pyrophosphate-dependent phosphofructokinase except in February. Most other tree species, such as sweetgum (*Liquidambar styraciflua* L.) (Sung and Kormanik 2000; Sung and others 1989a, 1993a) and pecan [*Carya illinoinensis* (Wangenh.) C. Koch] (Sung and others 1989a), and many annual plants (Sung and others 1989b, Xu and

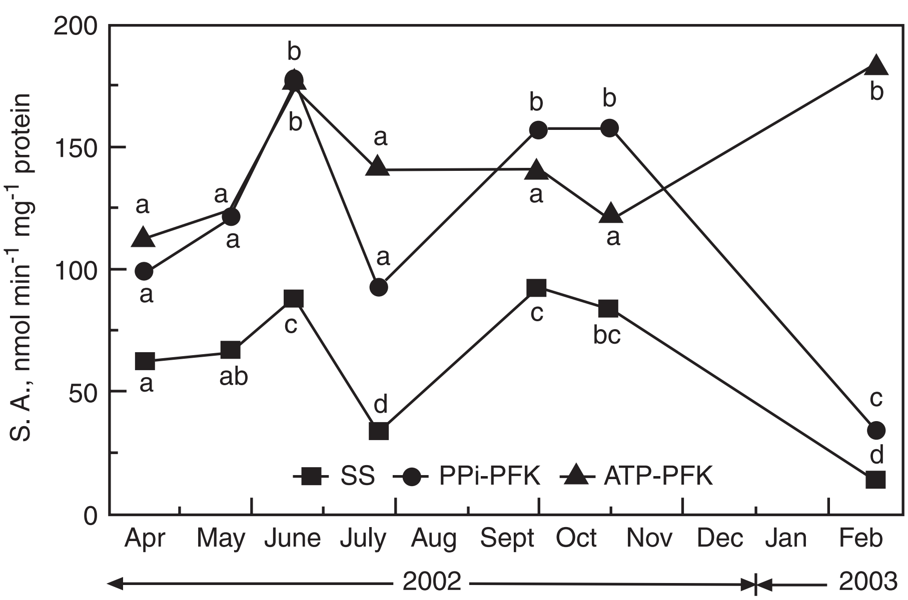

Figure 1—Seasonal patterns of sucrose synthase (SS), pyrophosphate-dependent phosphofructokinase (PPi-PFK), and ATP-dependent phosphofructokinase (ATP-PFK) specific activities (S.A.) in stem cambial tissues of 40+-year-old longleaf pine trees. For each enzyme, different letters indicate significance at the 0.05 level.

others 1989) have twofold to tenfold higher pyrophosphate-dependent phosphofructokinase activity than ATP-dependent phosphofructokinase. Nevertheless, as in other plants (Otrosina and others 1996; Sung and others 1989a, 1993b, 1996; Xu and others 1989), ATP-dependent phosphofructokinase in longleaf pine functioned as a maintenance enzyme that did not change its activity with seasons or environments. This was evident with the high level of ATP-dependent phosphofructokinase activity occurring in February when pyrophosphate-dependent phosphofructokinase was only 20 percent of the former (fig. 1).

Glucokinase activity was most active in spring and least active in summer and winter (fig. 2). Low levels of fructokinase also was observed in longleaf pine, loblolly pine (Sung and others 1993b, 1996), and Jeffrey pine (Otrosina and others 1996). Compared to activities of sucrose synthase and pyrophosphate-dependent phosphofructokinase (fig. 1), activities of UDP-glucopyrophosphorylase, phosphoglucomutase, and phosphoglucose isomerase were elevated (fig. 3). Monthly differences in these enzyme activities were not biologically significant, although some were statistically significant. These enzyme levels were

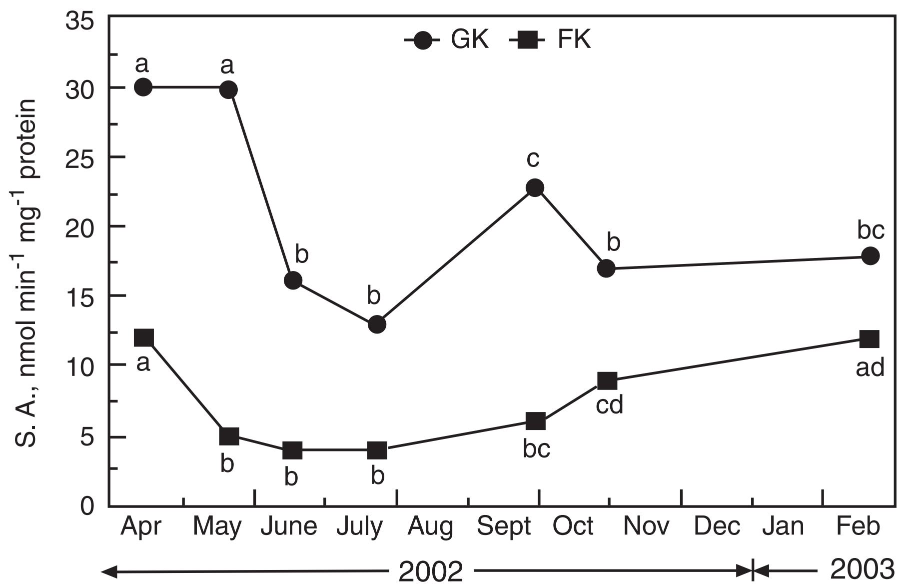

Figure 2—Seasonal patterns of fructokinase (FK) and glucokinase (GK) specific activities (S.A.) in stem cambial tissues of 40+-year-old longleaf pine trees. For each enzyme, different letters indicate significance at the 0.05 level.

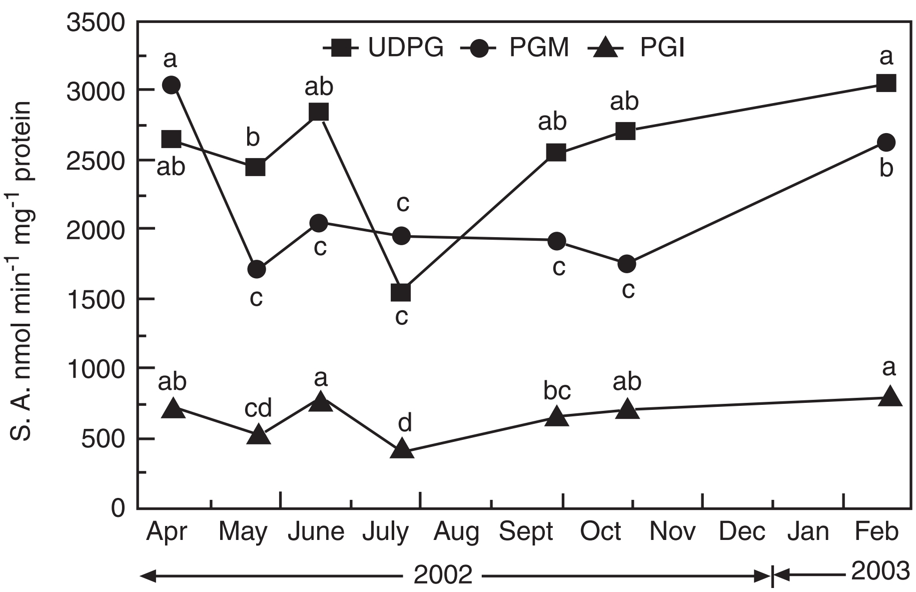

Figure 3—Seasonal patterns of UDP-glucopyrophosphorylase (UDPG), phosphoglucomutase (PGM), and phosphoglucose isomerase (PGI) specific activities (S.A.) in stem cambial tissues of 40+-year-old longleaf pine trees. For each enzyme, different letters indicate significance at the 0.05 level.

similar to values reported for other trees (Sung and others 1989a) and annual plants (Xu and others 1989).

CONCLUSIONS

Seasonal sucrose metabolism in longleaf pine tree stem cambial tissues resembled that of loblolly pine trees (Sung and others 1996). After low activity in February, sucrose synthase activity increased from April until June. In summer when rainfall was scarce, significant decreases in stem cambial sucrose synthase activity indicated that stem diameter growth may be affected. Sucrose synthase activity resumed in fall. The seasonal activity of pyrophosphate-dependent phosphofructokinase followed that of sucrose synthase. Activities of other enzymes in the sucrose meatabolic pathway did not have clear seasonal patterns, or their seasonal differences were biologically insignificant. Future studies involving annual sucrose metabolism and growth in longleaf pine roots will provide important information in determining the optimal window for reintroduction of fire into longleaf pine stands.

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