UNDERSTANDING SUCROSE METABOLISM AND GROWTH IN A DEVELOPING SWEETGUM PLANTATION

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ABSTRACT

Stem diameter growth of 9-year-old sweetgum (Liquidambar styraciflua) trees was measured and related with the activity of sucrose synthase (SS), an enzyme that has been associated with carbon sink strength in agriculture crops and tree seedlings. In 1984, 1-0 sweetgum seedlings were transplanted to control plots and plots amended with sewage sludge or nitrogen and phosphorus fertilizer. After 8 growing seasons, trees on sludge contained four times as much volume as controls and twice as much volume as those on fertilized plots. In 1991, sludge treated soil had about 2 to 5 times as much in N and P as soils from other treatments. There were no differences in the K levels between treatments.

Beginning in 1992, sucrose metabolizing enzymes were assayed monthly in both phloem- and xylem-side stem cambial tissues. In late April when stem bark became easy to peel, there was SS activity in phloem-side but not in xylem-side stem cambial tissues. Phloem SS activity remained constant throughout the season. In less than 2 weeks, SS in xylem cambium exceeded that in phloem cambium by 20 folds and reached highest levels in June through August. For most of the growing season, there were no differences among treatments in the patterns for SS activity in xylem-side stem cambial tissues. However, SS activity in cambial tissues of trees from the sludge sites remained high in late September and October, when cambium of trees from the other treatments was dry and inactive and no SS activity was measured. Seasonal treands in activity of pyrophosphate-dependent phosphofructokinase (PPi-PFK) were similar to those of SS, but activity of its alternative enzyme, ATP-PFK, was low and constant throughout the growing season. We concluded that: (1) application of sewage sludge enhanced tree growth for at least 9 years, (2) sucrose metabolism is similar in 9-year-old sweetgum trees and in seedlings, and (3) sludge application increases the annual duration of high SS and PPi-PFK activities.

INTRODUCTION

All plant cells except green ones that are photoautotrophic, live off sucrose which is the major translocated carbohydrate in plants (Zimmerman and Brown 1971). Since sucrose is the starting point of glycolysis and the termination of gluconeogenesis, sucrose metabolism (sucrolysis) is essential for plants to grow, adapt to environmental changes, and survive stresses (Sung et al. 1988). Of three alternative enzymes catalyzing sucrose breakdown, sucrose synthase (SS) is the dominant activity in organs that are actively growing and storing reserves (Sung et al. 1989a; Xu et al. 1989). Acid invertase (AI) is closely associated with elongating tissues (Xu et al. 1989) whereas the role of neutral invertase (NI) is yet to be clarified. In nursery-grown sweetgum seedling taproots, SS activity decreased to minimum after leaf abscission in fall and did not rise again until June of the next year when leaves were fully expanded (Sung et al. 1989b). The periodicity of

growth in loblolly pine seedling stem and root was supported biochemically by high SS activity in stem cambium during summer and fall and in root cambium during fall and winter (Sung et al. 1993). Furthermore, SS activity declined during brief periods of low temperature and drought, when growth was suspended. Although the temportal (seasonal) and spatial (stem vs. root) patterns of sucrolysis in transplanted loblolly pine seedlings were not different from those of the nonlifted controls, SS activity of transplanted seedlings was much less than the controls (Sung et al. 1993). In fact, the duration and extent of transplanting stress was quantified biochemically via SS activity.

It has been suggested that there are two sets of enzymes in plant glycolytic pathways, namely the adaptive and the maintenance enzymes (Mustardy et al. 1986). The adaptive enzymes have large (5- to 10-fold) and rapid changes in activity in response to environmental changes and to plant growth and development. The maintenance enzymes usually have either low or very high levels of activity regardless the environments or plant status. Sucrose synthase, AI, and pyrophosphate-dependent phosphofructokinase (PPi-PFK) were identified as the adaptive enzymes in bean seeds (Xu et al. 1989), potato tubers (Sung et al. 1989a) and sweetgum and loblolly pine seedlings (Sung et al. 1989b; Sung et al. 1993). In the same plant tissues, NI and ATP-PFK were the maintenance enzymes that did not change their activities more than two folds. All these studies, however, were with an agriculture crop or 1- to 2-year-old seedlings. We report here a biochemical assessment of growth and stress in large plantation trees.

Recently, an 8-year-old sweetgum plantation established to determine the growth responses of three fertility treatments became available. Individual plots received sewage sludge, fertilizer, or no amendments. For 8 growing seasons, these initial fertility treatments have strongly influenced tree growth and vigor. The long duration of these growth differences suggested basic differences in sucrose metabolism enzyme activities among treatments. We therefore tested the following hypotheses: (1) developing plantation trees and seedlings have similar sucrose metabolism enzymes, (2) there are adaptive and maintenance enzymes in the glycolytic pathway in plantation trees, and (3) sucrose metabolism is more active or lasts longer in fast-growing, sludge-treated trees than in slower-growing trees.

MATERIALS AND METHODS

Site History

The original study evaluated the effects of subsoiling, sewage sludge, and vesicular-arbuscular mycorrhizae (VAM) on sweetgum growth on high-quality soil located at the Savannah River Site, Aiken, SC. The experimental design was a split-split plot with four replicate blocks. Treatment variables were fertility, subsoiling, and VAM. In July 1983, sewage sludge from Athens, GA, was applied to one-third of each block at 34 dry metric tons/ha and then all plots were double disked. Another one-third of each block received fertilization treatment consisted of 280 kg/ha diammonium phosphate in 1985 and 240 kg/ha ammonium nitrate in 1986. The remaining one-third of each block received no amendments and served as the control. In September 1983, half of each block was subsoiled with furrows in parallel lines 122 cm apart and 76 cm deep. Sweetgum seedlings were produced at Whitehall Experimental Nursery in

Athens, GA. VAM seedlings had been inoculated with Glomus fasciculatum or G. macrocarpus. Both inoculated and noninoculated seedlings were grown in fumigated nursery beds with 75 to 100 ppm P. Sixteen seedlings with 6 or more first-order lateral roots were planted at a 3 x 3 m spacing in each plot in February 1984.

Tissue sampling and preparation

For the purpose of current study, there were three soil fertility treatments: sludge, fertilizer, and control. Cambium samples were taken monthly in 1992; and the same protocol will be continued in 1993. Two to three strips of 3.0 x 20 cm stem bark were removed from each tree at breast height (1.5 m aboveground). For each treatment, two trees from each of the two blocks were sampled in 1992. In 1993, dendrobands were put on the interior trees in the other two blocks. These trees will be sampled for enzyme activity and stem diameter growth. Phloem cambial tissues were scraped from the inside of the bark and xylem cambial tissues were scraped from the exposed surface of the stem. The scraped tissues were immediately frozen in liquid nitrogen and transported back to the lab. The next day tissues were homogenized for enzyme assays. The enzyme extraction and assay procedures followed Sung et al. (1993). Activities of sucrose synthase (SS), acid invertase (AI), neutral invertase (NI), pyrophosphate-dependent phosphofructokinase (PPi-PFK), and ATP-PFK were routinely assayed from the same extracts.

RESULTS AND DISCUSSION

In 1991, the eighth growing season after planting sweetgum plantation, leaves of trees grown in the sludge plots were retained and were functionally green until the frost late in November. Leaves of tree in the other plots became discolored in mid-September and fell soon after. In addition, all the trees in the sludge plots had bumper seed crop while other trees produced few if any seeds. In 1991 stems were measured. Since no important effects of the VA or subsoiling treatments were observed, data were compiled into three groups based on the initial fertility treatments - sludge, fertilizer, and control. Trees grown on sludge contained 4 times the volume of control trees and twice the volume of fertilizer trees. Trees on sludge were taller and larger in diameter at breast height (DBH) than the others. Trees grown on the fertilizer plots were taller and larger in DBH than control trees. It was reported that 5 years after sewage sludge treatment applied at pole-stage, sawlog volume increased 50% in Corsican pine (Pinus nigra var. maritima (Ait.) Melville) (Moffat et al. 1991).

In addition to measuring tree growth in 1991, we collected soil samples from each plot at two depths, 0 to 7.5 and 7.5 to 15 cm for soil nutrient analyses. The average concentrations of major element levels for the three fertility treatments were:

Treatment and depth	Concentration (ppm) N	P	K
Control			
0-7.5	370	29	29
7.5-15	267	24	24
Fertilizer			
0-7.5	389	36	25
7.5-15	289	28	23
Sludge			
0-7.5	1153	153	24
7.5-15	448	143	24

Eight years after treatment sludge plots contain 2- to 5-times as much total N and available P as the other soils. No differences in soil fertility level were found between soils of control and fertilizer treatments. Generally, there was more N in the top 7.5 cm soil than the next soil layer in all three treatments. In a study by Berry and Marx (1980), 3 years after the sludge treatment, there were 595 and 84 ppm of N and P in the soils whereas the control soils had 112 and 7 ppm on N and P, respectively.

Based on the 1991 soil fertility analysis the growth advantage for trees on sludge plots seems likely to continue for a few more years. In 1992, DBH increased 4.3, 2.1, and 1.1 cm, respectively, for trees in sludge, fertilizer, and control plots. Average increases in DBH among trees sampled for enzyme activity were 1.44, 0.99, and 0.97 cm during 1992. Reasons for the slower growth of sample trees are not clear. Bark strip removal may be the cause. Trees sampled before July produced some callus tissues along the cut edges, whereas no callus formation was noticed after June sampling.

As in sweetgum and loblolly pine seedlings (Sung et al. 1989b; Sung et al. 1993), sucrolysis with sucrose synthase was the dominant sucrose breakdown activity in the 9-year-old plantation sweetgum. No clear seasonal patterns in AI or NI activity were observed (data not shown). SS activity in phloem-side stem cambial tissues ranged from 5 to 35 nmol per mg protein per min throughout the sampling periods. In the first sampling in April 1992, no SS activity was detected in xylem-side cambium. The data suggested that phloem cambium became active earlier than xylem cambium in spring (Figure 1). Within 2 weeks, xylem cambium became active, and its SS activity was 10 to 20 times higher than that in phloem throughout the year. On a fresh weight basis, the xylem-side cambium has 4- to 10-fold as much soluble protein as that of phloem-side cambium (data not shown). Loblolly pine seedling stem phloem-side cambium also had low soluble protein and SS activity (Sung et al. 1993). It is generally acknowledged that for every cell division in phloem mother cell region there is at least 6 or 7 cell divisions taking place in xylem mother cell region.

Xylem cambium became active about 1 week earlier in 1993 than in 1992 (Figure 1). Except toward the end of the growing season, there were no differences in SS activity between treatments. From June to August, SS activity was highest in all trees. SS activity, however, remained high for at least 1 more month in sludge-treated trees than other trees (Figure 1). Therefore, an extended duration of high SS activity rather than high absolute activity gave sludge trees their growth advantage. Since the active cambial tissues were most scrapable, one needs to consider whether there were any differences in SS activity per unit of cambial area. Basically, the trends did not change when SS activity was expressed in nmol/cm^2.min (Figure 2) as in Figure 1. In both Figure 1 and 2, there was a second peak in SS activity of sludge trees in September. The functionally green leaves on trees of sludge plots in September certain can export sucrose for stem cambial SS to metabolize it for growth. The continuous declining SS activity in other trees in fall coincided with leaf discoloring and abscission.

SS activity was also assayed monthly in xylem-side cambial tissues of lateral roots. However, the variations within treatments were too large to draw meaningful conclusions. In fact, within the same piece of lateral root there were differences in the ease of bark peeling. In July and August, root SS activity averaged about 50 nmol/mg protein.min for all treatments. This rate was much lower than that of the seedling taproot which is the major belowground sink for sucrose (Sung et al. 1989b). A plantation tree has many lateral roots which probably are not equally competitive sucrose sinks at the same time.

As in earlier studies with seedlings (Sung et al. 1989a; 1989b; 1993), the PPi-PFK activity in plantation trees was that of an adaptive enzyme; it correlated well with growing season (Figure 3). Furthermore, the similarity betweeen seasonal patterns of PPi-PFK and SS in loblolly pine seedlings (Sung et al. 1993) was observed with sweetgum trees (Figure 2 and 3). ATP-PFK activity, on the contrary, was that of a maintenance enzyme. Like the invertases, ATP-PFK was more or less constant in activity throughout the year (Figure 4).

We concluded that sucrolysis in sweetgum plantation trees is similar to that in nursery seedlings. Throughout the growing season, SS is the dominant sucrose breakdown activity and it correlates well with physiological activity. Trees on sludge-treated soil grew more in diameter and had high SS and PPi-PFK activities for a longer-period than control and fertilized trees. The long-term positive effects of sludge on sweetgum tree growth and seed production are especially important for tree improvement. Monitoring of SS activity increases our understanding of the biochemical basis for the growth advantage.

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FIGURE LEGENDS

Figure 1. Seasonal changes in sucrose synthase specific activity, nmol/mg protein.min, in stem xylem cambial tissues of plantation sweetgum trees. Each data point was the average of four samples. The same plant extract was used for all enzyme assays.

Figure 2. Seasonal changes in sucrose synthase specific activity, $nmol/cm^2.min$, in stem xylem cambial tissues of plantation sweetgum trees. Data from Figure 1 was used to calculate these values.

Figure 3. Seasonal changes in PPi-dependent phosphofructokinase specific activity, $nmol/cm^2.min$, in stem xylem cambial tissues of plantation sweetgum trees. Each data point was the average of four samples.

Figure 4. Seasonal changes in ATP-dependent phosphofructokinase specific activity, $nmol/cm^2.min$, in stem xylem cambial tissues of plantation sweetgum trees. Each data point was the average of four samples.

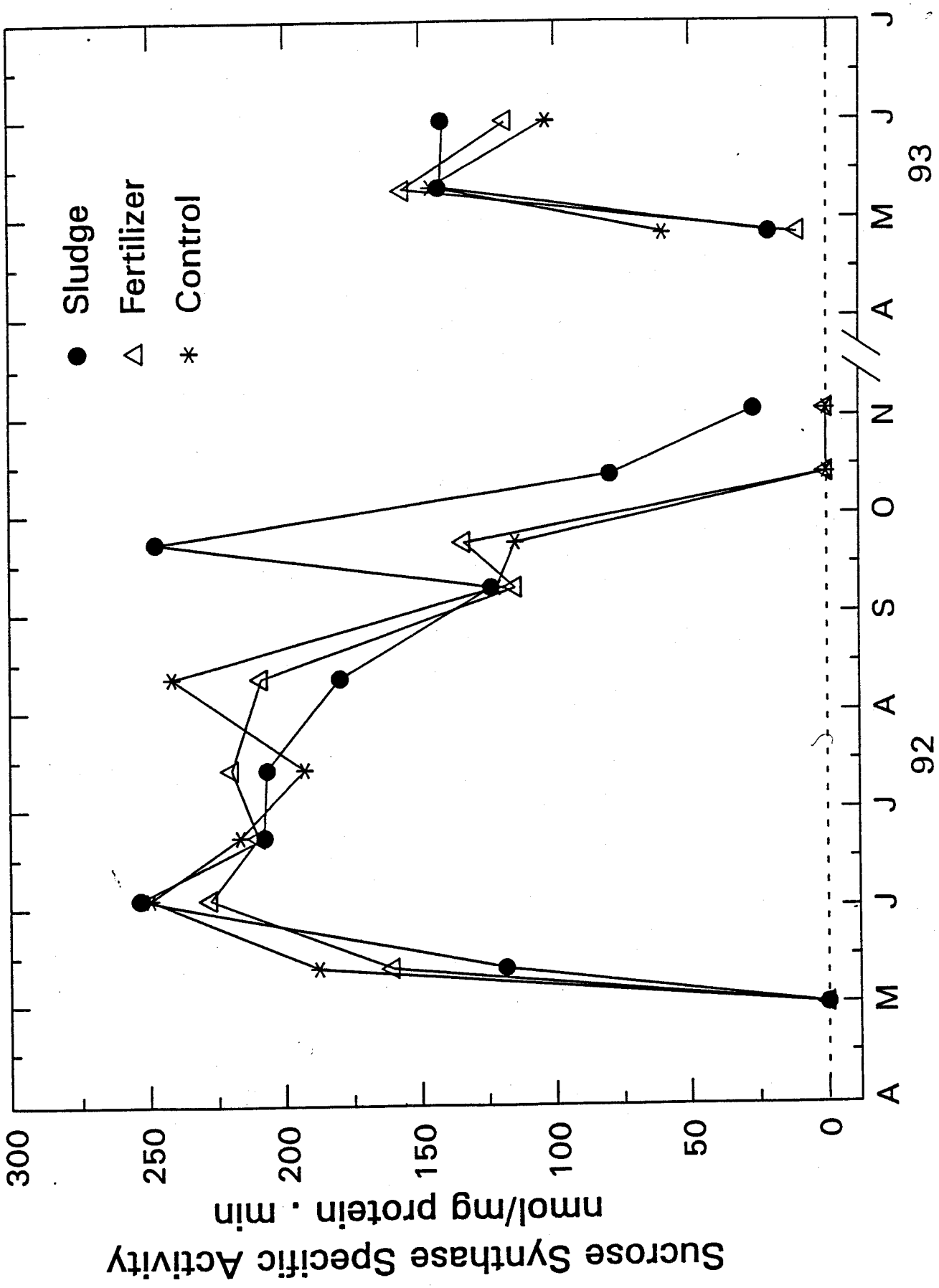
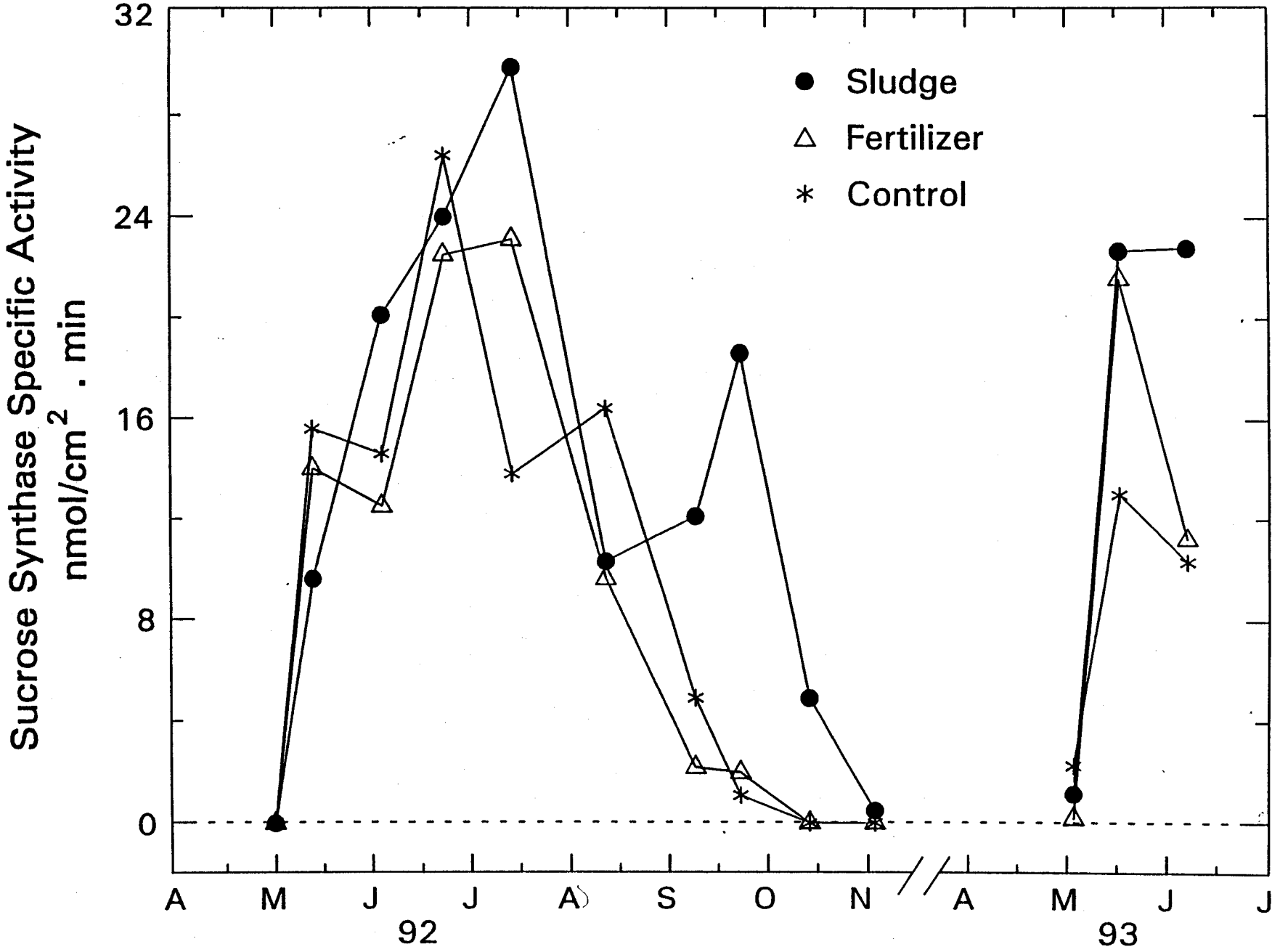

Sucrose Synthase Specific Activity
nmol/mg protein · min
300
250
200
150
100
50
0
Sludge
Fertilizer
Control
A M J J A S O N D J F M A M J J
92
93

Figure 1


Sucrose Synthase Specific Activity
nmol/cm^2 . min
32
24
16
8
0
Sludge
Fertilizer
Control
A
M
J
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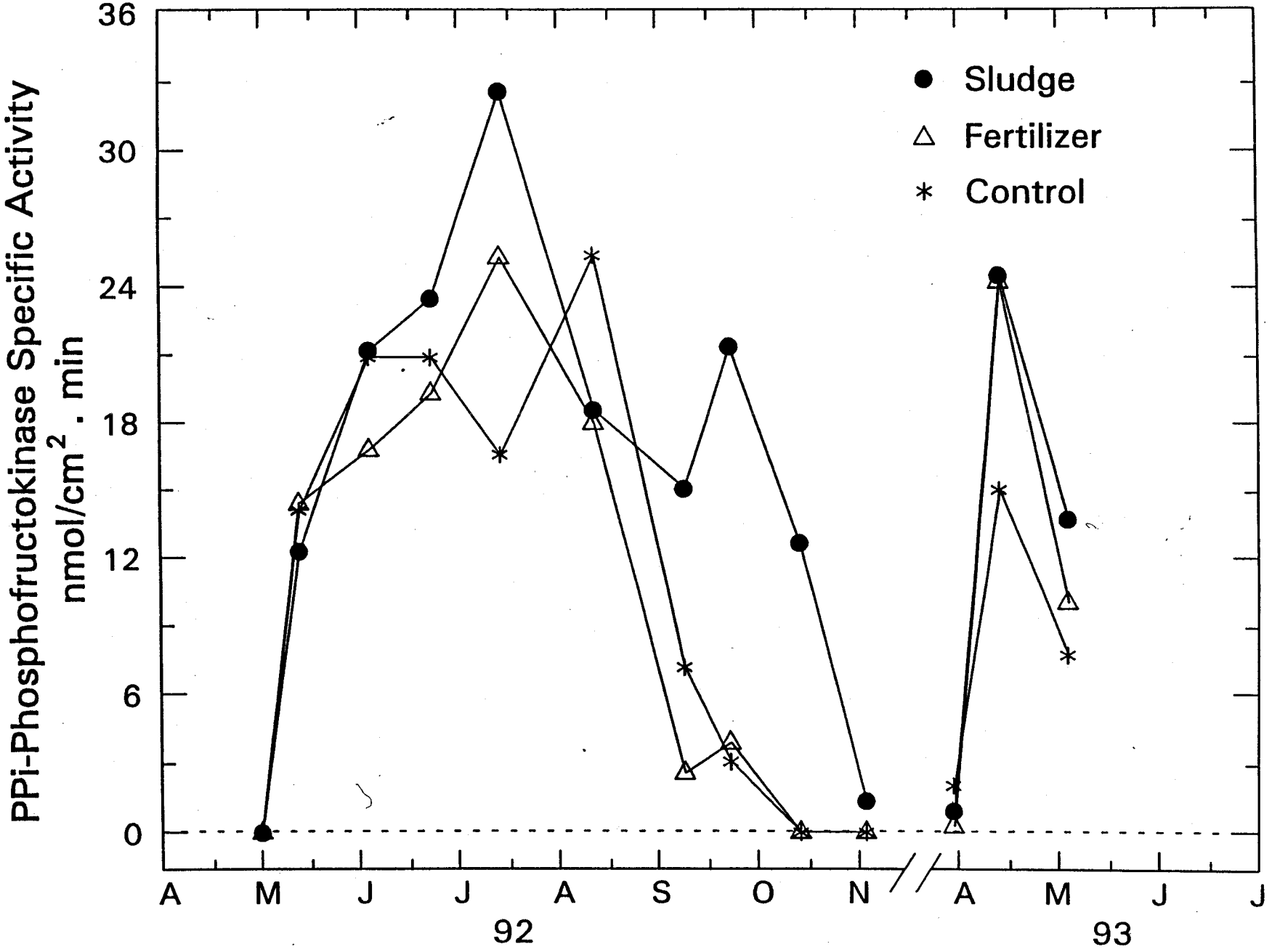
A
M
J
J
92
93

Figure 2


PPi-Phosphofructokinase Specific Activity
nmol/cm^2 . min
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30
24
18
12
6
0
Sludge
Fertilizer
Control
A
M
J
J
A
S
O
N
A
M
J
J
92
93

Figure 3

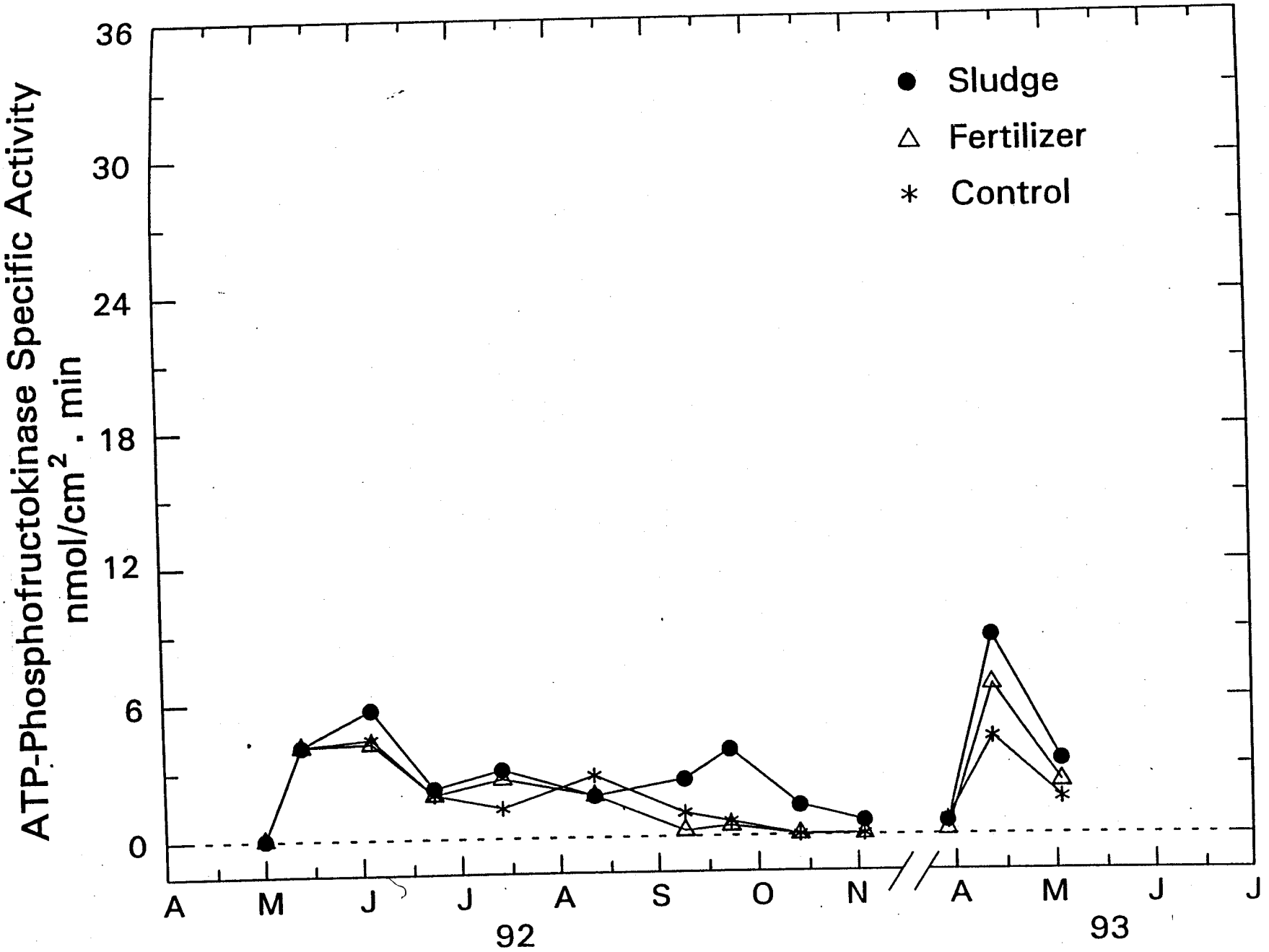
ATP-Phosphofructokinase Specific Activity
nmol/cm^2 . min
36
30
24
18
12
6
0
Sludge
Fertilizer
Control
A
M
J
J
A
S
O
N
A
M
J
J
92
93

Figure 4