Sulfur Deficiency Influences Vegetative Growth, Chlorophyll and Element Concentrations, and Amino Acids of Pecan

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Abstract. Greenhouse-grown pecan (Carya illinoensis (Wangenh.) C. Koch) seedlings were treated with S as SO$_2$ (0 to 4 m$^3$) to determine the effect of S on its vegetative growth, chlorophyll concentration, nutrition, and free and protein amino acid content. Sulfur deficiency symptoms occurred when leaf S was $\approx$1.5 mg·g$^{-1}$ dry weight or less. Chlorophyll concentration and growth increased curvilinearly with leaf S and had not peaked when leaf S was 2.7 mg·g$^{-1}$ dry weight. The N : S ratio in the leaf and leaf S were equally reliable indicators of the S status of the plant, but the optimum ratio was less than the value of 15 found in other plant species. Calcium and Mg decreased with S application, suggesting a direct SO$_2$ or NH$_3$ effect. The concentration of all other elements determined was elevated, in one or more plant parts, when S deficiency symptoms were visible. The most conspicuous effect of S deficiency on N metabolism was a greatly expanded free amino acid pool, mainly arginine. The concentration of free amino acids decreased exponentially with leaf S. Conversely, protein amino acid was inhibited by S deficiency and increased with leaf S concentration or, perhaps more specifically, with methionine.

In recent years, crop deficiencies of S have become more frequent and extensive due to increased use of high-analysis fertilizers that contain little or no S, increased crop yields, and decreased use of S as a pesticide. In addition, there has been a decreased gain of atmospheric S by soils and plants as a result of environmental regulations limiting SO$_2$ emission. Furthermore, there has been a decline in S reserves in surface soils caused by losses in organic matter through erosion and mineralization (Tabatabai, 1980; Tisdale et al., 1985).

Higher plants generally accumulate S and N in amounts proportional to that incorporated into protein (Friedrich and Schrader, 1978). Normally, $\approx$75% to 80% of the total N in plants is in protein (Bolton et al., 1976; Stewart and Porter, 1969) and $\approx$90% of the total S is in cysteine and methionine, of which nearly all is in protein (Giovanelli, 1987). Because of the close relationship between S and N, metabolism of N will be upset by S deficiency. Such is apparent in pecan, as severely S-deficient pecans accumulate an abnormally high concentration of N (D.S., unpublished data). The form of N accumulated in pecan is unknown, but in other plants, accumulation is nonprotein N in the form of arginine, amide, and nitrate (Bergmann, 1981; DeBoer and Duke, 1982; Dietz, 1989; Klapheck et al., 1982; Rendig et al., 1976). The critical leaf S concentration for pecan growth and fruiting is unknown. A preliminary study (Sparks, 1978) indicated that severe S deficiency symptoms can be expected when leaf S is $\approx$1.0 mg·g$^{-1}$ dry weight or less. The objectives of this study were to determine the relationship between S and vegetative growth, chlorophyll and element concentrations, and N metabolism of pecan.

Materials and Methods

Stratified ‘Curtis’ pecan seeds were planted (one per pot) on 21 Apr. into 1 l-liter plastic pots filled with perlite and placed in the greenhouse, with temperature controls set at 21°C night and 30°C day. Hoagland’s solution (Hoagland and Arnon, 1950) without S was applied on 21, 23, and 25 Apr. and then weekly until 16 May. At that time, the seedlings, which were 6 to 18 cm tall, were divided into 15 groups with relatively uniform-sized seedlings within a group. The seedlings within each group were randomly assigned to either 0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, or 4.0 mM S. The design was a randomized complete block.

Sulfur treatment began on 16 May and was applied as (NH$_4$)$_2$SO$_4$. Whenever S was applied, NH$_4$N$_2$O$_5$ was used to supply the S-deficient plants the correct equivalence of N, so that every treatment received the same amount of N. Initially, S was applied weekly for 3 weeks and then every 2 or 3 weeks. The interval between S applications was increased to induce a wider range of deficiency among the lower S treatments. Timing of application was based on the progression of deficiency symptoms.

On 4 Aug., 15 weeks after the seed had been planted and 10 days following a treatment, leaf samples from nodes 6 and 7 (numbered from the base of the shoot) were collected for analysis of chlorophyll, free and protein amino acids, total-N, NO$_3$-N, and S. Leaf samples were pooled into three replications with five plants per experimental unit. Except for those used for chlorophyll analysis, leaf samples were immediately freeze-dried after collection and ground to pass through a 30-mesh screen. Chlorophyll was extracted and determined in the fresh leaflet according to Arnon (1949).

The seedlings were visually evaluated just before harvest to assess the degree of S deficiency. Four months after planting, on 21 Aug., the plants were harvested. Plants were pooled into five replications with three plants per experimental unit. Leaves, trunk, and roots were separated, washed in deionized water, dried for 72 h at 70°C, and weighed. These plant parts were

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ground, redried, and analyzed for total N using a Technicon (Technicon Instruments Corp., Tarrytown, N.Y.) Auto Aria-
yzer (Isaac and Johnson, 1976); for P, K, Ca, Mg, Mn, B, Cu, and Zn, a Jarrell-Ash (Thermo Jarrell-Ash Corp., Frank-
lin, Mass.) plasma-emission spectrometer (Jones, 1977); and for S, a Leco S analyzer (Leco Corp., St. Joseph, Mich.) (Jones
and Isaac, 1972). As methionine and cysteine comprise up to
90% of the total S in most plants (Giovanelli, 1987) and as our
amino acid analysis showed that methionine is the major S amino
acid in pecan, purified commercial methionine was used as the
standard for S analysis instead of oil (Jones and Isaac, 1972).
Nitrate-N was determined calorimetrically according to Cataldo
et al. (1975).

Free amino acids were extracted from 500 mg of freeze-dried
and ground material suspended in 15 ml of distilled H2O. The
suspension was heated in a boiling water bath for 10 min with
intermittent shaking followed by centrifugation at 12,000 × g.
After decanting the supernatant, the residue was re-extracted as
above two more times, each time using 10 ml H2O. In the first
replication, the residue was extracted only twice. Results from
this replication suggested the need for an additional extraction;
consequently, the second and third replication were extracted
three times. Data from all three replications were used. The
supernatants from each extraction were pooled and used as the
free amino acid source. The residue after the last extraction was
used as the protein amino acid source. Both supernatant and
residue were freeze-dried.

Three ml of 1 N HCl was added to 45 mg of the dried free
amino acid residue and boiled in a water bath for 1 h to hydro-
lyze asparagine and glutamine to aspartic and glutamic acid,
respectively. The amino acid extract was dried under reduced
pressure, resuspended in 3 ml of distilled H2O, and dried again.
The extract was dissolved in 3 ml of sodium citrate buffer (pH
2.2), filtered through 1.4-µm filters, and analyzed for amino
acids.

Protein amino acids in 120 mg of dry residue were hydrolyzed
in 180 ml 6 N HCl heated at 100C for 24 h in a N2 atmosphere.
After filtration, the hydrolysate was dried under reduced pres-
sure, resuspended in 100 ml distilled H2O, and dried again. The
dried hydrolysate was dissolved in 15 ml of the sodium citrate
buffer, filtered, and the protein amino acids determined in a
Beckman model 121 (Beckman Instruments, Fullerton, Calif.)
automated amino acid analyzer using the method of Wilkinson
et al. (1968).

Data were subjected to regression analysis, (Helwig and
Council, 1979; Ezekiel and Fox, 1959). In the cases of nonlin-
ear models, the r values were calculated as 1.0 minus the ratio of
the residual sum of squares to the corrected total sum of
squares (Ware et al., 1982).

**Results and Discussion**

**Sulfur effects on vegetative growth.** In the O-mM S treatment,
deficiency symptoms were observed 5 weeks from planting the
seed. As a general rule, S deficiency symptoms (chlorosis and
necrosis) appeared on the youngest leaves first. The first symp-
tom of mild S deficiency was fading of the interveinal areas
in the regions between the minute veins. This fading continued
until the leaflet was uniformly yellow with only the main veins
remaining green. In some cases, but not all, the areas between
the minute veins regreened. Also in some, the yellowed areas
of the leaf developed a reddish-brown tinge in advanced cases.

Sulfur deficiency symptoms became progressively more se-
vere as a seedling elongated. Newly emerging leaves were golden
yellow and failed to turn green. In addition, the tips of the new
leaflets tended to curve downward, while, at the same time, the
margins of the leaflets tended to curve upwards. With further
seedling elongation, emerging leaves were progressively smaller
and the internodes became shorter until elongation ceased and
the apical meristem died. The veins of the smaller leaves be-
came necrotic, which started as a rusty discontinuous browning
but later became continuous. In severe cases, the midrib died
from the apical end, followed by death of the leaf blade.

Deficiency symptoms were severe in the O-mM S treatment,
and the apical portion of most of the leaflets had died before
harvest. With 0.2 mM S, leaflets were golden yellow and cupped,
and those with 0.4 mM S developed mild S deficiency symptoms
(pale-yellow to light-green leaflets). With 0.8 mM or more S,
the plants appeared normal (Table 1). Plants with 4.0 mM S,
double the S concentration in Hoagland’s solution, did not show
any toxicity. Very severe S deficiency symptoms occurred when
leaf S was >0.5 mg·g-1 or less, severe symptoms when leaf S
was >1.1 mg·g-1, and mild symptoms when S was >1.5 mg·g-1.

The leaf S value for severe deficiency is close to that previously
reported for severe S deficiency in pecan (Sparks, 1978).

Even though visible deficiency symptoms did not occur when
leaf S was >1.5 mg·g-1, growth continued to increase at higher

**Table 1. Effect of S on growth characteristics of seedling pecans
grown in perlite in the greenhouse.**

| S applied (mM) | S deficiency | Dry wt (g) | Root | Total | Leaf | Trunk |
|---------------|--------------|------------|------|-------|------|-------|
| 0.0           | Very severe  | 3.79       | 6.18 | 1.29  | 1.10 |
| 0.2           | Severe       | 13.49      | 20.56| 4.42  | 2.65 |
| 0.4           | Mild         | 18.07      | 28.33| 6.95  | 3.51 |
| 0.8           | None         | 19.74      | 31.10| 7.31  | 3.65 |
| 1.2           | None         | 22.13      | 35.81| 9.54  | 4.14 |
| 1.6           | None         | 24.83      | 40.01| 10.43| 4.75 |
| 2.0           | None         | 22.29      | 37.69| 11.02| 4.38 |
| 4.0           | None         | 28.39      | 46.63| 12.85| 5.39 |

*After 4 months of growth.

Root dry weight and total dry weight responded curvilinearly to
applied S; leaf and trunk dry weights were linear responses. P <0.05.

**Fig. 1.** Leaf chlorophyll and growth of pecan seedlings as a function
of leaf S. The relationship of chlorophyll, total dry weight, plant
height, and leaf number to leaf S is described by

\[ Y = 3.4128 \left(1 - 1.236e^{-0.8576S}\right), \]

\[ r^2 = 0.92; Y = 59.81 \left(1 - 1.145e^{-0.6999S}\right), \]

\[ r^2 = 0.86; Y = 50.68 \left(1 - 0.7382e^{-0.9397S}\right), \]

\[ r^2 = 0.78; Y = 5 + 14.76 \left(1 - 1.849e^{-1.997S}\right), \]

\[ r^2 = 0.85, \text{respectively. (P } < 0.01) \]
S levels. Leaf chlorophyll increased curvilinearly with leaf S and had not peaked when leaf S was >2.7 mg·g\(^{-1}\) (Fig. 1). Root dry weight increased curvilinearly and leaf and trunk dry weights increased linearly with leaf S (Table 1). The relationship of total dry weight to leaf S was also curvilinear (Fig. 1). Although the number of leaves per plant were near maximum when leaf S was >2.7 mg·g\(^{-1}\), dry weight accumulation had not peaked. Increased growth was associated with higher leaf dry weight (Table 1) and presumably improved leaf efficiency from the increase in chlorophyll (Fig. 1). As indices of growth continued to increase beyond the range of visible deficiency symptoms, we conclude that there is a relatively wide range of hidden S deficiency in pecan.

The N : S ratio has been proposed to better express the S status in the plants than total S alone (DeBoer and Duke, 1982; Dijkshoorn and van Wijk, 1967; Stewart and Porter, 1969). The \(r^2\) values of growth vs. leaf S (Fig. 1) and growth vs. the N : S (Fig. 2) are very similar. Although the N : S ratio does not express S status any better than S alone in pecan, the ratio can be used as an alternate method of expressing the S status in pecan, as in other plants (DeBoer and Duke, 1982; Dijkshoorn and van Wijk, 1967; Stewart and Porter, 1969). However, the ratio, >10, associated with the highest dry weight accumulation in pecan differs from the ratio of 15 found in lucerne (Medicago sativa L.) (DeBoer and Duke, 1982), wheat (Triticum aestivum), and bean (Phaseolus vulgaris) (Stewart and Porter, 1969). The critical N : S ratio for herbaceous plants seems not directly transferable to pecan.

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**Fig. 2.** Leaf chlorophyll and growth of seedling pecans as a function of the N : S ratio in the leaf. The relationship of chlorophyll, total dry weight, plant height, and leaf number to the N : S ratio is described by: \(1/Y = 3.2014 \times (1 - 1.0783 \times e^{-0.0664X}), r^2 = 0.88; 1/Y = 65.53 \times (1 - 1.0125 \times e^{-1.1164X}), r^2 = 0.86; 1/Y = 55.96 \times (1 - 0.5879 \times e^{-1.599X}), r^2 = 0.79; 1/Y = 19.9 \times (1 - 0.8683 \times e^{-0.798X}), r^2 = 0.83\), respectively. (\(P \leq 0.01\))
Sulfur effects on concentration elements. Nitrogen, P, and K concentrations were highest in the leaf, intermediate in the root, and lowest in the trunk, with the corresponding order for Ca, Mg, S, Mn, Cu, and B being leaf, trunk, and root (Figs. 3–6). The same order for the elements among the three organs was found in a P study with pecan (Sparks, 1988).

According to Mitscherlich's plant growth equation, increasing the amount of S applied increased the level of S in leaves, trunk, and roots (Fig. 3) but approached composition maximums before maximum dry-weight accumulation (Fig. 1 vs. 3). Sulfur application affected the concentration of all other elements in one or more plant parts (Figs. 3–6). Elements not affected in all plant parts were Fe in the leaf (Fig. 6), B and Fe in the trunk (Figs. 5 and 6), and Mg and B in the root (Figs. 4 and 5). With the exception of S (Fig. 3) and K in the trunk (Fig. 4), S application decreased the concentration of elements (Figs. 4-6) or, alternatively, the element concentrations increased with decreasing S in the nutrient solution.

The decrease in element concentration with applied S followed one of four basic equations: \( Y = a + bX; \ Y = a + b \ t/X; \ Y = a + b \ X + c \ t/X; \) or \( 1/Y = a(1 - bX)^{-1}; \) which is the inverse of Mitscherlich's growth curve. The linear equations and \( Y = a + bX + c \ t/X \) may indicate competitive absorption and, on this basis, suggest a direct \( SO_2^2 \) or \( NH_3 \) effect or both on Ca and Mg (Fig. 4). The equation \( Y = a + b \ t/X \) suggests element imbalances associated with severe S deficiency; such imbalances occurred only with K in the leaf (Fig. 4) and Cu in all plant parts (Fig. 5). The decrease in all other elements, except K in the trunk (Fig. 4), followed the inverse of Mitscherlich's plant growth equation (Figs. 4-6). Most of the decrease in element concentration occurred between 0 and 0.8 mM S (Figs. 4-6), which corresponds to the range of visible S deficiency symptoms (Table 1). This correlation between S deficiency and changes in element concentrations suggests element imbalances induced by S deficiency or growth-limiting levels of S as such. We attribute the decrease in the element concentrations to dilution from increasing dry matter with increased S. This hypothesis is supported by the inverse relationship of element concentration to dry-weight accumula-

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**Fig. 5.** Zinc, B, Mn, and Cu in organs of pecan seedling as a function of S in the nutrient solution. Zinc in leaf, trunk, and root is described by \( 1/Y = 0.06024 (1 - 0.5785e^{-1.4212X}), r^2 = 0.57; \) \( 1/Y = 0.03754 (1 - 0.6138e^{-0.4725X}), r^2 = 0.71; \) \( 1/Y = 0.0096 (1 - 0.78e^{-3.056X}), r^2 = 0.53, \) respectively. Boron in the leaf can be described by \( 1/Y = 0.03942 (1 - 0.6256e^{-1.3358X}), r^2 = 0.97; \) Manganese in the leaf, trunk, and root is described by \( 1/Y = 0.003231 (1 - 0.5229e^{-3.064X}), r^2 = 0.89; \) \( 1/Y = 0.01031 (1 - 0.7675e^{-0.4658X}), r^2 = 0.85; \) \( 1/Y = 0.02472 (1 - 0.8049e^{-3.1879X}), r^2 = 0.97, \) respectively. Copper in the leaf, trunk, and root is described by \( Y = 2.3131 + 0.0001691 \) \( 1/X, r^2 = 0.26, \) \( Y = 2.2998 + 0.0001747 \) \( 1/X, r^2 = 0.32, \) \( Y = 1.9504 + 0.0003129 \) \( 1/X, r^2 = 0.67, \) respectively. (\( P \leq 0.05 \))

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**Fig. 6.** Iron and total N in organs of pecan seedling as a function of S in the nutrient solution. Iron in root is described by \( 1/Y = 0.0209 (1 - 0.4369e^{-3.606X}), r^2 = 0.57; \) Nitrogen in leaf, trunk, and root is described by \( 1/Y = 0.0336 (1 - 0.3113e^{-3.4203X}), r^2 = 0.79; \) \( 1/Y = 0.0885 (1 - 0.6634e^{-1.16X}), r^2 = 0.92; \) \( 1/Y = 0.07412 (1 - 0.5129e^{-3.1708X}), r^2 = 0.89, \) respectively. (\( P \leq 0.05 \))
tion. These results indicate that, except for Ca and Mg, the effect of applied S in perlite on other elements is an indirect effect associated with S deficiency. The increase in N, P, and B concentration with S deficiency has also been reported for herbaceous plants (Adams and Sheard, 1966; DeBoer and Duke, 1982; Dietz, 1989; Klapheck et al., 1982; Smith et al., 1985; Stewart and Porter, 1969).

**Sulfur effects on free and protein amino acids.** Except for cystine/2 and glycine, all measurable free amino acids accumulated in severely S-deficient pecans (O mM S treatment) (Table 2). Arginine was the predominant form of the accumulated free amino acids, regardless of treatment, and with O mM S, arginine accounted for >93% of the total free amino acids. As S in the nutrient solution increased, free arginine dramatically decreased. Similarly, other measurable free amino acids, with the exception of glycine, decreased with S application. Glycine increased slightly as applied S increased from O to 0.8 mM and decreased at higher levels of S. The S-containing half cystine, which occurred in small amounts, did not correlate well with S application, nor did the other S-containing amino acid, methionine. Like methionine, proline, valine, isoleucine, leucine, tyrosine, and phenylalanine all occurred in trace amounts regardless of S treatment. Total free amino acids in the leaf decreased exponentially with applied S.

Total protein amino acids in the leaf increased with applied S (Table 3), indicating that S deficiency greatly inhibits protein accumulation. Except for half cystine and arginine, all protein amino acids increased with S application. Cystine/2 occurred only in low or trace amounts. The lack of an increase in protein arginine may have been due to incomplete extraction of free arginine, especially in the low S treatments. The protein amino acids in severely S-deficient plants correlated closely with those in normal plants (Fig. 7), indicating that the amount of protein, but not the composition, is altered by S deficiency.

Protein amino acid concentrations increased and those of free amino acids decreased with leaf S and dry weight (Fig. 8). The relationship of protein amino acids and free amino acids to dry weight paralleled the relationship to leaf S. The parallelism is due to the close relationship between growth and leaf S (Fig. 1). When free amino acids were near minimum, proteins were approaching a maximum (Fig. 8). These data indicate the interrelationships of amino acids, protein, S deficiency, and growth. Because free arginine was the main component of the free amino acid pool (Table 2), the relationship of free arginine to S or to

### Table 2. Effect of applied S on free amino acids in the pecan leaf (µmoles amino acid/gram dry leaf).²

| Amino acid | Sulfur in nutrient solution (mM) | 0 | 0.2 | 0.4 | 0.8 | 1.2 | 1.6 | 2.0 | 4.0 |
|------------|---------------------------------|---|-----|-----|-----|-----|-----|-----|-----|
| Lysine     |                                 | 12.8 | 6.66 | 3.51 | 1.58 | 1.03 | 0.79 | 0.75 | 0.66 |
| Histidine  |                                 | 1.31 | 1.14 | 0.54 | 0.19 | 0.24 | 0.17 | 0.14 | 0.12 |
| Arginine   |                                 | 547  | 202 | 93.5 | 27.1 | 17.9 | 11.1 | 10.4 | 5.45 |
| Cystine/2  |                                 | 0.90 | 0.77 | 0.52 | 0.65 | 0.83 | 0.62 | 0.42 | 0.17 |
| ASX        |                                 | 8.41 | 6.57 | 5.83 | 4.58 | 4.47 | 4.25 | 4.24 | 3.88 |
| Threonine  |                                 | 1.29 | 0.95 | 0.67 | 0.63 | 0.50 | 0.52 | 0.55 | 0.46 |
| Serine     |                                 | 3.84 | 3.24 | 2.20 | 1.47 | 1.23 | 1.14 | 1.12 | 0.95 |
| GLX        |                                 | 7.51 | 8.34 | 6.91 | 4.81 | 4.39 | 4.34 | 4.71 | 4.19 |
| Proline    | T T T T T T T T T T T T T T     |
| Glycine    |                                 | 0.85 | 1.00 | 1.04 | 1.38 | 1.28 | 1.15 | 1.12 | 1.02 |
| Alanine    |                                 | 2.46 | 2.12 | 1.67 | 1.50 | 1.52 | 1.41 | 1.40 | 1.44 |
| Valine     | T T T T T T T T T T T T T T     |
| Methionine |                                 | T T T T T T T T T T T T T T     |
| Isoleucine |                                 | T T T T T T T T T T T T T T     |
| Leucine    |                                 | T T T T T T T T T T T T T T     |
| Tyrosine   |                                 | T T T T T T T T T T T T T T     |
| Phenylalanine |                                 | T T T T T T T T T T T T T T     |
| Total      |                                 | 586.2 | 232.3 | 116.4 | 43.9 | 33.4 | 25.5 | 24.8 | 19.4 |

²The effect of applied S on alanine, threonine, ASX (asparagine and aspartic acid), serine, and GLX (glutamine and glutamic acid) followed the general equation, Y = a/(1 - be⁻α); arginine, lysine, and histidine followed Y = ae⁻α; and glycine followed Y = a + bX + cX². All relationships are statistically significant, P ≤ 0.05.

³T = trace amount.

### Table 3. Effect of applied S on protein amino acids in the pecan leaf (µmoles amino acid/gram dry leaf).²

| Amino acid | Sulfur in nutrient solution (mM) | 0 | 0.2 | 0.4 | 0.8 | 1.2 | 1.6 | 2.0 | 4.0 |
|------------|---------------------------------|---|-----|-----|-----|-----|-----|-----|-----|
| Lysine     |                                 | 32.9 | 53.7 | 64.3 | 72.8 | 78.8 | 78.2 | 81.4 | 82.6 |
| Histidine  |                                 | 9.1 | 17.7 | 21.9 | 25.0 | 27.2 | 26.6 | 28.2 | 29.4 |
| Arginine   |                                 | 75.9 | 59.5 | 57.2 | 57.6 | 64.9 | 61.0 | 63.6 | 63.4 |
| Cystine/2  |                                 | T T | T T | T T | T T | T T | T T | T T | T T |
| ASX        |                                 | 44.1 | 78.6 | 96.8 | 109 | 123 | 119 | 123 | 123 |
| Threonine  |                                 | 23.8 | 43.2 | 51.2 | 61.3 | 68.7 | 68.1 | 70.3 | 69.6 |
| Serine     |                                 | 33.6 | 50.5 | 60.7 | 69.2 | 76.8 | 76.0 | 78.4 | 78.9 |
| GLX        |                                 | 45.3 | 83.4 | 101 | 114 | 128 | 126 | 133 | 138 |
| Proline    |                                 | 29.3 | 50.6 | 60.8 | 67.9 | 75.4 | 74.5 | 78.1 | 77.8 |
| Glycine    |                                 | 44.0 | 81.5 | 101 | 115 | 127 | 126 | 129 | 129 |
| Alanine    |                                 | 39.7 | 73.6 | 91.4 | 104 | 115 | 118 | 124 | 117 |
| Valine     |                                 | 32.6 | 56.8 | 60.0 | 76.9 | 87.2 | 84.4 | 88.6 | 88.8 |
| Methionine |                                 | 7.2 | 15.4 | 16.7 | 20.3 | 22.7 | 21.5 | 23.7 | 21.8 |
| Isoleucine |                                 | 23.2 | 42.8 | 51.9 | 59.1 | 64.8 | 63.0 | 66.0 | 65.4 |
| Leucine    |                                 | 44.5 | 81.3 | 101 | 114 | 125 | 122 | 127 | 126 |
| Tyrosine   |                                 | 11.6 | 24.7 | 31.6 | 37.0 | 41.0 | 45.5 | 42.0 | 41.3 |
| Phenylalanine |                                 | 21.1 | 40.6 | 50.2 | 58.4 | 63.7 | 63.8 | 64.8 | 64.3 |
| Total      |                                 | 518 | 854 | 1027 | 1165 | 1289 | 1272 | 1322 | 1312 |

²The effect of applied S on all protein amino acids followed the general equation, Y = a(1 - be⁻α). The relationship was statistically significant, except in the case of arginine and half cystine, P ≤ 0.01.

³T = trace amount.
Fig. 7. Relationship of protein amino acids in normal (4 mM S in the nutrient solution) vs. S-deficient (0 mM S in the nutrient solution) pecan seedlings. The relationship is described by $Y = 3.889 + 2.688X$, $r^2 = 0.94$ ($P \leq 0.01$). Arginine is not included in relationship, see text.

Fig. 8. Relationship of protein amino acids and total free amino acids to total dry weight and leaf S. The relationship between total free amino acids and total dry weight, total free amino acids and leaf S are described by $Y = 975.5 \ e^{-0.841X}$, $r' = 0.95$; $Y = 1420\ e^{-0.875X}$, $r' = 0.97$, respectively the relationship between protein amino acids and total dry weight, protein amino acids and leaf S are described by $Y = 1664 \ (1 - 0.8413e^{-0.841X})$, $r' = 0.89$; $Y = 1522 \ (1 - 0.9707e^{-0.875X})$, $r' = 0.96$, respectively. ($P \leq 0.01$)

dry weight was similar to the responses of total free amino acids (Fig. 8) and for this reason is not presented.

The relationship between S application and N partitioning is summarized in Fig. 9. Plants in the O-mM treatment accumulated the highest concentration of total N. As S in the nutrient solution increased, total N in the leaf rapidly decreased and leveled off when S was applied between 0.8 to 4.0 mM At O mM S,

free amino acid-N represented 65% of the total N, thereby accounting for higher than normal total N associated with severe S deficiency in pecan. As S in the nutrient solution increased, the free amino acid-N dramatically decreased to $\approx 2\%$ of the total N. However, the reverse is true for protein amino acid-N, which constituted only $\approx 24\%$ of the total N in the O mM S treatment. As S in the medium increased, protein amino acid-N increased to $\approx 80\%$ of the total N in the 2- and 4-mM S treatments. Although the concentrations of NO$_3$-N in the O- and 0.2-mM treatments differed significantly from all other treatments, this N form was not highly correlated with S application, and the proportion of NO$_3$-N to total N is essentially the same among the treatments. This relationship suggests that NO$_3$-reduction is not significantly inhibited by S deficiency in pecan.

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This study clearly shows that free amino acid and protein accumulations are greatly altered by S deficiency in pecan, as in many other plant species (Bolton, 1976; Dietz, 1989; Eppendorfer, 1968; Klapcheck et al., 1982; Macnicol, 1983; Rendig et al., 1976; Stewart and Porter, 1969). The tremendously expanded free amino acid pool, mainly arginine, is the most conspicuous effect of S deficiency on N metabolism in pecan. Because the amino acid composition of the proteins did not change appreciably among treatments (Fig. 7), the accumulation of free amino acids under S deficiency may have been from that produced by de novo synthesis (Bergmann, 1981; Klapcheck et al., 1982); Free arginine accumulation under S deficiency has also been reported in other plants (Bergmann, 1981; DeBorer and Duke, 1982; Dietz, 1989; Klapcheck et al. 1982; Macnicol, 1983), and S deficiency may stimulate its synthesis (Klapcheck et al., 1982). In our study, the N sources were NH\(^+\), and NO\(^-\). Arginine accumulation was apparently not due to NH\(^+\), as such, because free arginine also accumulated when NO\(^-\), was the sole N source (Dietz, 1989).

Although asparagine and glutamine may be major N forms in transport of N in herbaceous plants, arginine appears to be the most important N storage form in pecan, as in other fruit trees (Bidwell and Durzan, 1975). This situation is suggested because plants receiving the highest S treatment, which had the lowest free amino acid pool, still had arginine constituting the highest percentage (≈28%) of the total free amino acids. Similarly, arginine is high in pecan kernel, a storage organ (Elmore and Polles, 1980; Meredith, 1974). Arginine is especially adapted to N storage because the proportion of N to C, 4:6, is higher than in other amino acids.

The lack of a correlation between the S-containing cystine and protein in pecan leaf and the linear relationship between protein and the S-containing methionine (Fig. 10) suggest that the reason for the reduced protein level under S deficiency may be the limited pool of free methionine for protein synthesis. However, Macnicol (1983) reported that in developing pea (Pisum sativum L.) seed, although free cystine level was greatly reduced under S deficiency, the relative levels of aminoaoyl pool of cystine or methionine were not affected. A further study is needed to determine if the immediate cause for the lower protein level is due to the unavailable methionine or a lower total aminoaoyl pool (Macnicol, 1983) or mRNA (Chandler et al., 1984) for protein synthesis.

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