

Zinc Deficiency Inhibits Chlorophyll Synthesis and Gas Exchange in 'Stuart' Pecan

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Abstract. Leaves of 'Stuart' pecan [*Carya illinoensis* (Wangenh.) C. Koch] with various levels of Zn deficiency were analyzed for physiological indicators of leaf vigor. Leaf chlorophyll content, stomatal conductance, and net photosynthesis were adversely affected by Zn deficiency. In leaves with severe Zn deficiency, each of these indicators increased 3- to 5-fold with a doubling of leaf Zn concentration, but stabilized as leaf Zn approached the sufficiency range (14 μg·g⁻¹). High intercellular CO₂ associated with low net photosynthesis indicates that stomatal aperture was not the cause of the reduction of net photosynthesis under Zn deficiency.

Inadequate Zn levels severely limit crop productivity in pecan (Brooks, 1964; Worley et al., 1972). Zinc deficiency remains a recurrent problem in pecan orchards in spite of extensive research documenting critical leaf values (Amling, 1965; Sparks and Payne, 1982; Worley et al., 1972) as well as methods of prevention and correction (Payne and Sparks, 1932; Sparks, 1976; Worley et al., 1981) for proper growth of the pecan tree. The morphological abnormalities that occur in both the vegetative and reproductive growth of Zn-deficient trees have been described (Hu and Sparks, 1990; Rand, 1922). However, the impact of Zn deficiency on the physiology of the pecan tree has not been examined. Photosynthesis would be expected to be affected because chlorosis and distorted leaf growth are prominent symptoms of Zn deficiency. Our objective was to quantify the effects of Zn deficiency on chlorophyll concentration and stomatal conductance of pecan leaves and their relationship to photosynthesis.

The study was conducted in a pecan orchard located on the Southeastern Fruit and Tree Nut Laboratory, Byron, Ga., with 'Stuart' trees ≈60 years old. Normal and Zn-deficient shoots were selected from eight trees when degrees of Zn deficiency could be clearly distinguished (13 May). The shoots were divided into five groups according to rosette and extent of leaf crinkling: 1, rosette; 2, very severe crinkling but no rosette; 3, severe crinkling; 4, moderate crinkling limited to apical leaves only; and 5, no visible symptoms of Zn deficiency.

Net photosynthesis and stomatal conduct-

ante were determined using a LI-COR 6000 portable photosynthetic system with a 4-liter leaf chamber (LI-COR, Lincoln, Neb.). Evaluations were made in mid-August using the apical leaflet of the middle leaf on shoots exposed to the sun. Measurements were made between 9:00 AM and 12:00 noon at a photosynthetic photon flux density of 1860 (±180) μmol·s⁻¹·m⁻², and at ambient atmospheric CO₂ levels (385 ± 2.3 μmol·mol⁻¹). Each leaflet was measured 10 consecutive times on each of the 10 leaves in each category of Zn deficiency. During the period of measurement, leaf temperature increased ≈1.07C, while relative humidity increased ≈1.1%. Leaf area was determined with a LI-COR 3100 area meter. For the two most severe Zn-deficient groups, intercellular CO₂ values, determined with the LI-COR 6000, were higher for some leaves than ambient CO₂ levels even though positive net photosynthetic rates were detected. As a result, intercellular CO₂ values were calculated by the method of von Caemmerer and Farquhar (1981). Except for the two most severe Zn-deficient groups, the calculated intercellular CO₂ values were within a 2.5% difference of those determined with the LI-COR 6000 meter.

Leaflet samples for chlorophyll and Zn analysis were taken on 29 July according to prescribed sampling procedures (Sparks, 1970). Chlorophyll was extracted and determined in the fresh leaflet (Arnon, 1949). Air-dried leaflets were oven-dried for 72 h at 70C, ground, redried, and analyzed for Zn by inductively coupled plasma argon spectrophotometry (Isaac and Johnson, 1983).

Data were subjected to regression analysis (Helwig and Council, 1979). In cases of nonlinear models, the r² 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).

Severity of Zn deficiency was inversely related to Zn concentration in the leaf. Zinc concentration in leaves with rosette, very severe deficiency, severe deficiency, moderate

deficiency, and no visible symptom of Zn deficiency was 3.8, 4.4, 4.9, 6.1, and 14.3 μg·g⁻¹, respectively. The values of 6.1 and 14.3 associated with Zn deficiency and no deficiency, respectively, are close to those reported by Finch (1936) and Finch and Kinison (1933). The leaf Zn concentration of ≈14 μg·g⁻¹ is also the value associated with normal fruit growth (Hu and Sparks, 1990).

Chlorophyll concentration increased with leaf Zn (Fig. 1), corresponding to morphological observations that leaf chlorosis decreases with increasing leaf Zn. The response curve of stomatal conductance to leaf Zn concentration (Fig. 1) was very similar to that for chlorophyll as indicated by the correlation (r = 0.976) between the two factors. As expected from the chlorophyll and stomatal conductance response curves, net photosynthesis (Fig. 2) was also very sensitively regulated by Zn when Zn was within the deficiency range. The response of intercellular CO₂ was the reverse of net photosynthesis and decreased as leaf Zn increased (Fig. 2). The response of net photosynthesis and intercellular CO₂ had not stabilized at Zn rates of 14 μg·g⁻¹, in contrast to chlorophyll and stomatal conductance, which were near maximum at Zn rates of 14 μg·g⁻¹ (Fig. 1).

Net photosynthesis increased and intercellular CO₂ decreased with chlorophyll concentration (Fig. 3). A first order derivative indicated a two-phase increase in net photosynthesis with chlorophyll. Thus, the changes in net photosynthesis were more sensitive to the changes in chlorophyll content at low and high chlorophyll contents than at intermediate levels. This differential response may be associated with the transition from disintegrated chloroplasts in severe Zn deficiency to normal chloroplasts in nondeficient leaves (Rand, 1922). Likewise, a report in kidney bean plant (*Phaseolus vulgaris* L.) with varying chlorophyll contents at different stages of greening, demonstrated a two-phase increase of photochemical reactions

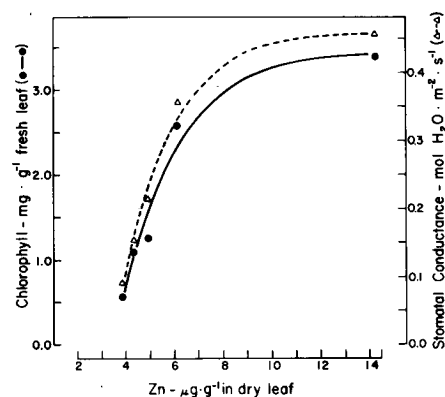


Fig. 1. Chlorophyll concentration and stomatal conductance of 'Stuart' pecan leaves vs. Zn concentration in the leaf. The relationship of chlorophyll vs. Zn and stomatal conductance vs. Zn follows Mitscherlich's plant growth model as follows: $Y = 3.4575 (1 - 4.278 e^{-0.4203x})$, $r^2 = 0.958$; and $Y = 0.4621 (1 - 5.0906e^{-0.4770x})$, $r^2 = 0.931$; respectively, $P \leq 0.01$.

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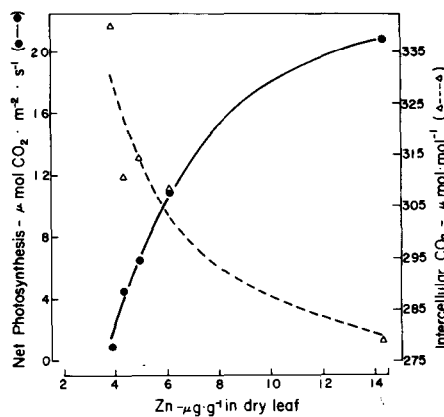


Fig. 2. Relationship of net photosynthesis and intercellular CO_2 in 'Stuart' pecan leaves to Zn in the leaf. The relationship between net photosynthesis and Zn follows Mitscherlich's plant growth model, $Y = 22.1587(1 - 2.5587e^{-0.2637X})$, $r^2 = 0.965$; while the relationship of intercellular CO_2 vs. Zn is described by an equilateral hyperbola curve, $1/Y = 0.00377 - 0.002884(1/X)$, $r^2 = 0.820$. $P \leq 0.01$.

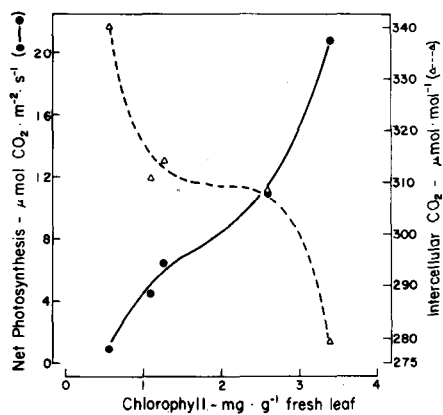


Fig. 3. Net photosynthesis and intercellular CO_2 of 'Stuart' pecan leaves as affected by the leaf chlorophyll content. The relationship of net photosynthesis to chlorophyll and intercellular CO_2 to chlorophyll follows third order equations described by $Y = -7.7693 + 20.2562X - 9.7776X^2 + 1.8533X^3$, $r^2 = 0.980$; and $Y = 397.88 - 140.592X + 72.272X^2 - 12.116X^3$, $r^2 = 0.902$, respectively. $P \leq 0.01$.

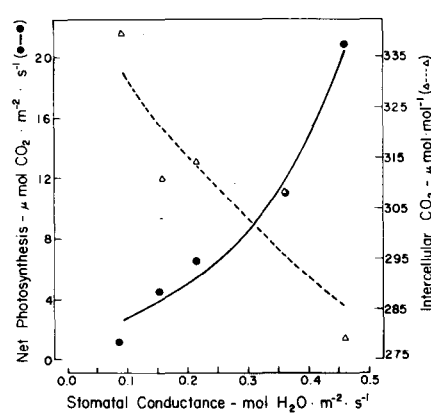


Fig. 4. Relationship of net photosynthesis and intercellular CO_2 to stomatal conductance in 'Stuart' pecan. The relationship of photosynthesis to stomatal conductance is described by $Y = 142.4469/(1 + 90.0871 \times 0.002799X)$, $r^2 = 0.946$; while the relationship of intercellular CO_2 to stomatal conductance follows $\sqrt{Y} = 18.5444 - 3.5988X$. $r^2 = 0.714$. $P \leq 0.01$.

with the increase of chlorophyll (Koizumi et al., 1990). The first phase of the increase in chlorophyll content was mainly associated with reaction centers, and the second phase with the formation of light-harvesting pigments.

Net photosynthesis increased and intercellular CO_2 decreased with increasing stomatal conductance (Fig. 4). Wong et al. (1979) proposed that stomatal aperture was regulated by carbon fixation in mesophyll cells of N- and P-deficient plants. Their hypothesis was based on the fact that intercellular CO_2 remained constant regardless of the severity of these nutrient deficiencies. In our study, intercellular CO_2 was not constant and the relationship between photosynthesis and stomatal conductance was nonlinear (Fig. 4). These results suggest that the capacity of stomatal response to carbon fixation may be partially lost under Zn deficiency. These data also suggest that stomatal aperture is not the mechanism restricting photosynthetic capacity in Zn-deficient pecans. This situation differs from water-stressed plants (Sharkey and Seemann, 1989), where decreased photosynthesis was accounted for by reduced CO_2 availability due to stomatal closure.

Limited photosynthesis in Zn-deficient plants may be due to lesions in metabolic pathways regulating photochemical activity and/or carbon reduction. Zinc deficiency resulted in a partially irreversible loss of chloroplast photochemical activity in safflower (*Carthamus tinctorius* L.) (Pandey and Sharma, 1989) and reduced the Hill reaction in corn (*Zea mays* L.) (Shrotri et al., 1981). In addition, Zn deficiency reduced carbonic anhydrase activity in peach [*Prunus persica* (L.) Batsch.] (Basiouny and Biggs, 1976) and pecan (Stir, 1983). Reductions in light and dark reactions of photosynthesis might be expected because Zn deficiency decreases RNA and protein synthesis (Mazus et al., 1986; Schneider and Price, 1962).

Chlorophyll and stomatal conductance were near maximum when leaf Zn was $14 \mu\text{g}\cdot\text{g}^{-1}$. Photosynthesis was still increasing at values

$<14 \mu\text{g}\cdot\text{g}^{-1}$, but at a diminishing rate. These results indicate that on a shoot basis, optimum leaf Zn is at some value near $14 \mu\text{g}\cdot\text{g}^{-1}$. However, on a whole-tree or orchard basis, pecan will often respond to leaf diagnostic values $>14 \mu\text{g}\cdot\text{g}^{-1}$. This discrepancy occurs, as previously discussed (Hu and Sparks, 1990), because of the extreme variation in Zn deficiency within and among trees and the method of leaf sampling.

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