

## Growth of Honey Locust Seedlings during High Root-zone Temperature and Osmotic Stress

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**Abstract.** Growth of honey locust (*Gleditsia triacanthos* var. *inermis* Willd.) seedlings was studied during exposure to reduced osmotic potential ( $\psi_{\pi}$ ) and high temperature in the root zone. Half-sib plants were cultured in solution. Root-zone temperature was increased from ambient (23C) to 35C for 0, 6, 12, or 24 hours·day<sup>-1</sup>. Within each temperature treatment, solution  $\psi_{\pi}$  of -0.05, -0.10, and -0.20 MPa were maintained by additions of polyethylene glycol (PEG) 8000. Root and shoot dry weights decreased with increasing exposure to 35C among seedlings in -0.05-MPa solution and decreased for seedlings in -0.10- and -0.20-MPa solutions in all temperature regimes. Growth of epicotyls displayed similar trends, but epicotyls of plants in -0.20-MPa solution were longest with 6 hours·day<sup>-1</sup> at 35C. Significant interactions between effects of temperature and osmotic regimes indicated that water-stressed honey locust seedlings are relatively insensitive to elevated root-zone temperatures. However, related studies showed that PEG caused reductions in growth that could not be explained by decreases in  $\psi_{\pi}$  and suggested that responses of honey locust to PEG differed from those when drought was imposed by withholding irrigation in an aggregate medium.

Temperatures exceeding 30C have been documented in the root zones of street trees in the Midwestern United States (Graves and Dana, 1987b). Although data on plant responses to high root-zone temperature are limited, continuous exposure to temperatures of 30C and higher has been shown to restrict the growth of several temperate tree species

(Barney, 1951; Graves et al., 1989a, 1989b; Graves et al., 1991; Gur et al., 1972). In contrast, Graves et al. (1991) found the shoot extension of honey locust seedlings with the root zone constantly at 34C exceeded that of seedlings grown at 24C.

Resistance to elevated root-zone temperatures may contribute to the survival of landscape trees in urban environments. Other environmental factors can restrict the life spans of city trees; therefore, data on the interactions between high temperature and other potential strains are needed. Water is often considered limited in urban tree root zones (Foster and Blaine, 1978; Spire, 1984), and frequency of water deficit maybe greater in soils at high temperature. The objective of

this project was to characterize the growth of honey locust seedlings exposed concurrently to high root-zone temperature and water deficit.

Honey locust seeds collected in 1989 from a tree in College Park, Md., were scarified in sulfuric acid and germinated for 7 days between moist paper towels at 25C. Seedlings with fresh weights of 0.5 to 0.6 g were established in a growth chamber adjusted to 16-h photoperiods of  $280 \pm 30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  photosynthetically active radiation (PAR) from cool-white fluorescent lamps,  $23 \pm 1\text{C}$ , and  $50\% \pm 10\%$  relative humidity. Thirty-six plants were grown singly in aerated, 50% Hoagland solution #1 with Fe supplied as Fe-EDTA (pH 5.8 to 6.0) (Hoagland and Arnon, 1950) in 1.2-liter stainless steel vessels. Each vessel was surrounded by a polyvinyl chloride jacket through which heated water was circulated to raise root-zone temperature (Graves and Dana, 1987a).

Treatments began 3 days after placing plants in the chamber. Nine seedlings were assigned at random to each of four treatments (0, 6, 12, and 24 h·day<sup>-1</sup>, when root-zone temperature was increased from ambient to  $35 \pm 0.5\text{C}$ . Daily temperature episodes were applied during hours 8 through 13 and 5 through 16 of the photoperiod for the 6- and 12-h·day<sup>-1</sup> treatments, respectively. Less than 30 min were required to attain 35C, and  $\approx 4$  h were required for temperature to return to ambient. Within each temperature regime, three plants were assigned randomly to nutrient solutions with osmotic potentials ( $\psi_{\pi}$ ) of -0.05, -0.10, and -0.20 MPa. Quantities of polyethylene glycol (PEG) 8000 needed to attain these  $\psi_{\pi}$  (0, 78.95, and 126.3 g·liter<sup>-1</sup> of nutrient solution, respectively) were calculated from the relationship between PEG concentration and  $\psi_{\pi}$  at 23C (Fig. 1), which was determined with a Wescor 5500 vapor pressure osmometer (Wescor, Logan, Utah). Solutions were replenished daily with deionized water and replaced every 5 days. After 20 days, epicotyl length was measured, and roots and

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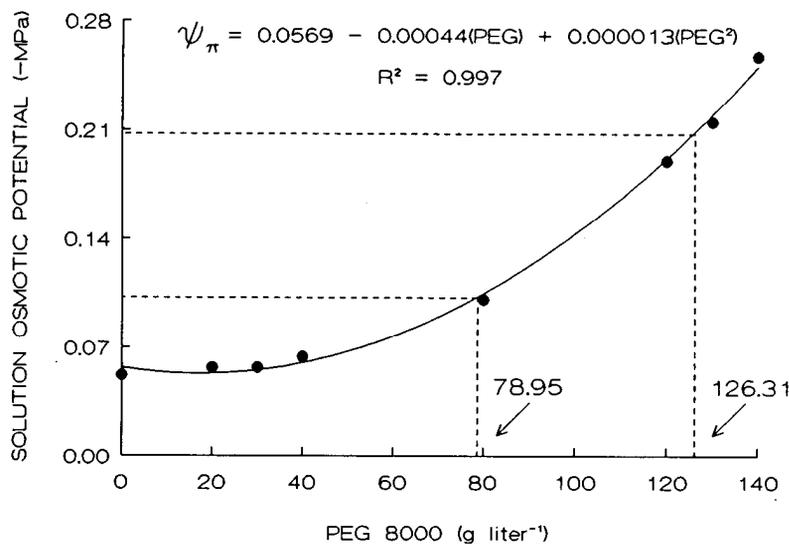


Fig. 1. Osmotic potential ( $\psi_{\pi}$ ) of 50% Hoagland solution #1 at 23C with 0 to 140 g polyethylene glycol (PEG) 8000/liter. Dashed lines indicate PEG concentrations used to attain solution  $\psi_{\pi}$  of  $-0.10$  and  $-0.20$  MPa.

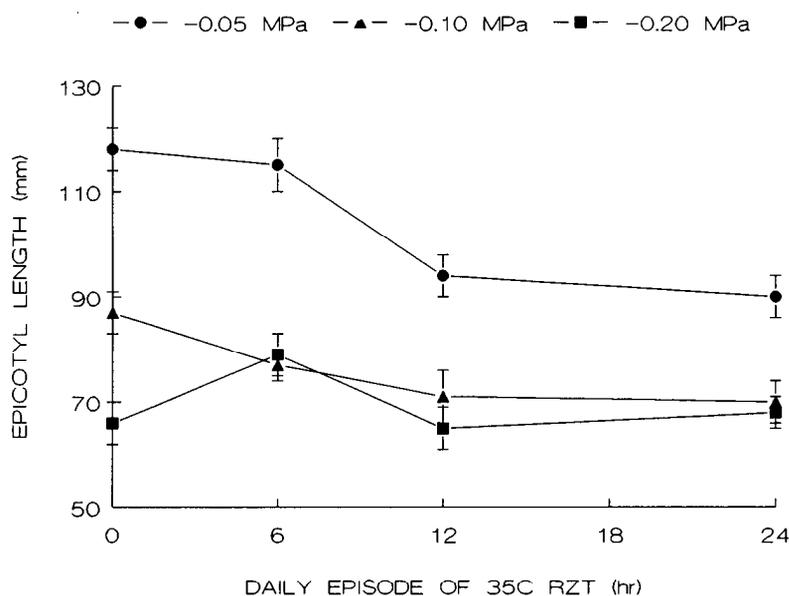


Fig. 2. Mean ( $\pm$ SE) epicotyl length ( $n = 9$ ) of honey locust seedlings after 20 days in osmotic and root-zone temperature (RZT) treatments. The LSD for these means ( $\alpha = 0.05$ ) is 11.

shoots were dried at 65C for 48 h and weighed.

The experiment was performed three times, and all data were combined for analysis. Interactions involving replicate experiments were homogeneous and pooled to provide the error term to test the effects of the independent variables. Second-order polynomial response surfaces were fitted to the variables. Polynomials of higher order were not examined because reasonable biological interpretations of these terms were not apparent. The goodness of fit of each response surface model was assessed by comparing the residual mean square to the error mean square of the analysis of variance (ANOVA) fitting the factorial treatment structure. Residual mean square values were considerably larger than the error mean square values, indicating that the polynomials were not representative of the data. Therefore, we

considered ANOVA and the presentation of means and standard errors the most appropriate way to interpret the results of the experiment. Least significant difference values ( $\alpha = 0.05$ ) were determined for epicotyl length and shoot dry weight (Steel and Tome, 1980).

Two related experiments were conducted. In the first, 12 seedlings were grown singly in 1-liter polyethylene containers under chamber conditions. Half the seedlings were grown in 50% Hoagland solution as described for the previous experiment. The other six plants were grown in a peatmoss-based medium (Sunshine Mix; Fisons Horticulture, Vancouver, B. C., Canada). A pencil-type tensiometer (Soil Measurement Systems, Tucson, Ariz.) was installed in the center of each container with Sunshine Mix, and these plants were irrigated to saturation with Hoagland solution and allowed to drain to

container capacity daily for the first 3 days in the chamber. Daily irrigation then was withheld from three randomly selected plants, and the other three were irrigated as usual every other day. On the same day treatments began on plants in Sunshine Mix, three of the solution-cultured plants were placed in PEG-amended Hoagland solution with an  $\psi_{\pi}$  of  $-0.20$  MPa. Nutrient solutions were replenished with deionized water daily and replaced every 7 days. We wanted the degree of drought stress of plants in PEG-amended solution to be similar to that of plants in Sunshine Mix from which irrigation was withheld. Therefore, we measured the epicotyl length of all plants every other day and compared the percent reduction in epicotyl length for plants stressed osmotically in solution with the reduction in epicotyl length for nonirrigated plants in Sunshine Mix, relative to the irrigated controls. Moisture tension in all containers with Sunshine Mix also was recorded every other day. After 28 days, the root : shoot weight ratio was determined after weighing tissues dried for 48 h at 65C.

In the second related study, 24 seedlings were grown singly under chamber conditions in 1-liter plastic containers of aerated Hoagland solution. Plants were transferred after 3 days to solution with 0, 10, 20, 40, 80, or 160 g PEG/liter. Four seedlings were assigned at random to each PEG concentration. Cultures were replenished daily with deionized water, and solutions were replaced every 7 days. Epicotyl lengths were determined after 21 days.

There was a significant ( $\alpha = 0.01$ ) interaction between root-zone temperature and osmotic effects for all plant traits measured. Epicotyl length of seedlings in  $-0.05$ -MPa solution was similar with 35C for 0 and 6 h-day<sup>-1</sup>, but was reduced among plants in the 12- or 24-h-day<sup>-1</sup> treatments (Fig. 2). Length of epicotyls was lower in cultures with solution  $\psi_{\pi}$  of  $-0.10$  and  $-0.20$  MPa than in those with  $-0.05$  MPa. The influence of temperature on epicotyl length of seedlings in  $-0.10$ -MPa solution was similar to that observed in  $-0.05$ -MPa solution. At  $-0.20$  MPa, however, epicotyls were longer for seedlings with 35C for 6 h-day<sup>-1</sup> than for those in the 0- or 12-h-day<sup>-1</sup> treatments (LSD = 11).

Dry weights decreased with exposure to 35C for 12 or 24 h-day<sup>-1</sup> among seedlings in the  $-0.05$ -MPa solution (Fig. 3 A and B). The reduction in root weight between 6 and 24 h-day<sup>-1</sup> at 35C was linear [root weight =  $185.3 - 5.5$  (hours of temperature treatment);  $R^2 = 0.99$ ], but there was no difference in shoot weight of seedlings exposed to 35C from 12 to 24 h-day<sup>-1</sup> (LSD = 64). Temperature had little influence on the weights of osmotically stressed seedlings. Plants in solutions with reduced  $\psi_{\pi}$  had less biomass (Fig. 3 A and B) and reduced root : shoot weight ratios (Fig. 3C).

In the study to compare methods of inducing drought, mean ( $\pm$ SE) moisture tensions in containers with Sunshine Mix after 28 days of treatment were  $-4.0$  (0.5) and  $-47.9$  (5.7) kPa for irrigated and nonirri-

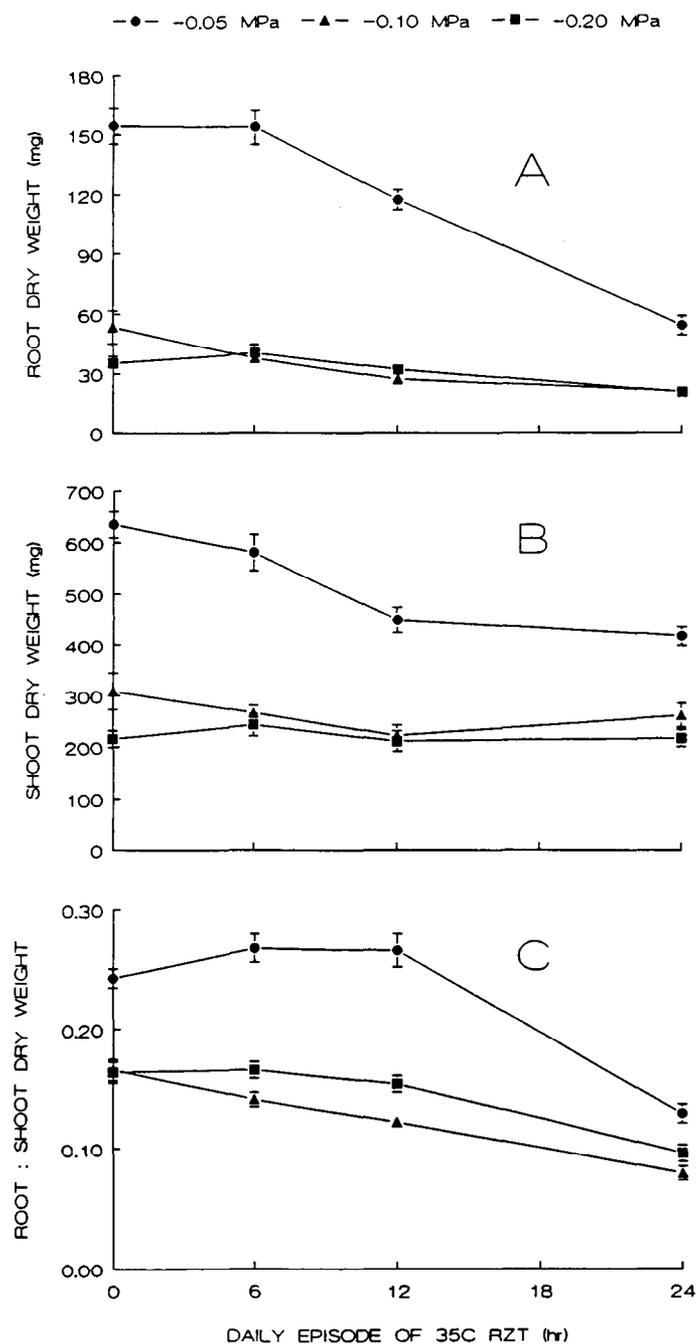


Fig. 3. Mean root dry weight (A), mean shoot dry weight (B), and mean root : shoot dry weight ratio (C) of honey locust seedlings ( $\pm$ SE;  $n = 9$ ) after 20 days in osmotic and root-zone temperature (RZT) treatments. The LSD for shoot dry weight means ( $\alpha = 0.05$ ) is 64.

gated plants, respectively. Compared to their respective controls, mean epicotyl length was reduced by 23% for plants in solution amended with PEG and 20% for nonirrigated plants in Sunshine Mix. The reduction in root : shoot ratio among plants in solution with  $\psi_{\pi}$  lowered to  $-0.20$  MPa with PEG was similar to that illustrated in Fig. 3C. However, the mean root: shoot weight ratio ( $\pm$ SE) for irrigated and nonirrigated seedlings in Sunshine Mix was 0.211 (0.002) and 0.529 (0.079), respectively. Total dry weight of seedlings treated with PEG was less than that of seedlings in solution without PEG, but total dry weight of irrigated and nonirrigated plants in Sunshine Mix was not different.

During the final experiment, as little as 10 g PEG/liter of solution reduced honey locust epicotyl length (Fig. 4).

Our experiments show that the growth of honey locust seedlings in solution is greatly diminished by small reductions in the  $\psi_{\pi}$  of the medium with PEG. These findings are consistent with those of Zekri and Parsons (1989), who reported that root and shoot growth of *Citrus aurantium* L. and *C. jambhiri* hybrid were reduced in sand cultures irrigated with solutions of PEG 4000 at  $-0.10$  MPa. Gergely et al. (1980) found a linear decrease in the mass of *Malus domestica* Borkh. in solutions of PEG 4000 with  $\psi_{\pi}$  between 0 and  $-0.75$  MPa. Other species,

however, appear more resistant to PEG-induced stress. Radicle growth of the legume *Lespedeza stipulacea* Maxim. at 22 and 27C was not reduced when media  $\psi_{\pi}$  was lowered to  $-0.32$  and  $-0.77$  MPa with PEG 20,000 (Masiunas and Carpenter, 1984). Radicles of another legume, *Lens culinaris* Medik. cv. Castellana, were longer for seedlings cultured in a PEG 4000-amended solution with a  $\psi_{\pi}$  of  $-0.30$  MPa than for controls (Moran et al., 1989). The large reduction in biomass of honey locust seedlings in  $-0.10$ -MPa solution suggests this species is comparatively sensitive to PEG-induced stress.

We did not determine the turgor and osmotic potentials of seedlings in this study. It is possible, however, that the interaction between high root-zone temperature and drought effects on honey locust growth were the result of an increased resistance to heat that can result from osmotic adjustment in water-stressed tissues. Moran et al. (1989) found radicle cells of plants grown in PEG 4000 solutions had lower  $\psi_{\pi}$  than those of plants grown without osmoticum, suggesting osmotic adjustment had occurred. Studies by Seemann et al. (1986) and Hellmuth (1971) suggest that lower tissue  $\psi_{\pi}$  correlates positively to high-temperature resistance. Whether drought-induced reductions in tissue  $\psi_{\pi}$  account for the relative insensitivity to heat of osmotically stressed honey locust (Fig. 3) or the temperature-dependent differences in epicotyl elongation among plants in solution with a  $\psi_{\pi}$  of  $-0.20$  MPa is not known. Comparisons of means using LSD values indicated that, for seedlings in  $-0.20$ -MPa solution, treatment with 35C for 6 h-day<sup>-1</sup> increased epicotyl length significantly (Fig. 2), but there were no differences in the dry weight of shoots of these seedlings (Fig. 3B). This difference in response suggests that temperature affected cellular elongation to a greater extent than carbon fixation among plants subjected to the greatest osmotic stress. Further research is needed to address the possibility that osmotic adjustment contributed both heat resistance and the capacity to maintain expansive growth in osmotically stressed honey locust seedlings. Martin et al. (1989) found that shoot extension was similar for plants of *Ulmus parvifolia* Jacq. cv. Drake grown in an aggregate medium held at 28 and 35C for 6 h-day<sup>-1</sup> despite a 39% reduction in shoot dry weight at 35C. Whether root-zone temperature affected the quantity of water available to plants in that study is not known.

Graves et al. (1991) found greater shoot elongation in honey locust seedlings held at a constant 34C root-zone temperature than in those at 24C. In the current study, however, the epicotyl length of seedlings not stressed osmotically was reduced considerably by continuous exposure to 35C (Fig. 2). Several factors may account for the differences in results of these two studies. First, rates of metabolic processes in plants generally have been observed to increase gradually up to a critical threshold temperature above which metabolism rapidly diminishes (Hale and Orcutt, 1987). Root-zone temper-

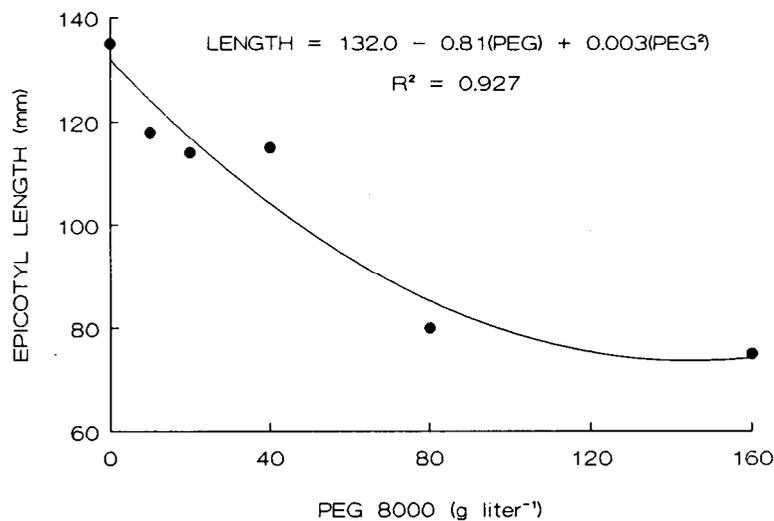


Fig. 4. Mean epicotyl length ( $n = 4$ ) of honey locust seedlings with 0 to 160 g polyethylene glycol (PEG) 8000/liter in Hoagland solution.

atures as high as 34C may promote growth in honey locust, but development is inhibited greatly at temperatures > 34C. Graves et al. (1991) cultured plants under fluorescent and incandescent lamps in an aggregate medium. Radiation quality and intensity, oxygen availability in the root zone, and the mechanical resistance of the media also may account for the differences between the results of these studies.

Agitated solution cultures and PEG were used in this study to achieve precise temperature and osmotic conditions, but we urge caution in making broad conclusions on heat and drought responses based solely on data using these methods. Inducing drought stress osmotically caused a marked reduction in root : shoot ratio (Fig. 3C), but this ratio more than doubled among nonirrigated plants in Sunshine Mix. Withholding irrigation in the mix and reducing solution  $\psi_{\pi}$  to  $-0.20$  MPa caused similar reductions in epicotyl length, our non-destructive estimate of stress response; also, when destructive harvests were completed, PEG-induced stress was found to have reduced seedling dry weight. Concomitant with a large increase in root : shoot ratio, however, total dry weight of irrigated and nonirrigated seedlings in Sunshine Mix was similar. Thus, it is questionable whether nonirrigated plants in the mix were stressed and, if they were, whether the extent of stress was similar to that imposed using PEG in solution culture. Nonetheless, the results of this experiment heighten our concern that responses of honey locust to PEG may not mimic those caused by droughts in nature. Apprehension about the use of PEG to induce stress was increased further by our

finding that as little as 10 g PEG/liter in solution restricted shoot expansion (Fig. 4). We were unable to detect any change in  $\psi_{\pi}$  when PEG was used at this rate (Fig. 1). Something other than a reduced  $\psi_{\pi}$  apparently inhibited growth in PEG solutions. Contaminants have been reported in commercial sources of PEG (Lagerwerff et al., 1961; Reid, 1978), but Lawlor (1970) concluded that uptake of PEG, not impurities, was responsible for toxic effects on plants. Most workers, however, have considered PEG to be nonpenetrating (Krizek, 1985). The use of PEG with a large molecular weight and the care taken to avoid damage to root tissues should have reduced the possibility for uptake in these experiments (Krizek, 1985). Additional studies to test how media and drought induction methods influence responses to root-zone temperature and moisture deficit are warranted.

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