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Influence of Reduced Leaching on Growth of Seedlings of Black Locust Inoculated with Rhizobia

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Seedlings of black locust (*Robinia pseudoacacia* L.) were inoculated with compatible rhizobia and irrigated twice each week with solution containing either 50 or 150 mg nitrogen/L. Leaching fraction treatments of 0, 0.2, or 0.4 were maintained for 6 weeks. Seedlings supplied 50 mg nitrogen/L grew poorly compared to plants supplied 150 mg nitrogen/L regardless of leaching fraction. Dry masses of shoots and of entire plants were lower for seedlings in the 0 and 0.2 leaching fraction treatments compared with those in the 0.4 treatment. Neither leaching fraction nor applied nitrogen concentration appeared to affect the number of nodules per plant, but nodule diameter seemed greater on plants irrigated with the more dilute solution. We conclude that reducing leaching can reduce shoot growth of containerized black locust, but the monetary and environmental benefits of lower water and fertilizer use should be considered before the commercial significance of the growth reduction is judged.

INDEX DESCRIPTORS: *Robinia pseudoacacia* L., sustainable agriculture, nitrogen fixation, salinity

Reducing leaching is a way producers of nursery crops might respond to increasing pressure to decrease the use and runoff of irrigation solutions. The growth and acceptability of some floricultural crops are unaffected by reducing or eliminating leaching of irrigation solution (Ku and Hershey, 1991; Yelanich and Biernbaum, 1990), while other crops are sensitive to the resulting build-up of salts in the rooting medium (Ku and Hershey, 1992).

There is little information on the response woody nursery crops to reduced leaching. *Robinia* species grow rapidly and are considered relatively salt tolerant (Flint, 1983). Therefore they serve as useful model species for studies on the feasibility of reducing leaching during production of containerized nursery stock. In addition, *Robinia* species form symbioses with nitrogen-fixing rhizobial bacteria. Development of these symbioses might permit production with unusually dilute irrigation solutions because the amount of nitrogen required from fertilizers might be relatively small for nodulated plants.

This experiment was conducted to determine the effect of reducing the leaching fraction on growth of seedlings of black locust (*Robinia pseudoacacia* L.) inoculated with compatible rhizobia using two irrigation solutions containing different concentrations of fertilizer.

MATERIALS AND METHODS

Seeds of black locust were scarified in 18 M sulfuric acid for 60 min. on 18 May 1993 and germinated in horticultural perlite and screened sphagnum peat moss mixed in equal volumes. Seedlings were irrigated with tap water as needed until 14 June when 24 uniform plants were transplanted singly to 15-cm by 15-cm standard plastic pots (Belden Plastics, St. Paul, Minn.). These seedlings constituted plants in block one. On the following day, 24 additional seedlings that comprised block two were planted using identical procedures. Fisons Special Blend Sunshine Bark Mix (Fisons, Warwick, N.Y.) was used as the growing medium. Its components, by volume, were 50% Sphagnum peat moss, 40% composed pine bark, and 10% fine-screened horticultural perlite, and the mix was amended with trace nutrient elements. The medium in each container was inoculated at the time of transplanting by dribbling 1.5 ml of a culture of USDA strain 4246 rhizobia (*Rhizobium* collection, Beltsville, Md.), prepared as described by Batzli et al. (1992), into the hole where roots were placed.

Plastic mesh was placed over the drainage holes in the bottom of each container to prevent loss of medium during the experiment. Each pot was filled uniformly with medium leached with 550 ml distilled water three times. Leaching caused the electrical conductivity of the leachate to be reduced from 2.7 dS/m after the first irrigation to 0.7 dS/m after the third. Pots were arranged on 24-cm by 24-cm centers in a glasshouse on a raised bench. To facilitate leachate collection, each pot was placed within a 1.9-L plastic specimen container (Fisher Scientific, Pittsburgh, Penn.) such that pot bottoms were not in contact with specimen containers. Pots in the two blocks were placed on opposite ends of the bench. Within both blocks, four pots were assigned randomly to a three-by-two factorial of treatments. The 48 pots were considered individual experimental units. Three leaching fraction treatments, maintained as described below, were applied using two irrigation solutions containing different amounts of fertilizer, 50 (low fertility) and 150 (high fertiilty) mg nitrogen/L.

After transplanting, seedlings were irrigated with 300 ml of nutrient solution at the concentration to which they had been assigned using Peter's Excel All Purpose (Grace-Sierra Horticultural Products Co., Milpitas, Calif.) with 21N-5P-20K-0.02B-0.01Cu-0.1Fe-0.05Mn-0.01Mo-0.07Zn. Nitrogen was 6.51% ammonium, 12.60% nitrate, and 1.89% urea. Throughout the experiment, the low-fertility solution was treated with 100 microliters of 18 M sulfuric acid to lower the pH to 6.0 to 6.6, the range obtained without treatment for the high-fertility solution. Excess solution was allowed to drain so that pots were at container capacity. Initial mass of individual pots at container capacity (M_a) was determined using an electronic balance.

Irrigation treatments were applied twice weekly. Plants in block one were treated each Monday and Thursday. Those in block two were treated each Tuesday and Friday. Before each irrigation, the mass of individual pots (M_b) was determined. Irrigation volume to be applied (V_a) was determined using the formula $V_a = [(LF \times ET)/(1 - LF)] + ET$, where ET, the evapotranspiration, was determined by subtracting M_b from M_a, and LF was the assigned leaching fraction. To ensure that all containers were at container capacity after irrigation, additional solution was applied to plants in the 0 leaching fraction treatment as needed until a drop of solution was visible at the drainage holes. Volume of leachate from plants in the 0.2 and 0.4 treatments was determined after each irrigation. The mass of the pots then was determined to obtain an updated M_a for the subsequent irrigation.

Plant development was assessed on 26 (block 1) or 27 (block 2) July. Length of the longest stem and the number of stems at least 3 cm long were determined for each plant in random order. Stems were severed at the cotyledon scars, and shoots were held in a drying oven at 67C for 2 days before their masses were determined. Root systems were separated from the medium and assessed visually for apparent trends in the number and size of root nodules. Dry masses of the cleaned root systems then were determined as described for shoots. The significance of blocking, leaching fraction, irrigation solution concentration, and the interaction of leaching and solution treatments was assessed using analysis of variance (ANOVA).

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Table 1. Influence of leaching fraction and rate of applied nitrogen on growth of containerized seedlings of black locust. Leaching fraction values are means of 16 replicate plants representing both nitrogen rates. Nitrogen rate values are means of 24 replicate plants representing the three leaching fraction treatments. The LSD values are for the $p=0.05$ level.

	Leaching fraction				Nitrogen rate applied (mg/L)		
Dependent variable	0	0.2	0.4	LSD	50	150	LSD
Total dry mass (g)	3.9	4.0	4.9	0.6	2.3	6.2	0.5
Root dry mass (g)	0.9	0.9	1.0	0.2	0.6	1.2	0.1
Shoot dry mass (g)	3.1	3.1	3.9	0.5	1.7	5.0	0.4
Number of stems ≥3 cm	1.0	1.3	2.2	0.9	1.0	2.0	0.7
Length of primary stem (cm)	39.1	41.1	46.7	4.4	29.2	55.5	3.6
Total stem length (cm)	41.7	43.1	55.0	9.0	29.2	64.1	7.4

RESULTS

Blocks did not differ significantly, and data from both were combined for all dependent variables. Interactions between fertility and leaching fraction were not significant. Means of all dependent variables were greater for plants in the higher fertility regime than for those that received the more dilute solution (Table 1).

Total plant dry mass was reduced by decreasing the leaching fraction, but there was no difference in total dry mass between plants treated with leaching fractions of 0 and 0.2 (Table 1). Root mass was similar at different leaching fractions, and the reduction in total dry mass as leaching fraction decreased resulted primarily from effects on shoot mass (Table 1). The number of stems, the length of the primary stem, and the total stem length tended to decrease with decreasing leaching fraction (Table 1). However, differences between plants in the 0 and 0.2 leaching fraction treatments were not significant.

Most plants were observed to have nodulated profusely, and no trends on the influence of leaching fraction were apparent. Fertility did not appear to affect the number of nodules, but nodule diameter appeared much greater for plants in the low fertility treatment.

DISCUSSION

Reducing the leaching fraction from 0.4 to 0.2 or 0 altered the development of shoots of black locust in ways that could be significant commercially. Mean shoot dry mass was about 20% lower for plants grown with reduced leaching than for those in the 0.4 leaching fraction treatment. The number of stems per plant tended to decrease as leaching fraction decreased (Table 1). The fact that mean shoot dry mass was the same among plants in the 0 and 0.2 leaching fraction treatments suggests that lateral branching was inhibited by the accumulation of salts in the root zone. Although further study is needed to clarify the relationship between leaching and lateral branching, we can conclude that reduced leaching can retard overall shoot growth of black locust. The growth inhibition was significant statistically, but whether a decrease in shoot dry mass of about 20% is significant commercially should be determined by growers who must consider potential monetary and environmental benefits from the reduction in fertilizer and water use (Ayers and Westcot, 1976; Hershey and Ku, 1991).

The relationships between fertility treatment, root nodulation, and plant growth provide insights into the feasibility of using nitrogen fixation to reduce fertilizer use during production of leguminous nursery crops. The size of plants in the lower fertility regime likely was too small to be commercially acceptable. The tremendous difference in growth between plants in the two fertility treatments was interesting because it indicates that growers of black locust would be unable to rely on nitrogen fixation to supply all of the nitrogen required for rapid plant development. The higher fertility regime apparently did not inhibit root nodulation but did reduce nodule development. Further studies should investigate further the extent to which the need for applied nitrogen differs between nodulated and non-nodulated plants. Additional work also could explore the symbiotic efficiency of different rhizobia effective with black locust. Such differences have been observed previously (Batzli et al., 1992) but have not been characterized under nursery conditions using media containing high concentrations of fertilizer salts.

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