Influence of Inoculant Form and Applied Nitrogen on Growth and Root Nodulation of *Maackia amurensis*[1]

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Abstract

We studied growth and nodulation of *Maackia amurensis* Rupr. & Maxim. treated with three forms of an inoculant of *Bradyrhizobium* (USDA 4349) and irrigated with solutions containing N at 1.8, 3.6, 7.2, or 14.3 mol·m^{-3} (25, 50, 100, or 200 ppm). One inoculant (arabinose-gluconate liquid) was prepared in our laboratory, whereas the other two (Cell-Tech liquid and Cell-Tech peat powder) were obtained from a commercial source. Nodule dry mass of 10-week-old seedlings was similar regardless of inoculant form. Only plants supplied N at 1.8 and 3.6 mol·m^{-3} (25 and 50 ppm) nodulated consistently, and nodule dry mass of plants in these two treatments was not different. Laminar area and plant dry mass were highest among plants provided the two highest concentrations of N. Shoot N content was lowest and highest for plants provided N at 3.6 mol·m^{-3} (50 ppm) and 14.3 mol·m^{-3} (200 ppm), respectively, and it was not affected by form of inoculant. We conclude that liquid and peat-based inoculants cause a similar degree of nodulation. This study also demonstrated that providing N at concentrations of 7.2 mol·m^{-3} (100 ppm) or higher inhibits nodulation, and that inoculation with USDA 4349 does not substitute for applied N if maximal early seedling growth is desired.

Index words: Amur maackia, nitrogen fixation, sustainable production.

Significance to the Nursery Industry

Maackia amurensis is receiving increasing recognition as a small to medium-sized tree suitable for small and urban landscapes. Unlike most temperate legumes common in nursery production, *M. amurensis* forms root nodules with bacteria that fix N gas (N_2). This trait may allow trees to be produced with little N fertilizer if plants or root media are inoculated with the specific bacteria compatible with this species. In this experiment, a peat-based inoculant and two forms of liquid inoculant evoked similar degrees of nodulation on seedlings grown in containers with soilless medium. We also found that N applications at concentrations of 7.2 mol·m^{-3} (100 ppm) or higher prevent nodule formation on *M. amurensis*. Plants provided N at lower concentrations nodulated but grew less than plants that received more N and did not nodulate. Therefore, the time required to produce saleable plants probably will increase if growers induce nodulation during early seedling development.

Introduction

The leguminous tree *Maackia amurensis* has landscape attributes suitable for urban planting sites (1, 2). The first evidence that *M. amurensis* forms root nodules in symbiotic association with *Bradyrhizobium* that fix N_2 recently was reported (1). The capacity of *M. amurensis* to associate with N_2-fixing bacteria might reduce the need to fertilize with N during nursery production (1, 3). This could reduce production costs and the potential for excess N to leach into surface and ground water supplies. N_2 fixation also might improve the long-term survival of trees installed at sites with low soil fertility.

Establishing N_2-fixing symbioses during nursery production requires inoculation with compatible *Bradyrhizobium*. Eleven isolates of *Bradyrhizobium* compatible with *M. amurensis* (1) have been isolated and are available from the U.S. Department of Agriculture *Rhizobium* Collection in Beltsville, MD. These have been used to prepare inoculants in liquid media under laboratory conditions (1), but few nurseries would have access to the equipment necessary to follow these protocols. Therefore, we cooperated with Liphatech, Inc., Milwaukee, WI, to develop liquid and peat-moss-based inoculants that could be marketed to nurseries. One objective of this research was to compare these commercial preparations with a liquid inoculant we prepared for their effects on root nodulation, plant growth, and shoot N content. Because research with other legumes shows that applied N can inhibit nodulation (8, 10), the second objective of our experiment was to determine how different concentrations of applied N applied would affect nodule and plant development.

Materials and Methods

Seedlings of *M. amurensis* were grown in 15 cm (6 in) diameter plastic pots (volume = 1840 ml, Belden Plastics, St. Paul, MN) in a glass-glazed greenhouse for 10 weeks during each of two replicate experiments. Air temperature and relative humidity, monitored by using a hygrothermograph (Serdex Bacharach 22-7009, Bacharach, Pittsburgh, PA), were 24 ± 6C (75 ± 11F) and 25 ± 10%, respectively. Natural irradiance was supplemented by use of high-pressure sodium lamps from 0600 to 2100 HR CST. Midday irradiance at the tops of plants, measured with a quantum sensor (LI 185A, LI-COR, Lincoln, NB), was 725 ± 250 µmol/s·m^2. Plastic screens were placed over the drainage holes of pots before they were filled with Fisons Special Blend 1 (Fisons Horticulture, Vancouver, B.C.), which contained sphagnum peat:composted pine bark:perlite (5:4:1 by vol).

Cell-Tech liquid and peat powder inoculants containing *Bradyrhizobium* USDA 4349 from cultures we supplied were

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obtained from Liphatech, Inc. A liquid inoculum of the same bacterium in arabinose-gluconate medium (AG liquid) was prepared at our laboratory (5). The density of USDA 4349 was 10^8 cells/g in the peat powder, 10^9 cells/ml in the Cell-Tech liquid, and 10^8/ml in the AG liquid. Inoculants were applied (1 ml of liquid or 1 g of peat) to a 2 cm (1 in) deep planting hole in the medium. Seeds of *M. amurensis* from one tree at the U.S. Dept. of Agriculture National Arboretum in Washington, DC, were scarified in 18M sulfuric acid for 1 hr and rinsed in deionized water. Two seeds were sown on the inoculum in each pot. Seedlings were thinned to one per pot by using sterile tools 4 weeks after sowing. Seeds were sown on January 26 and April 20, 1994, for the first and second replications of the experiment, respectively.

During both experiments, 24 pots were assigned randomly to each of the three inoculants and to an uninoculated control treatment. Six replicates in each inoculant treatment were assigned randomly to each of four N treatments that commenced 1 week after seeds were sown. Pots were arranged in six randomized complete blocks on greenhouse benches. Each block contained one plant in each of the 16 factorial combinations of inoculant and fertilizer treatments. Fertilizer solutions that contained N at 1.8, 3.6, 7.2, or 14.3 $mol \cdot m^{-3}$ N (25, 50, 100, and 200 ppm) were prepared in tap water with Peters Excel 15-2.2-12.5 Cal-Mag Special fertilizer (The Scotts Co., Marietta, GA). N in the fertilizer was in the forms of nitrate (60%), ammonium (31%), and urea (9%). The pH of fertilizer solutions was adjusted to between 6.2 and 6.8 by using HCl and KOH, and 500 ml of solution was applied to each pot once weekly. Pots were placed individually in high-density polyethylene containers (volume = 1900 ml, Fisher Scientific, Pittsburgh, PA) to collect leachate and reduce the potential for contaminating uninoculated control plants with *Bradyrhizobium*.

Seedlings were harvested 10 weeks after seeds were sown. Laminar area was measured on a leaf area meter (LI-3100, LI-COR). The planting mix was gently washed off the roots with tap water, and the nodules were separated from the roots. All tissues were dried in an oven at 67C (153F) for 48 hr before mass was determined. Total N content of the shoot of each plant was measured by using a Lachat autoanalyzer (Lachat Instruments, Milwaukee, WI). Data were analyzed by using an analysis of variance for both experiments combined with a factorial model in which means from inoculation and fertilizer treatments were treated as replications. Mean separations were performed by using Fisher's LSD ($\alpha = 0.05$).

Results and Discussion

There was no difference in nodule dry mass among plants inoculated with *Bradyrhizobium*, and no nodules formed on uninoculated control plants (data not shown). Inoculation treatments did not affect laminar area or mass of plants, and there was no interaction of inoculation treatment and concentration of applied N for these traits (data not shown). Seedlings provided N at 7.2 and 14.3 $mol \cdot m^{-3}$ (100 and 200 ppm) had greater laminar area and plant dry mass than seedlings provided N at 1.8 and 3.6 $mol \cdot m^{-3}$ (25 and 50 ppm) (Table 1). All inoculated plants irrigated with solutions containing N at 1.8 and 3.6 $mol \cdot m^{-3}$ (25 and 50 ppm) nodulated, and nodule dry mass of plants in these treatments was similar (Table 1). Only four of the 96 plants treated with N at 7.2 and 14.3 $mol \cdot m^{-3}$ (100 and 200 ppm) over both replicate experiments formed nodules, and the mean nodule dry mass of these plants was less than that of plants provided N at 1.8 and 3.6 $mol \cdot m^{-3}$ (25 and 50 ppm) (Table 1). Shoot N content ranged from 1.57% for seedlings provided N at 3.6 $mol \cdot m^{-3}$ (50 ppm) to 3.09% for seedlings provided N at 14.3 $mol \cdot m^{-3}$ (200 ppm) (Table 1). Shoots of uninoculated seedlings contained lower percentages of N than shoots of seedlings in medium inoculated with Cell-Tech liquid and peat (Table 2). Shoot N content of inoculated seedlings was similar regardless of inoculant form (Table 2).

Table 1. **Effect of concentration of applied N on laminar area, nodule and seedling dry mass, and shoot N content of 10-week-old seedlings of *Maackia amurensis*. Seedlings either were treated with one of three inoculants of *Bradyrhizobium* USDA 4349 or were uninoculated controls. Values are means of data combined from two replicate experiments (see text).**

Concentration of applied N [$mol \cdot m^{-3}$ (ppm)]	Laminar area (cm^2)	Dry mass (mg)		Shoot N content (%)
		Nodule	Plant	
1.8 (25)	65	23.8	583	1.80
3.6 (50)	92	19.6	996	1.57
7.2 (100)	278	0.3	2285	2.43
14.3 (200)	266	0.2	2148	3.09
LSD ($\alpha = 0.05$)	95	17.5	611	0.20

Growers can use either liquid or peat-based inoculants to induce nodulation during production of *M. amurensis*. Growth of plants in medium with the two commercial inoculants did not differ from that of plants with *Bradyrhizobium* 4349 in AG medium. We prepared the AG medium immediately before both replications of the experiment. In contrast, the commercial inoculants were prepared about 1 month before beginning the first replication of the experiment and were held at 4C (39F) until first use and between replications. This indicates that growers can store inoculants for at least 3 months without loss of efficacy.

This study provides new information on the influence of N fertilizer on nodulation in *M. amurensis*. Consistent nodulation occurred only among plants provided N at 1.8 and 3.6 $mol \cdot m^{-3}$ (25 and 50 ppm). N at 7.2 and 14.3 $mol \cdot m^{-3}$ (100 and 200 ppm) strongly inhibited nodule development (Table 1). This finding is consistent with reports that N suppresses nodulation of other legumes (8, 10). Growers wishing to produce nodulated plants must manage N applications carefully to prevent inhibition of nodulation. N applied after nodulation also is likely to affect nodule longevity (9) and activity (10), but the influence of applied N on N_2 fixation of well-nodulated *M. amurensis* awaits characterization.

Table 2. **Effect of form of inoculant of *Bradyrhizobium* USDA 4349 on shoot N content of 10-week-old seedlings of *Maackia amurensis*. Values are means of data combined from two replicate experiments (see text).**

Inoculant treatment	Shoot N content (%)
Uninoculated control	2.05
Arabanose-gluconate liquid	2.21
Cell-Tech liquid	2.26
Cell-Tech peat powder	2.38
LSD ($\alpha = 0.05$)	0.20

Despite having nodules, the laminar area and mass of plants provided the two lower N concentrations were less than one-third and one-half, respectively, of plants provided N at 7.2 $mol\cdot m^{-3}$ (100 ppm) (Table 1). The influence of N on growth was not different among uninoculated seedlings, which lacked nodules in all N treatments, and inoculated plants that nodulated when N at the two lower concentrations was applied. This suggests there was no net effect of nodulation on growth. Any growth enhancement from N_2 fixation apparently was not sufficient to overcome the energy cost of establishing and maintaining nodules, which can use from about 4% to 40% of host-plant photosynthates for respiration (4, 6, 7). Growers should not expect N_2 fixation to replace the need to apply N at concentrations above 3.6 $mol\cdot m^{-3}$ (50 ppm) if near-optimal growth of young seedlings is desired.

Inoculation did not affect laminar area and dry mass of plants, but shoot N content of plants inoculated with Cell-Tech liquid and peat powder was greater than that of uninoculated plants, and the shoot N content of all inoculated plants did not differ (Table 2). This is consistent with a previous study in which *M. amurensis* inoculated with *Bradyrhizobium* 4349 had a higher N content without an increase in dry mass compared to uninoculated controls (1). Long-term studies could be used to determine whether the growth of nodulated plants eventually exceeds the growth of unnodulated plants produced with low N. Our findings and the results of previous work (1) suggest that growers wishing to produce saleable plants rapidly must rely on applications of N at rates that will suppress nodulation. A strategy for producing nodulated plants rapidly might be to supply high concentrations of N during most of the production cycle, and then reduce N fertilization and inoculate shortly before plants are marketed. Subsequent studies should document the survival and growth of nodulated plants installed at sites with soils low in N. N_2 fixation may have a greater practical impact on long-term tree survival in poor soils than on reducing N inputs during production.

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