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cantly lower levels of euonymus scale on *E. kiautschovicus* 'Manhattan' in the field study make this a desirable selection for landscape production. In contrast, the use of *E. japonicus* 'Albo-marginatus' and *E. japonicus* 'Microphyllus' in landscape plantings would likely be more susceptible to euonymus scale infestation. These susceptible cultivars can still be successful in the landscape, as euonymus scale can be suppressed using integrated pest management practices including the release of *Chilocorus kuwanae* (Silvestri), an introduced ladybird beetle, and applications of horticultural oils, insecticidal soap, or conventional insecticide.

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Seed Source Affects Seedling Development and Nitrogen Fixation of *Maackia amurensis*[1]

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Abstract

Seeds of *Maackia amurensis* Rupr. & Maxim. were obtained from 38 sources, and 2,393 seedlings were examined for variation in plant growth and development. Mean stem length and number of leaves per plant after 21 months ranged from 10 to 40 cm (4 to 16 in) and from 6 to 13, respectively, among seedlings from different sources. The mean product of length and width of a representative leaflet was 362 to 1510 mm^2 (0.6 to 2.3 in^2) among sources. A subset of seven seed sources was used to determine how seedling growth, root nodulation, and N content of shoots are influenced by applied N and inoculation with *Bradyrhizobium*. Plants from the seven sources varied in nodule dry mass and shoot N. Mean stem length, laminar area, and dry mass of plants provided N and grown in uninoculated medium were higher than those of plants not provided N regardless of inoculation. When N was not provided, inoculation increased N in shoots but did not affect growth. Nodule dry mass of plants in inoculated medium was correlated positively with surface area of lamina. Variation among seedlings provides a basis for selecting genotypes that produce high nodule mass and grow rapidly.

Index words: Amur maackia, *Bradyrhizobium*, sustainable production.

Significance to the Nursery Industry

Maackia amurensis has potential for increased use in small and urban landscapes, but little attention has been given to selecting superior forms. This project focused on variation among plants we grew from seeds collected from 38 sources at 17 locations in the United States. Variation in growth among seedlings from all sources was determined in the first experiment because slow growth of plants in nurseries may be discouraging production of this species. In a second experiment, variation in root nodulation, N content of shoots, and plant growth were assessed among seedlings from seven sources. Both experiments demonstrated potential to select genotypes that grow quickly and nodulate well with rhizobial bacteria. This project provides the basis for long-term research we are conducting to identify genotypes of *M. amurensis* that are particularly well-adapted for low-input nursery production and survival in landscapes with infertile soils.

Introduction

Maackia amurensis is a tree species in the Fabaceae with traits desirable for landscaping (3, 7), and this species forms root nodules in which nitrogen gas (N_2) is fixed by rhizobial bacteria (2). Management of N_2 fixation by growers of *M. amurensis* might facilitate nursery production with low inputs of N fertilizer and improve the performance of trees at landscape sites with infertile soils.

Commercial production and use of *M. amurensis* are limited, perhaps because plants of this species grow slowly. Dirr (4) noted that shoot height of trees has been reported by others to increase by only 3.7 m (12 ft) over 20 years. Seedlings also grow slowly. Seedling mass of two other legumes, *Gleditsia triacanthos* L. var. *inermis* Willd. (thornless honey locust) and *Sophora japonica* L. (Japanese pagoda tree), was

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more than three times higher than seedling mass of *M. amurensis* after the first 6 weeks of development (6). Producing genotypes selected to grow rapidly could shorten nursery production time, reduce costs, and increase the appeal of this species to consumers. Identifying patterns of variation in growth of seedlings would facilitate selection efforts. Thus, one objective of this project was to study variation in development among seedlings grown from seeds from numerous sources.

Selection of genotypes to maximize biological N_2 fixation has been reported recently for other woody legumes (12, 13). Variation in nodulation among inoculated seedlings of *M. amurensis* has not been evaluated but could indicate whether genotypes differ in the benefit they derive from rhizobial N_2 fixation. Therefore, the second objective of this project was to assess variation in root nodulation, N content of shoots, and growth of seedlings that either were dependent on N_2 fixation or were irrigated with a solution that contained N.

Materials and Methods

Traits of plants from 38 seed sources. Seeds of *M. amurensis* were obtained from 17 locations in the U.S. (Table 1). Each location provided one to seven groups of seeds that we considered individual sources (Table 1). Seeds within sources were from the same maternal parent (half sibs) except for sources 4–3 and 17 (Table 1).

The number of seeds germinated from each source was based on the number of seeds available and ranged from six to 92. Half of the seeds to be germinated from each source were scarified in 18-M sulfuric acid for 1 hour and rinsed in deionized water on August 30, 1992. Procedures were repeated on September 9, 1992, for the remaining seeds. Scarified seeds were placed in paper towels moistened with tap water and held in darkness at 20C (68F). After 1 week, germination was determined by inspecting each seed for emergence of the radicle, and all seedlings with normal develop-

Table 1. Information on the sources of seed used to produce plants of *Maackia amurensis*. Seeds within sources were half-sibs in all cases where the number of sources from a location matches the number of trees at a source from which seeds were collected.

Seed source number	Location of parent tree(s)	No. of trees from which seeds were collected	Miscellaneous
1	Madison, WI	1	Tree obtained as seedling in 1957 from Scanlon Nursery in Ohio.
2–1, 2–2	Lisle, IL	2	Seeds in 2–1 were from a tree grown from seed collected in Russia. Both trees were at Morton Arboretum.
3–1 through 3–4	Lincoln, NE	4	Trees were obtained from the Univ. of Nebraska-Lincoln nursery in 1990.
4–1 through 4–3	Chanhassen, MN	9	Sources 4–1 and 4–2 contained seed from single trees. Seeds in 4–3 were from seven half-sib trees. All trees were at the Univ. of Minnesota Landscape Arboretum.
5	Portland, OR	unknown	Seeds were collected at Hoyt Arboretum.
6	Brookfield, IL	1	Trees from Hooks Nursery (IL) were planted at Brookfield Zoo in 1978.
7–1 through 7–7	Clermont, KY	7	Trees from Scanlon Nursery were planted at Bernheim Forest Arboretum in 1961.
8–1 through 8–3	Jamaica Plain, MA	3	Trees were at Arnold Arboretum.
9	Philadelphia, PA	1	Trees has been at the Morris Arboretum since 1964.
10	Mentor, OH	1	Tree was grown at the Holden Arboretum from seed received in 1955 from the Gothenburg Botanic Garden in Sweden.
11	New London, CT	1	Tree was at Connecticut College and was purchased from Scanlon Nursery in 1978.
12	Swarthmore, PA	1	Tree was grown at the Scott Arboretum from a seedling obtained in 1964 from J. Frank Schmidt and Son Co.
13	Seattle, WA	1	Tree was *M. amurensis* var. *buergeri* (Maxim.) C.K. Schneid. It was at the Washington Park Arboretum and had been obtained from Arnold Arboretum.
14	Wallingford, PA	1	Tree was at the Taylor Memorial Arboretum and had been obtained from Kingsville Nursery in 1957.
15–1 through 15–5	Washington, DC	5	Trees were located at the U.S. Dept. of Agriculture National Arboretum.
16–1 through 16–4	Silver Spring, MD	4	Trees were located at Sligo Creek Park.
17	Ames, IA	unknown	Seeds were collected in the 1920s from China and provided by the North Central Region Plant Introduction Station.

Table 2. Stem length, leaf number, and product of length and width of a representative leaflet of seedlings of *Maackia amurensis* grown from seeds sown in Aug. and Sept. of 1992 from 38 sources. Stem length data were collected during May of 1994. Leaflet data were collected during May and June of 1993.

Seed source	n	Mean ± SE stem length (cm)	Mean ± SE leaf no.	Mean ± SE product of leaflet length and width (mm^2)
1	108	20 ± 1	8 ± 0.3	936 ± 38
2–1	12	21 ± 2	8 ± 0.7	1006 ± 114
2–2	20	19 ± 2	6 ± 0.5	869 ± 77
3–1	45	25 ± 1	8 ± 0.4	743 ± 57
3–2	23	19 ± 2	7 ± 0.5	537 ± 52
3–3	6	23 ± 2	9 ± 0.9	697 ± 118
3–4	4	21 ± 3	10 ± 3.9	523 ± 153
4–1	52	15 ± 1	6 ± 0.4	618 ± 40
4–2	53	19 ± 1	6 ± 0.4	807 ± 35
4–3	49	32 ± 2	10 ± 0.6	862 ± 59
5	86	23 ± 1	7 ± 0.3	1116 ± 40
6	65	30 ± 2	11 ± 0.4	362 ± 19
7–1	40	40 ± 2	11 ± 0.7	515 ± 35
7–2	27	36 ± 3	11 ± 0.9	574 ± 50
7–3	33	37 ± 2	11 ± 0.6	597 ± 43
7–4	58	31 ± 1	11 ± 0.5	605 ± 30
7–5	80	25 ± 2	10 ± 0.7	591 ± 32
7–6	62	36 ± 2	13 ± 0.6	535 ± 27
7–7	63	31 ± 1	11 ± 0.4	572 ± 28
8–1	73	20 ± 1	6 ± 0.3	992 ± 38
8–2	152	18 ± 1	6 ± 0.4	1153 ± 40
8–3	90	21 ± 1	6 ± 0.2	1310 ± 46
9	46	19 ± 1	8 ± 0.4	1131 ± 61
10	84	10 ± 1	6 ± 0.2	654 ± 40
11	8	30 ± 3	11 ± 0.9	444 ± 50
12	43	19 ± 1	9 ± 0.4	1510 ± 105
13	124	20 ± 1	8 ± 0.2	655 ± 25
14	12	22 ± 3	8 ± 0.9	924 ± 90
15–1	170	32 ± 1	9 ± 0.2	1054 ± 31
15–2	18	27 ± 3	8 ± 0.7	1089 ± 98
15–3	163	26 ± 1	8 ± 0.2	971 ± 30
15–4	74	24 ± 1	7 ± 0.4	1201 ± 52
15–5	166	25 ± 1	8 ± 0.6	1036 ± 26
16–1	113	27 ± 1	8 ± 0.4	538 ± 27
16–2	62	29 ± 1	8 ± 0.4	525 ± 30
16–3	35	27 ± 2	7 ± 0.5	625 ± 35
16–4	18	26 ± 3	8 ± 0.6	669 ± 71
17	56	21 ± 1	8 ± 0.4	978 ± 42

ment were planted singly in plastic pots that were 6 cm (2.4 in) wide and had a volume of 325 cm^3 (20 in^3) (Belden Plastics, St. Paul, MN). The root medium was soil:perlite:*Sphagnum* peat moss (2:3:5 by vol). Seedlings were placed in random order on greenhouse benches under incandescent lamps that extended natural photoperiods to 16 hours. Pots were irrigated to container capacity with tap water as needed to keep the surface of the medium moist. Seedlings were stored at 4C (39F) from December, 1992, until April, 1993, when they were arranged randomly in a greenhouse without supplemental irradiance. Beginning in April, 1993, plants were fertilized once monthly with Peters Excel Cal-Mag Special 15N–2.2P–12.5K (Scotts, Marietta, GA) at 7.2 mol N/m^3 (100 ppm). Length and width of the terminal leaflet on the most basipetal leaf of each seedling were measured in random order in May and June, 1993. As data were collected, seedlings were repotted by using the same medium in round plastic pots that had a top diameter of 11 cm (4 in), a bottom diameter of 8 cm (3 in), a height of 10 cm (4 in), and a volume of 700 cm^3 (43 in^3).

Seedlings were vernalized after the 1993 growing season by adjusting the greenhouse thermostat to prevent heating until air was < 3C (37F). Shoot growth resumed with incipient bud break ≈ March 1, 1994. Stem length, defined as the distance from cotyledon scars to the apex of the longest stem, and the number of leaves on all seedlings were measured in random order during May, 1994. Means and standard errors (SE) within sources were determined for 1993 leaflet size data and for 1994 stem length and leaf number data. Large differences in the number of replicate seedlings among sources (Table 1) precluded use of a mean separation test.

N source effects on seedlings from seven sources. Amber glass Leonard jars (10) with a diameter of 10 cm (4 in) and a volume of 600 cm^3 (37 in^3) were filled with soil:perlite (1:4 by vol) and autoclaved for 1.5 hours. The soil, which was from a fallow field plot at Boone, IA, contained 7 mg N/m^3 (7 ppm) and was sieved through a 5-mm (0.2-in) screen before use. Sterile deionized water was added to the jars before seeds were sown until the medium was at container capacity.

Seeds from sources 1, 4–3, 6, 8–2, 12, 13, and 15–3 (Table 1) were scarified in 18-M sulfuric acid for 1 hr and rinsed in

sterile deionized water. Two seeds per jar were planted with sterile tools on October 29, 1993. There were 18 jars assigned to each source. Six jars per source were assigned to one inoculated and two uninoculated treatments. Seedlings in one uninoculated treatment received complete 25% Hoagland solution #2, whereas seedlings in the second uninoculated treatment and the inoculated treatment received N-free Hoagland solution (8). Both solutions contained 22 µmol Fe-EDDHA and were sterilized before application. USDA 4349 *Bradyrhizobium* in arabinose-gluconate (AG) medium (9) was used as inoculum. There were 10^8 cells/ml, and 2 ml were applied aseptically to a planting hole that was 1 cm (0.5 in) deep in all jars in the inoculated treatment. AG medium (2 ml/jar) without rhizobia was applied to planting holes of jars in the other treatments.

The 126 jars in the experiment were arranged as six completely randomized blocks on greenhouse benches. Air temperature was 24 ± 6C (75 ± 11F), and midday irradiance (400–700 nm) was 600 ± 285 $\mu mol \cdot s^{-1} \cdot m^{-2}$. We used sterile tools to thin seedlings to one per jar 4 weeks after planting. A second inoculation was done 6 weeks after planting by placing 2 ml of inoculum or AG medium without rhizobia on the medium at the base of the stem of each seedling. Sterile nutrient solutions were added to the reservoir of jars every 7 to 10 days to maintain a volume of ≈ 350 ml. Seedlings were harvested on January 22, 1994. Medium was washed from roots, and nodules were removed from roots. Surface area of lamina was measured on a leaf area meter (LI-3100, LI-COR, Lincoln, NE), and plant tissues were dried at 67C (153F) for 48 hr before mass was measured. N content of shoots was determined by using a Lachat autoanalyzer (Lachat Instruments, Milwaukee, WI).

This experiment was repeated February 8 to May 3, 1994. All procedures were duplicated except the second inoculation was done 2 weeks after seeds were sown. Data from the first and second replications were combined. Analysis of variance was done by using a factorial model for inoculation treatments and half-sib families. Mean separations were performed by using Fisher's LSD at $P = 0.05$.

Results and Discussion

Seed germination was 67 to 100% among sources, and the overall mean rate was 91% (5). Mean stem length and number of leaves after 21 months were 10 to 40 cm (4 to 16 in) and 6 to 13, respectively, among different sources (Table 2). The mean product of the length and width of representative leaflets of seedlings ranged from 362 mm^2 (0.6 in^2) for source 6 to 1510 mm^2 (2.3 in^2) for source 12 (Table 2). Leaf shape and color varied among sources, but these parameters were not quantified.

These results show growth of *M. amurensis* is influenced by seed source. Interestingly, SE of means for stem length, number of leaves, and leaflet size were of similar magnitude regardless of whether the seeds were confirmed half-sibs (Table 2). Uniformity of seedlings from source 4–3, which were not half-sibs, might have been because the seeds were from seven half-sib trees (Table 1). These trees are planted within 12 m (40 ft) of one another and were the most likely sources of pollen for cross pollination (S. McNamara, personal communication). The findings of this experiment indicate further efforts to identify fast-growing genotypes can be focused on sources such as 7–1, 7–2, 7–3, 7–6, 4–3, and 15–1. The tallest of our 2,393 seedlings was from source 7–1 and had a stem length of 73 cm. We have selected it and over 400 other relatively tall seedlings for long-term evaluation.

The second experiment revealed interactions ($P = 0.05$) between inoculation treatment and seed source for stem length, surface area of lamina, plant dry mass, and N in shoots. The magnitude of differences between inoculation treatment means among sources accounts for these interactions. Stem length of uninoculated plants given N ranged from 1.7 (source 12) to 3.3 (source 15–3) times that of inoculated plants not provided N (Table 3). Area of lamina on uninoculated plants given N ranged from 3.3 (source 1) to 5.9 (source 6) times that of inoculated plants not given N (Table 3). Dry mass of uninoculated plants provided N was 2.5 (source 1) to 5.3 (source 8–2) times that of inoculated plants not provided N (Table 3). Inoculated plants not given N had more N in their shoots than did uninoculated plants not given N (Table 3). Shoot N contents of inoculated plants not provided N and uninoculated plants supplied N were not different for most sources. Shoot N of plants from source 6, however, was higher among uninoculated plants given N than inoculated plants not given N (Table 3). The opposite was true for source 15–3.

There was no interaction between seed source and inoculation treatment for nodule dry mass. Mean nodule dry mass with inoculation treatments combined for sources 1, 4–3, 6, 8–2, 12, 13, and 15–3 were 15, 29, 7, 11, 13, 12, and 30 mg, respectively (LSD = 9 mg). Mean nodule dry mass for all

Table 3. Influence of seed source and inoculation treatment on growth and shoot N content of 12-week-old seedlings of *Maackia amurensis*. Values are means of nine to 12 replications.

Variable	Seed source	Inoculation treatment: –N Uninoculated	–N Inoculated	+N Uninoculated
Stem length (mm) (LSD = 44)	1	39	43	70
	4–3	98	76	214
	6	45	60	191
	8–2	71	66	153
	12	60	60	101
	13	45	51	125
	15–3	68	60	200
Laminar area (cm^2) (LSD = 67)	1	19	31	101
	4–3	86	64	306
	6	24	29	172
	8–2	40	45	241
	12	42	41	160
	13	22	37	189
	15–3	47	63	316
Plant dry mass (mg) (LSD = 683)	1	428	614	1560
	4–3	1002	780	2972
	6	396	390	1919
	8–2	795	709	3733
	12	659	662	1961
	13	375	555	1956
	15–3	653	794	3270
Shoot N content (%) (LSD = 0.36)	1	2.06	2.46	2.27
	4–3	1.74	2.42	2.33
	6	1.32	1.68	2.22
	8–2	1.12	1.87	1.93
	12	1.50	1.87	1.92
	13	1.88	2.35	2.20
	15–3	1.73	2.39	1.92

families combined for uninoculated plants not supplied N, inoculated plants not supplied N, and uninoculated plants supplied N was 18, 30, and 3 mg, respectively (LSD = 6 mg).

Results of the second experiment show the source of N affects growth of *M. amurensis*. Seedlings irrigated with N generally had longer stems, lamina with more surface area, and higher mass than seedlings inoculated with *Bradyrhizobium* and not provided N. This might have been because 2.9 to 6.1 g carbon/g N is assimilated during N fixation, whereas assimilation of mineral N requires 0.8 to 2.4 g carbon/g N (1). The efficiency of N_2 assimilation by *Bradyrhizobium* USDA 4349 should be compared under nursery conditions with that of other rhizobia (2). Greater differences in growth between inoculated and uninoculated plants not given N might have been detected if uninoculated plants were free of nodules. Nodulation of uninoculated plants shows there was contamination of the root zone with *Bradyrhizobium*, but nodule mass of uninoculated plants was lower than that of inoculated plants, which validates comparisons of plants in these treatments.

There are at least two explanations for the differences in nodule mass among the seven groups of half-sib seedlings. First, nodule development may have been a function of photosynthetic productivity, which presumably varied with the lamina surface area (Table 3). Up to 10% of the photosynthates of legumes may be used by rhizobia during symbioses, and inadequate production of photosynthates can limit nodule function (1). We found a highly positive correlation ($r^2 = 0.88$, $P < 0.0001$) between lamina area and nodule mass. Therefore, area of lamina may be an important selection criterion if optimizing nodulation is desired. Data on plants from source 8–2, however, indicate the extent of nodulation is not influenced solely by area of lamina. When provided N, the area of lamina of plants from sources 4–3 and 8–2 was not different, but nodule mass of plants from source 8–2 was 62% less than that of plants from source 4–3. This finding underscores a second potential explanation for differences in nodule mass among the half-sib groups. It is possible that half-sib groups differed in their compatibility with USDA 4349 *Bradyrhizobium*. Our results provide justification for further work to identify genotypes of both *M. amurensis* and rhizobia that form particularly efficient symbioses, which has been accomplished for other woody legumes (12, 13).

It has been suggested that inoculation of *M. amurensis* with rhizobia might allow production in low-N media without use of mineral N (2, 5, 11). Data from this study and a previous experiment (11) do not support this concept. Well-nodulated plants not provided N consistently have had < 50% of the dry mass of plants supplied N. The slow growth of nodulated plants not given N indicates commercial growers of *M. amurensis* must supply mineral N to achieve near-maximal growth rates. Inoculation did not increase growth but did increase N in shoots among plants not given N (Table 3). Leaves of inoculated plants were dark green, whereas leaves of uninoculated plants were chlorotic. Sustaining the N content and healthy appearance of leaves of trees at landscape sites with poor soils may be the most horticulturally significant role for N_2 fixation in *M. amurensis*.

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