

Response of Grafted *Juglans nigra* to Increasing Nutrient Availability: Growth, Nutrition, and Nutrient Retention in Root Plugs

K. Francis Salifu and Douglass F. Jacobs

Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907

Guillermo Pardillo and Mary Schott

American Forestry Technologies Inc., 7852W 2005, West Point, IN 47992

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Abstract. We examined growth and nitrogen (N), phosphorus (P), potassium (K), and microelement nutrition of grafted black walnut (*Juglans nigra* L.) seedlings exposed to increasing nutrient supply and grown in the greenhouse for 18 week. Plants were potted and grafted within the first 4 week, then fertigated once each week for a 7-week period with a varying nutrient solution of 20N-4.4P-16.6K that delivered 0, 1160, 2320, and 4620 mg N/plant. Plants were harvested at week 18. There was a positive mean growth response to increased fertilization, although trends were statistically similar across treatments. Leaf nutrient concentration ranged from 22 to 31 g • kg⁻¹ N, 5 to 14 g • kg⁻¹ P, and 19 to 25 g • kg⁻¹ K. The 2320 mg N/plant treatment increased leaf nutrient content 18% to 86% for N, 33% to 303% for P, and 23% to 58% for K compared with the control. Nitrogen efficiency decreased with increased N supply. Increased nutrient retention in the growing medium at higher fertility suggests root plugs could serve as immediate critical nutrient sources for grafted black walnut seedlings after outplanting. Study results suggests nursery fertilization can be used to improve the nutritional quality of grafted black walnut as well as store nutrients in root plugs for later utilization to benefit early establishment success.

Black walnut (*Juglans nigra* L.) is considered among the most valuable hardwood tree species in North America because of its high-quality timber and multiple uses (Ponder, 2004; Williams, 1990). Black walnut typically occurs as a minor component of mixed deciduous forests in central and eastern parts of the United States and in the deciduous forest region of southern Ontario (Williams, 1990). Although judicious nursery fertilization can lead to the production of high-quality seedlings with improved field performance (Landis et al., 2005; Salifu and Timmer, 2003; Smith et al., 2005), nutritional information is relatively unavailable for black walnut seedlings. Lack of nursery fertilization data for black walnut may be related to

the assumption that the large seed may provide sufficient nutrition for seedlings during the nursery stage. Similarly, internal remobilization of nutrients from rootstock could provide adequate nutrition to fuel new growth of grafted plants. Although field studies suggest black walnut seedling growth may increase (Jacobs et al., 2005; Ponder, 1998) or decrease (Beineke, 1986; Ponder, 1996, 2004) with fertilization, we are not aware of any published information to explain how walnut might respond to nutrient inputs at the nursery stage, especially for grafted stock. A clear understanding of how grafted black walnut plants respond to nutrient inputs may provide useful information to improve nutritional stock quality for better field performance. Additionally, a balance sheet of N input, uptake, and use efficiency in nursery fertilization programs will be useful to help guide decisions regarding efficient use of fertilizer (Dumroese and Wenny, 1992). Such information is not available for walnut.

Furthermore, it has been suggested that root plugs of container plants may serve as nutrient sinks and store excess nutrients not exploited by plants (Idris et al., 2004). These plugs may act as critical nutrient sources to meet early nutrient demand of newly transplanted seedlings (Idris et al., 2004; Timmer and Teng, 2004). In the present study, we examined this hypothesis by quantifying

macro- and microelement status of the growing substrate used for growing grafted walnut under four fertility levels to characterize nutrient retention capacity of the medium. This information allowed us to evaluate the potential of the root plugs to serve as sinks for nutrients at the nursery stage and provide critical nutrient sources to help meet early plant demand after field transplant.

Thus, we examined growth and nutrition of grafted walnut seedlings in relation to increased nutrient supply over a 7-week fertigation period when grown for 18 week in the greenhouse. Additionally, we computed a balance sheet of N input, uptake, and use efficiency across the N availability gradient to help understand N dynamics in this plant-soil system. We tested the hypotheses that: 1) grafted black walnut plants will respond positively to increased nutrient availability; 2) seedling root plugs act as sinks for excess nutrients not exploited by plants, which will be demonstrated by increased nutrient retention in the growing medium at higher fertility; and 3) a nitrogen balance sheet will show the appropriate levels for N input, uptake, and use efficiency by plants.

Materials and Methods

Growth conditions. Scion material from one single clone (expired patent Purdue #1) was grafted onto 2-year-old rootstock seedlings in Mar. 2005. The rootstock were raised from nuts collected locally in Indiana and sown in nursery beds in Fall 2002, germinating in Apr. 2003, and grown in the nursery for two summers (2003 and 2004). In Fall 2004, the rootstock seedlings were lifted from the nursery beds and potted into 2.8-L Treepots (Stuewe and Sons, Corvallis, Ore.) filled with Scotts Metro-Mix 560 growing medium (The Scotts Co., Marysville, Ohio). This medium contains 35% to 54% composted pine bark, 20% to 30% processed coconut cow pith, 10% to 20% sphagnum peatmoss, 5% to 15% processed bark ash, and 5% to 15% horticultural perlite. Additional information on media physical properties and chemistry are presented and discussed in Salifu et al. (2006). The dormant rootstocks were kept in the greenhouse until Apr. 2005, when the grafting process took place. Plants were top-grafted at the stage of leaf burst and expansion. The grafting phase was accomplished within 4 week (Table 1). Subsequently, grafted plants were exposed to four fertility treatments. One treatment was a non-fertilized control. The other three fertility treatments consisted of weekly fertigation with Peat-Lite Special 20N-4.4P-16.6K plus microelements (The Scotts Co.). Within each fertigation treatment, the amount of N in the nutrient solution increased over time, resulting in a total N application over the 7-week period of 0, 1160, 2320, and 4620 mg N/plant (Table 1). Total N consisted of NH₄-N (7.97%) and NO₃-N (12.03%). Each treatment replication contained 24 seedlings grown in 2.8-L pots. Thus, the experiment consisted of a total of 96 plants per treatment. The four

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Table 1. Amount of nitrogen fertilizer supplied to grafted black walnut seedlings in irrigation for 7 weeks when grown for 18 weeks in a greenhouse. The same volume of irrigation (1.6 L) was supplied to each seedling at each fertigation event.

Fertilizer rate (mg/plant)	Amount of fertilizer in irrigation (mg)									Total 7 Weeks
	April		May			May/June			Week 11	
	Weeks 1 to 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10			
0 (control)	Grafting phase	0	0	0	0	0	0	0	0	0
1160	Grafting phase	120	120	120	160	160	240	240	1160	
2320	Grafting phase	240	240	240	320	320	480	480	2320	
4620	Grafting phase	480	480	480	640	640	950	950	4620	

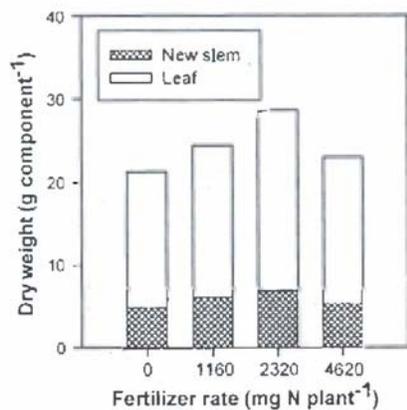


Fig. 1. Dry weight for scion components of grafted black walnut plants in relation to increased nitrogen supply at four fertility levels for 7 weeks when grown for 18 weeks in a greenhouse (see Table 1 for complete description of treatments).

fertility treatments were installed as a completely randomized design with four replications (384 plants total) and arranged on a greenhouse floor (mean temperature of 25 °C) under ambient light conditions at the American Forestry Technologies Enc. greenhouse facility in West Point, Ind. (40°25'N, 86°55'W). Fertilization commenced at week 5, immediately after the grafting phase and continued for another 7 week (Table I). Each plant was hose-irrigated with L of the nutrient solution with pH corrected to 6.5 through injection with sulfuric acid at each fertigation event. Nitrogen supplied to plants (mg) was computed as the average ppm N of solution x total liters of irrigation applied as detailed in Dumroese and Wenny (1992). This information was used to construct a balance sheet of N input, uptake, and use efficiency.

Plant sampling, chemical and statistical analyses. Two pots were randomly sampled from each replicate (a total of eight plants) per treatment 18 week after planting. After harvest, roots were washed free of media, and seedlings were partitioned into leaves (part of scion), new stems (part of scion), old stem (part of rootstock), and roots (from rootstock). All samples were composited by treatment replication for nutritional analysis but measured individually and averaged for growth analysis. Plant material was oven dried for 48 h at 70 °C and ground. A complete nutritional analysis

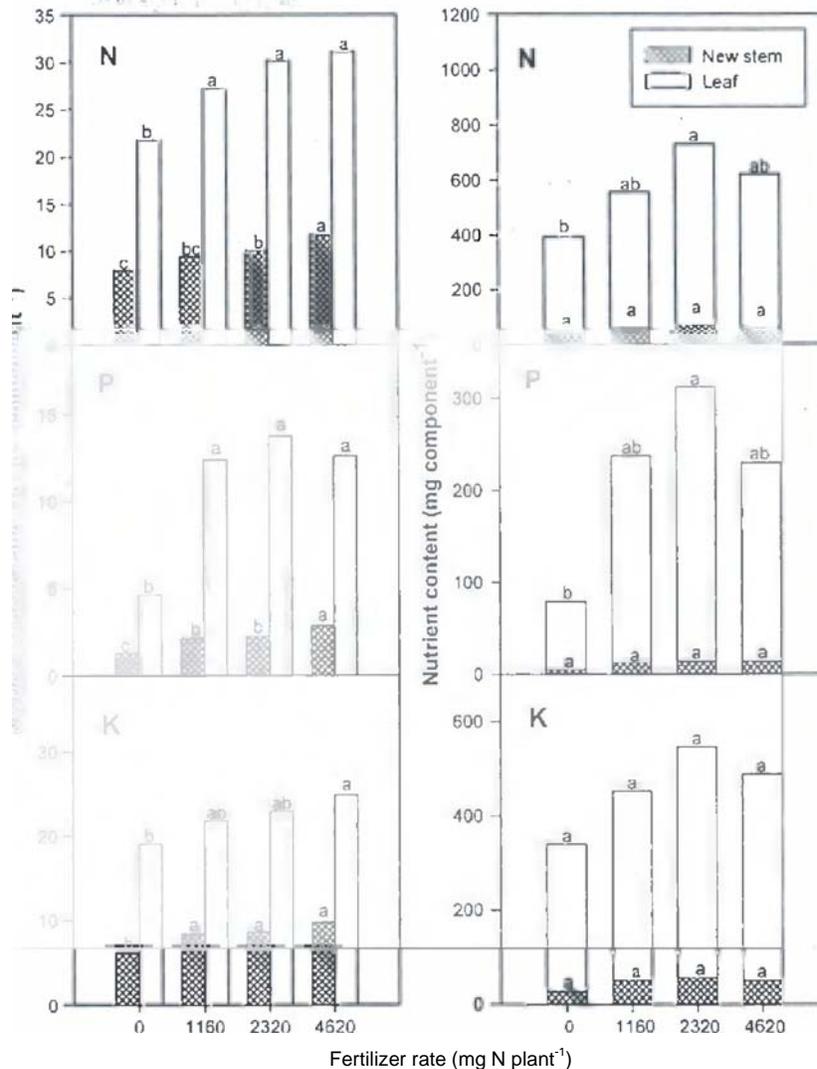


Fig. 2. Nutrient concentration (left) and nutrient content (right) for scion components of grafted black walnut plants in relation to increased nitrogen supply at four fertility levels for 7 weeks when grown for 18 weeks in a greenhouse (see Table 1 for a complete description of treatments). Similar bars marked with different letters differ statistically according to Tukey's honestly significant difference test at $\alpha = 0.05$.

determined by combustion "Dumas" procedure (Association of Official Analytical Chemist [AOAC] 968.06) using a LECO nitrogen analyzer (LECO Corp., St. Joseph, Mich.). Additionally, plant samples were digested in nitric + perchloric acids (AOAC 935.13) and the other elements determined using inductively coupled argon plasma (ICAP) analysis (AOAC 985.01). EC (EC), NO₃-N, Nas-N, P,

and K of growing media sampled at harvest were determined from saturated aqueous extracts following procedures described in Brown (1997). Growth and nutritional data were subjected to one-way analysis of variance and significant ($P < 0.05$) means were ranked according to Tukey's honestly significant difference test at $\alpha = 0.05$ using SAS software (SAS Institute, 2001).

Table 2. Plant (mot, old stem, new stem and leaf) N content, and microelement concentration in root plugs and in leaves of grafted black walnut plants exposed to increased fertility levels for 7 weeks when grown for 18 weeks in a greenhouse in 2005.

Fertilizer rate (mg N plant ⁻¹)	N content (mg)	Microelement concn (mg · kg ⁻¹)							
		Growing media				Leaf			
		B	Fe	Mn	Zn	B	Fe	Mn	Zn
0 (control)	1460 b	0.10 c	0.35 b	0.10	0.10 b	52.25	144.75 b	123.75	51.00
1160	2640 a	0.15 bc	0.55 b	0.10	0.10 b	69.75	162.75 ab	153.75	50.75
2320	2710 a	0.25 b	0.38 b	0.10	0.10 b	66.25	166.75 ab	169.25	56.00
4620	3050 a	0.48 a	1.10 a	0.38	0.20 a	69.50	190.50 a	220.50	41.00
ANOVA <i>P</i> < <i>F</i>	0.0084	0.0001	0.0014	0.1506	0.0097	0.0606	0.0077	0.2334	0.6233

Column means followed by different letters differ statistically according to Tukey's honestly significant difference test $\alpha = 0.05$.

Results and Discussion

Growth and nutrition. Growth of the new scion is partitioned into leaf and new stem as shown in Figs. 1 and 2. Seedlings fertilized at 2320 mg N/plant accumulated greater mean dry weight in leaves and new stems than the others, although the observed differences were nonsignificant ($P > 0.05$) (Fig. 1). This growth response is likely associated with induced luxury consumption in walnut seedlings, which is demonstrated by significant leaf N uptake (Fig. 2, right), but no differences ($P > 0.05$) in dry weight (Fig. 1). Such trends have been previously noted with fertilization of black spruce (*Picea mariana* Mill. BSP) seedlings (Salifu and Timmer, 2003). Decreased component dry weight at the highest fertility level suggests potential over fertilization. Total root dry weight was similar across all fertility treatments with means (SE) varying from 95 (11) g for the control, 89 (4) g for the 1160 mg N/plant treatment, 96 (20) g for the 2320 mg N/plant treatment, and 84 (7) g for the 4620 mg N plant⁻¹ treatment.

Leaves acted as sinks for nutrients as demonstrated by greater allocation to these tissues (Fig. 2). New stem and leaf N, P, and K concentrations increased with increasing N supply (Fig. 2, left). Nutrient levels in leaves of black walnut seedlings may range from 25 to 30 g · kg⁻¹ for N, 2 to 3 g · kg⁻¹ for P, and 13 to 15 g · kg⁻¹ for K (Frak et al., 2005; Ponder, 2004). Additionally, over 26 g · kg⁻¹ N, 2.5 g · kg⁻¹ P, and 13 g · kg⁻¹ K in leaves may be considered adequate for black walnut seedling growth (Ponder, 2004). Leaf nutrient concentrations ranged from 22 to 31 g · kg⁻¹ N, 5 to 14 g · kg⁻¹ P, and 19 to 25 g · kg⁻¹ K in this study (Fig. 2, left). This observation suggests nutrient levels were adequate in fertilized seedlings based on the recommendations by Ponder (2004). The similarity in N status noted in leaves of 2-year-old hybrid walnut (*Juglans nigra* × *regia*) trees (25-29 g · kg⁻¹) (Frak et al., 2005) with results of our study (Table 2) and those documented by (Ponder, 2004) support this contention. Nutrient levels in controls were below those recommended as adequate for growth of walnut seedlings (Fig. 2). This implies that native fertility of the media was inadequate for plant growth even in the short term, suggesting the need for nutrient supplementation when using such medium for raising plants.

Fertility treatments significantly affected plant (leaf, new stem, old stem and root combined) total N (Table 2). Similarly, leaf N and P contents increased with fertility, contrasting markedly with the observed similar uptake in new stems (Fig. 2, right). The 2320 mg N/plant fertility treatment was also associated with greater nutrient retention in

associated with greater nutrient acquisition. For example, nutrient content increased 18% to 86% for N, 33% to 303% for P, and 23% to 58% for K in leaves in the 2320 mg N/plant treatment compared with the others. The 2320 mg N/plant fertility treatment was also associated with greater nutrient retention in

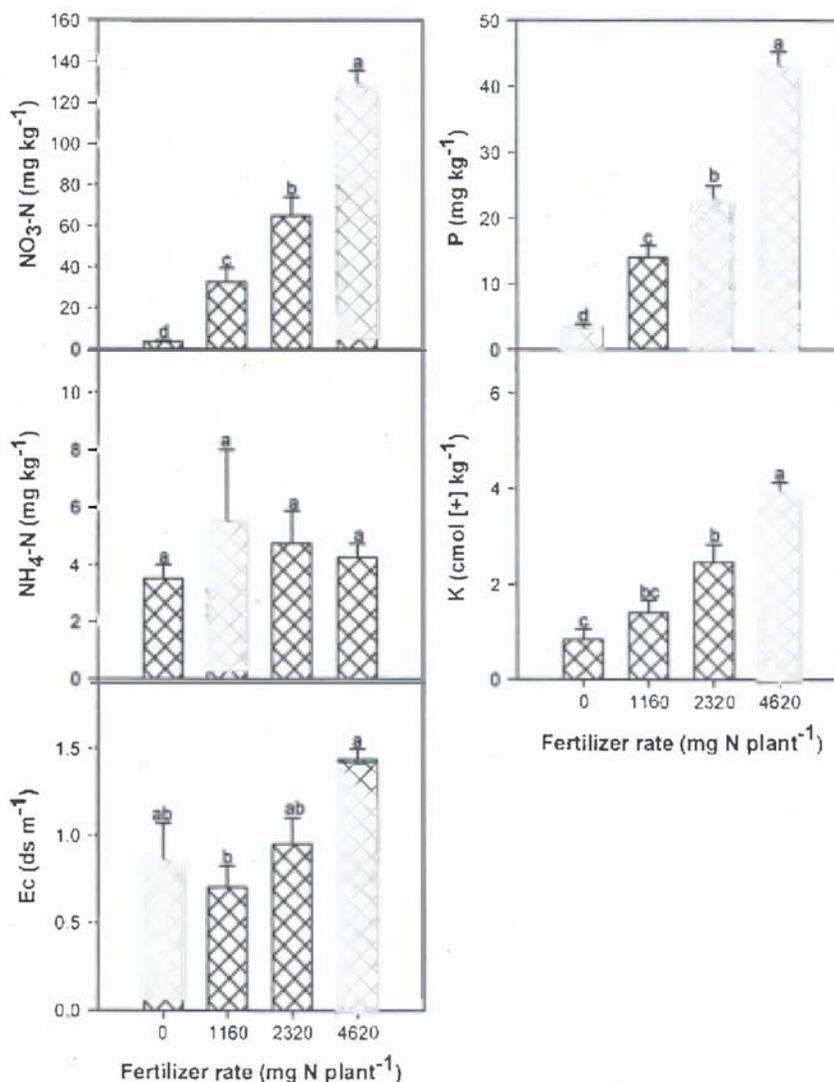


Fig. 3. Chemical characteristics of root plugs sampled from grafted black walnut seedlings exposed to increased nitrogen supply at four fertility levels for 7 weeks when grown for 18 weeks in a greenhouse (see Table 1 for a complete description of treatments). Similar bars marked with different letters differ statistically according to Tukey's honestly significant difference test $\alpha = 0.05$.

Table 3. Balance sheet of total irrigation, N input from irrigation, N uptake from fertilizer and use efficiency in grafted black walnut seedlings exposed to increased fertility levels for 7 weeks when grown for 18 weeks in a greenhouse in 2005.

Fertilizer rate (mg N/plant)	Irrigation (l)	N Supplied (mg plant ⁻¹)	Total N content (mg)		Fertilizer N efficiency (%)	Unaccounted N (g)
			Whole plant	Fertilizer		
0 (control)	11.12	0	1460	0	0	0
1160	11.12	1160	2630	1170	102	-0.01
2320	11.12	2320	2710	1250	54	1.08
4620	11.12	4620	3050	1590	35	3.02

Note: N uptake from fertilizer obtained by deducting N content of the controls (0) from the other treatments.

the growing medium (Fig. 3). Thus, the 2320 mg N/plant treatment may represent the desired dose rate under the prevailing cultural conditions in this current study. However, we recommend further studies to examine a greater range of fertilizer inputs as well as to refine analysis of fertilizer responses between 1160 and 2320 mg N/plant and between 2320 and 4620 mg N/plant, which could improve diagnostic precision.

Generally, foliar microelement concentrations increased at higher nutrient inputs and differed for iron (Fe) (Table 2). Similarly, microelement concentrations of media increased with fertility and differed for boron (B), zinc (Zn), and Fe. Published foliar microelement concentrations considered adequate for growth of hardwood species may range from 80 to 90 mg·kg⁻¹ for Fe and from 25 to 50 mg·kg⁻¹ for Zn (Stone, 1968). (Ponder 2004) documented ranges from 69 to 129 mg·kg⁻¹ for Fe and from 33 to 55 mg·kg⁻¹ for Zn in leaves of black walnut seedlings. Thus, the results of our study (Table 2) are in general agreement with this published information.

Nutrient retention in root plugs and N uptake efficiency. Increased substrate fertility with nutrient supply (Fig. 3) promoted nutrient uptake by plants at higher availability (Fig. 2, Table 3). However, N uptake efficiency decreased with increased substrate fertility (Table 3) concurring with results noted elsewhere (Dumroese et al., 2005). The N unaccounted for by the plants at higher fertility (Table 3) was reflected in greater N retention in the growing media. For example, electrical conductivity (EC), a reflection of soluble salts and substrate fertility, increased at higher fertility (Table 3) confirming greater nutrient availability in media (Fig. 3). EC is useful for monitoring fertilization protocols (Dumroese et al., 2005; Jacobs and Timmer, 2005; Landis et al., 1989). The EC range found in this study (Fig. 3) is within the range of substrate EC recommended (0.302-5.0 ds m⁻¹) for optimum growth of conifer container stock (Phillion and Bunting, 1983; Timmer and Parton, 1984) and below the level (2.60 ds m⁻¹) at which Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] root penetration was inhibited (Jacobs et al., 2003).

The greater nutrient retention in media at higher fertility (Fig. 3) suggests seedling root plugs acted as sinks for nutrients by storing excess nutrient resources not captured by plants. Such nutrient pools in root plugs could serve as important nutrient sources to fuel new growth of outplanted seedlings (Idris

et al., 2004; Timmer and Teng, 2004). Grafted walnut seedlings responded positively to increased nutrient availability, suggesting that fertilization can be used to improve the nutritional quality of grafted black walnut plants in the nursery, which could benefit outplanting performance. Further studies are needed to examine how nursery fertility treatments affect field performance of grafted black walnut stock. Additionally, the hypothesis that root plugs could serve as critical nutrient sources to fuel new growth of outplanted grafted black walnut plants warrants further testing.

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