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Growth, Nutrition, and Photosynthetic Response of Black Walnut to Varying Nitrogen Sources and Rates

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ABSTRACT

Black walnut (*Juglans nigra* L.) half-sib 1+0 seedlings were exponentially fertilized with ammonium (NH_4^+) as ammonium sulfate [$(NH_4)_2SO_4$], nitrate (NO_3^-) as sodium nitrate ($NaNO_3$), or a mixed nitrogen (N) source as ammonium nitrate (NH_4NO_3) at the rate of 0, 800, or 1600 mg N plant^{-1} and grown for three months. One month following the final fertilization, N concentration, growth, and photosynthetic characteristics were assessed. Compared with unfertilized seedlings, N addition increased plant component N content, chlorophyll content, and photosynthetic gas exchange. Net photosynthesis ranged from 2.45 to 4.84 μmol m^{-2} s^{-1} for lower leaves but varied from 5.95 to 9.06 μmol m^{-2} s^{-1} for upper leaves. Plants responded more favorably to NH_4NO_3 than sole NH_4^+ or NO_3^- fertilizers. These results suggest that N fertilization can be used to promote net photosynthesis as well as increase N storage in black walnut seedlings. The NH_4NO_3 appears to be the preferred N source to promote black walnut growth and physiology.

Keywords: photosynthesis, chlorophyll, nitrate, ammonium, nitrogen, *Juglans nigra*

INTRODUCTION

Black walnut (*Juglans nigra* L.) is one of the most valuable species of the Central Hardwood Forest Region of the United States. The species is used for high quality lumber and veneer (Cassens, 2004) and has additional value for

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nut production and wildlife (Williams, 1990). Total volume of black walnut in the United States increased from 45.3 million m^3 in 1989 to 68.0 million m^3 in 1999 and saw log volume increased from 10.1 million m^3 to 17.0 million m^3 (Shifley, 2004). Considering the value and increasing harvested volume of black walnut, optimizing growth and physiology of planting stock used for plantation establishment is important for maximizing production.

Nitrogen (N) fertilization of tree crops is often used to promote growth and survivability. In the case of black walnut, field fertilization has generally led to decreases in growth because competing vegetation pre-empts growth resources and suppresses development of target trees (Williams, 1974; Ponder, 2004). Especially on marginal lands, such as reclaimed surface-mined lands, nutrient resources may not be sufficient to meet growth demand of tree seedlings (Lea and Brockway, 1986; Andersen et al., 1989). Thus, vegetation control used in combination with field fertilization may benefit walnut growth and nutrition, as noted for several hardwood species (Jacobs et al., 2005).

Recent advances in black walnut genetics will continue to progress in the next few years (Bosela et al., 2004; Michler et al., 2005). For such advances in hardwood genetics to be meaningful, they need to be coupled with an understanding of silvicultural techniques (Michler et al., 2005) and seedling physiology (Wilson and Jacobs, 2006). Because nutritional studies in black walnut have generally focused on effects of fertilization in the field, much less is known about the physiological, nutritional, and growth responses of black walnut to fertilization in an environment without competing vegetation. It is important to determine how black walnut seedlings respond to N fertilization and particularly to varying N sources to help optimize response and maximize potential gains from genetic manipulation.

Nitrogen is the primary nutrient element needed in the greatest quantities for plant growth and physiology, hence the focus on N in the current study. Nitrogen is a constituent of proteins, amino acids, RNA, DNA, and many other essential molecules in plants. Perhaps the greatest impact that N has on plant nutrition is in relation to photosynthesis. The largest portion of N allocated to leaves is invested in ribulose 1,5-bisphosphate carboxylase-oxygenase (RUBISCO). Nitrogen is also a necessary component of other enzymes involved in carbon assimilation reactions of photosynthesis and in the light-energy harvesting machinery of the pigment complexes (Lawlor, 1994).

Additionally, the source of N can have a strong impact on the energy demand for N uptake, transport, and incorporation into plant proteins. Nitrogen is generally available in soils in inorganic form as nitrate (NO_3^-) or ammonium (NH_4^+) (Lips et al., 1990). Ammonium is converted into glutamine and glutamate in energy-requiring reactions catalyzed by the enzymes glutamine synthetase and glutamate synthase (Stewart et al., 1980). Nitrate must be converted into nitrite and then NH_4^+ in energy-requiring reactions catalyzed by nitrate reductase and nitrite reductase before it can be assimilated into amino acids. Therefore, NO_3^- requires more energy for assimilation than does NH_4^+.

Unlike NH_4^+ however, NO_3^- can be transported to the leaves before it is assimilated, where the reactions that convert it into amino acids take place using the direct products of the light reactions of photosynthesis, reducing energy costs to the plant (Lawlor, 1994). The location of NO_3^- assimilation varies widely among plant species, and so the question of which source of N supplies the plant with the least expense of energy is species-specific.

The N source used by the plant will determine how much photosynthetic energy is devoted to producing growth and how much must be expended on the physiology of N assimilation. Information on responses to N source is very limited for black walnut. For example, Gray and Garrett (1998) found increased flowering and nut production in adult black walnut trees with fertilization, but no significant effects between ammonium nitrate (NH_4NO_3) or with sodium nitrate ($NaNO_3$). Additionally, although net N transfer or retranslocation within the plant can provide significant quantities of N to promote new growth at a lower cost to the plant, the importance of retranslocation in walnut nutrition is not well elucidated.

In an earlier study, we found a greater increase in nitrate reductase activity with NH_4NO_3 than with $NaNO_3$ in 1+0 bareroot seedlings, but greater tissue N concentration with $NaNO_3$ than with NH_4NO_3 (Nicodemus et al., 2006). However, we were unable to identify the preferred N source that will promote walnut growth and nutrition. This current study was designed to determine the preference of black walnut seedlings to N source supplied as NH_4NO_3, $NaNO_3$, or ammonium sulfate [$(NH_4)_2SO_4$] at three different levels (0, 800, and 1600 mg N plant^{-1} season^{-1}). Fertilizer was applied exponentially in order to match N supply with plant demand, which increases N uptake efficiency and minimizes N leaching losses (Dumroese, 2003; Dumroese et al., 2005). We expected (1) improved growth, nutrition, and photosynthetic response in fertilized walnut seedlings compared to unfertilized plants, (2) NH_4NO_3 will promote greater growth, nutrition and photosynthetic response in walnut seedlings than NH_4^+ [as $(NH_4)_2SO_4$] or NO_3^- (as $NaNO_3$) alone (Brix, 1981; Kim et al., 2002), (3) higher leaf chlorophyll levels in response to increased N supplementation will be associated with greater photosynthetic production in walnut seedlings, and (4) net N transfer will benefit walnut seedling growth and nutrition.

MATERIALS AND METHODS

Plant Material and Treatments

Open-pollinated (from expired patent Purdue #1 mother tree) black walnut (*Juglans nigra* L.) seedlings were grown under operational bareroot production systems for one season in the Indiana State Nursery at Vallonia (38°85′N, 86°10′W). Open-pollinated seedlings from expired patent Purdue #1 were used

because of their high genetic quality and frequency of use in black walnut plantings (Beineke, 1989). In October 2004, the seedlings were lifted and over-wintered at 2°C in a cooler at the Purdue University Horticulture Farm. In April 2005, the seedlings were removed from cold storage and potted into 10.65 l Treepot(tm) containers (Steuwe and Sons, Corvallis, OR, USA) at the Purdue University Horticultural Plant Growth Facility (40°4′N, 86°30′W). Berger BM-6(tm) (Berger Peat Moss, St. Modeste, Quebec, Canada), which consists of 4:1 peat:perlite, was the planting medium used in this study. Plants were watered equally as needed (~1 l plant^{-1} week^{-1}) with clear water with pH adjusted to ~6.0, which is known to be within the ideal range for growing walnut seedlings (Williams, 1990). The pH was maintained at a steady level because N form preference has been shown to be pH specific in some species (van den Driessche, 1978). Air temperature ranged from 20 to 24°C and supplemental light (350 to 400 μmol m^{-2} s^{-1}) was used to provide 16 h of daylight.

Plants were fertilized with N as NH_4NO_3, $NaNO_3$, or $(NH_4)_2SO_4$ at 0, 800, or 1600 mg plant^{-1} season^{-1}. Thus, the experimental design was a 3 × 3 (N source × N rate) factorial design with 8 replications. Initial N content (N_s = 612 mg N plant^{-1}, Table 1) was determined from 5 plants, which was used to compute exponential fertilization regimes as detailed elsewhere (Timmer, 1997). The amount of fertilizer applied over the season (N_T) was 0, 800, or 1600 mg N plant^{-1}, each supplied over seven applications. Exponential fertilization was used to help maximize plant N uptake and physiological response to experimental treatments. Exponential fertilization has been successfully applied to one-year-old conifer seedlings (Dumroese, 2003). The approach has demonstrated merit for optimizing N uptake and minimizing losses (Timmer, 1997; Dumroese et al., 2005). One month following the final N application, physiological measurements were taken and seedlings were processed to determine dry mass of components and N concentration. Component N content was computed as concentration multiplied by dry mass. Nitrogen retranslocation is accurately determined using ^{15}N, but in this study, we estimated net N transfer as the differences in total N content in old shoots before and after treatment (Peuke et al., 1994; Salifu and Timmer, 2003).

Photosynthesis and Chlorophyll Measurements

Photosynthesis was measured in situ with a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). It has been shown that leaf rank affects physiological measurements in *Juglans* species (Frak et al., 2005). Therefore, photosynthesis was consistently measured on the oldest and youngest fully expanded leaves of each seedling on the second leaflet from the terminus. Measurements were taken at 1000 nmol light intensity and 400 ppm carbon dioxide (CO_2) from 1000 to 1400 h solar time on two subsequent days as μmol

Table 1
Component N content by N level and N source, and net N transfer within old stems (retranslocation). Net N transfer is computed as the differences in net N in old stems before and after planting. Negative values for net N transfer signify an increase in N uptake into old shoots (assimilation). Lower case letters signify significant differences within columns at $\alpha = 0.10$ as determined by Tukey's highly significant difference test

Treatment	N content (mg)					Net N transfer(mg)
	Old stem	New Stem	Roots	Leaves	Total	Old stem
Initial	58.80	0.00	552.98	0.00	611.78	—
End of season						
N level						
0 mg N	31.64b	10.87b	395.55b	186.41b	624.47b	27.16a
800 mg N	53.80a	23.53a	572.27ab	406.61a	1056.20a	5.00b
1600 mg N	59.54a	24.51a	682.09a	428.61a	1194.75a	−0.74b
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
N source						
$(NH_4)_2SO_4$	45.34	20.71ab	515.15	344.89a	928.71	13.46
$NaNO_3$	47.74	14.87b	534.04	303.60b	900.26	11.06
NH_4NO_3	51.88	23.33a	600.72	373.14a	1046.46	6.92
P-value	0.4576	0.0328	0.3568	0.0715	0.2002	0.4576

CO_2 assimilated m^{-2} s^{-1}. Leaf area was measured on a LI-3000 leaf area meter (Li-Cor Inc., Lincoln, NE, USA).

Leaf chlorophyll was measured using the method of Arnon (1949) as modified to use dimethyl sulfoxide (DMSO) (Hiscox and Israelstam, 1979). Dimethyl sulfoxide has been shown to be as reliable a solvent as acetone for chlorophyll extraction (Tait and Hik, 2003), but does not require maceration. Leaf disks of 100 mg were cut from the second leaflet from the terminus of the youngest and oldest fully formed leaf with a hole punch. Leaf discs were placed in test tubes with 7 mL of DMSO and transferred to a water bath at 60°C for 90 minutes, with a marble on the top of each tube to prevent solvent evaporation. The sample was then removed from the bath and made up to a total volume of 10 mL with DMSO. A 3 mL aliquot was transferred to a cuvette to measure absorbance. A Perkin-Elmer LC-95 UV/Visible spectrophotometer (Perkin-Elmer Inc., Norwalk, CT, USA) was used to measure the absorbance of the solution at 645 and 663 nm. Chlorophyll *a* (C_a), chlorophyll *b* (C_b), and total chlorophyll (C_T) were determined from the absorbance at 645 (D_{645}) and 663 (D_{663}) according to the following formulae (Arnon 1949):

$$C_a = 0.0299D_{645} - 0.0046D_{663}$$

$$C_b = 0.0127D_{663} - 0.00269D_{645}$$

$$C_T = C_a + C_b$$

Growth, Chemical, and Statistical Analysis

Plants were separated into leaves, roots, new and old stems following physiological measurements. New stems were those that were taken from the stem between the location of the initial terminus (at planting) and the final terminus of the stem (at harvest). These were placed in an oven for at least 72 hours at 70°C and component dry mass determined. Samples were weighed and ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass a 20 mesh sieve. For carbon (C) and N determination, 200 mg of sample was used. Carbon and N were determined by combustion using a LECO CNS-2000 analyzer (Leco Corporation, St. Joseph, MI, USA).

Analysis of variance (ANOVA) was conducted on measured data and where significant, treatment means were ranked according to Tukey's highly significant difference test at $\alpha = 0.10$ using SAS version 9.1 (SAS Institute Inc, Cary, NC, USA). The data were tested and found to meet the ANOVA assumptions for normality (Shapiro-Wilks test) and homogeneity of variances (Bartlett test). The Anderson and McLean's (1974) linear model for the ANOVA

is given as:

$$Y_{ijk} = \mu + S_i + R_j + SR_{ij} + \epsilon_{(ij)k}$$

Where:
Y_{ijk} is plant component dry mass, or measured physiological response of the *k*th replicate (k = 1, 2, 3,. . ., 8), estimated from the *j*th N rate (j = 1, 2, 3) from the *i*th N source (i = 1, 2, 3); μ = overall mean; S_i = fixed effect of the *i*th N source; R_j = fixed effect of the jth N rate; followed by the interaction effects and ε is the associated error from bulk replicates. Significant N source and rate interaction effects for leaf dry mass and leaf area were separated by orthogonal contrasts (Nogueira 2004), which examined N source effects within the 800 and 1600 mg N plant^{-1} levels. This approach has been successfully applied by other researchers to separate interaction means (Corrente et al., 2001).

RESULTS

Nitrogen Level Effects on Growth and Physiology

Unfertilized plants acquired little N from the medium, about 12.69 mg N plant^{-1}, which represents about 2.1% more N at final harvest compared to status at planting (Table 1). Net N transfer within the plant may have accounted for a greater proportion of the N demand in new growth in control plants. For example, average net N transfer was 27 mg in controls and 5 mg at the 800 mg N plant^{-1} level (Table 1). However, N was assimilated in the 1600 mg N treatment, suggesting improved uptake into old shoots, which diminished the need for retranslocation. Thus, as N was added, less N was depleted from old shoots, or more N was replenished. Net N transfer was not significantly affected by source effects (Table 1).

Black walnut component N concentration increased ($P < 0.0001$) with N level (Table 2). Similarly, N fertilization had a highly significant effect ($P < 0.0001$) on component C:N. Nitrogen level did not significantly affect total dry mass of the plant, largely owing to non-significant effects on the roots (Table 3; Figure 1). Compared to unfertilized plants, leaf biomass increased ($P < 0.0001$) by 54.0% in the 800 mg N plant^{-1} level and by 55.6% in the 1600 mg N plant^{-1} level (Table 3). Stem dry mass followed similar trends as observed for leaf dry mass (Table 3; Figure 1).

Mean net photosynthesis ranged from 2.45 to 4.84 μmol m^{-2} s^{-1} in lower leaves and from 5.95 to 9.06 μmol m^{-2} s^{-1} in upper leaves. Thus, net photosynthesis increased 43.8% at the 800 level and 47.2% at the 1600 level when compared with unfertilized plants for lower leaves (Figure 2). By contrast, net photosynthesis increased 31.0% only in the 1600 mg N treatment relative to unfertilized plants for the upper leaves (Figure 2). Fertilization increased (P

Table 2
Effects of N rate (mg N supplied plant^{-1}) and source on total N and C:N in components of walnut seedlings grown in a controlled greenhouse environment for 3 months

Treatment	Measured Parameter	Plant component			
		Leaf	Root	Old stem	New stem
N rate					
	C (%)				
0		48.00*b*	45.67a	49.26a	49.24a
800		48.11b	46.32a	48.92a	49.41a
1600		46.33a	45.80a	48.77a	49.60a
	P value	0.0075	0.6828	0.3211	0.1490
	N (%)				
0		1.70b	0.72*c*	0.51*c*	0.58*c*
800		2.50a	1.00b	0.65b	0.73b
1600		2.62a	1.44a	0.72a	0.82a
	P value	0.0001	0.0001	0.0001	0.0001
	C:N				
0		29.03a	69.84a	101.17a	87.40a
800		19.56b	47.93b	77.30b	69.47b
1600		18.15b	33.44*c*	68.18b	61.86b
	P value	0.0001	0.0001	0.0001	0.0001
	N (g)				
0		0.19b	0.40b	0.032b	0.01b
800		0.41a	0.57a	0.054a	0.02a
1600		0.43a	0.68a	0.06a	0.03a
	P value	0.0001	0.0001	0.0001	0.0001
N source					
	C (%)				
(NH4)2SO4		46.44b	45.09a	48.71b	49.76a
NaNO3		47.54ab	46.30a	48.70b	49.41ab
NH4NO3		48.45a	46.41a	49.54a	49.07b
	P value	0.0067	0.1830	0.0173	0.0017
	N (%)				
(NH4)2SO4		2.19b	0.97b	0.61a	0.71a
NaNO3		2.21b	1.04ab	0.64a	0.71a
NH4NO3		2.42a	1.15a	0.63a	0.71a
	P value	0.0452	0.0535	0.4931	0.9751
	C:N				
(NH4)2SO4		22.63a	54.60a	85.63a	73.17a
NaNO3		22.57a	53.17a	78.67a	71.66a
NH4NO3		21.54a	43.44b	82.35a	73.90a
	P value	0.5356	0.0126	0.2098	0.8421
	N (g)				
(NH4)2SO4		0.35ab	0.52a	0.045a	0.023a
NaNO3		0.30b	0.53a	0.048a	0.015b
NH4NO3		0.37a	0.60a	0.052a	0.02ab
	P value	0.0715	0.3568	0.4576	0.0328

Non-italicized values in bold are significant at $P < 0.05$. Italicized values in bold are significant at $P < 0.10$.

Table 3
Analysis of variance testing effects of N source and N rate and their interactions on component dry mass, leaf chlorophyll and photosynthesis of walnut seedlings grown in a controlled greenhouse environment for 3 months. Non-italicized values in bold are significant at $P < 0.05$. Italicized values in bold are significant at $P < 0.10$

Sources of variation	Dry mass						Leaf area	Leaf					
								Chlorophyll			Photosynthesis		
	Leaf	Root	Old stem	New stem	Shoot (total)	Plant		*a*	*b*	Total	Upper	Lower	Total
N source	0.3998	0.9628	0.5086	*0.0848*	0.3620	0.8539	0.3113	*0.0582*	0.4741	0.1080	*0.0569*	0.0383	0.5317
N rate	0.0001	0.6155	0.0167	0.0165	0.0001	0.2666	0.0001	0.0001	0.0001	0.0001	0.0128	0.0127	0.0020
Source × rate	0.0089	0.8240	0.1803	0.6713	0.0187	0.5810	0.0311	0.0205	0.3723	0.0428	0.5360	0.7140	0.7806

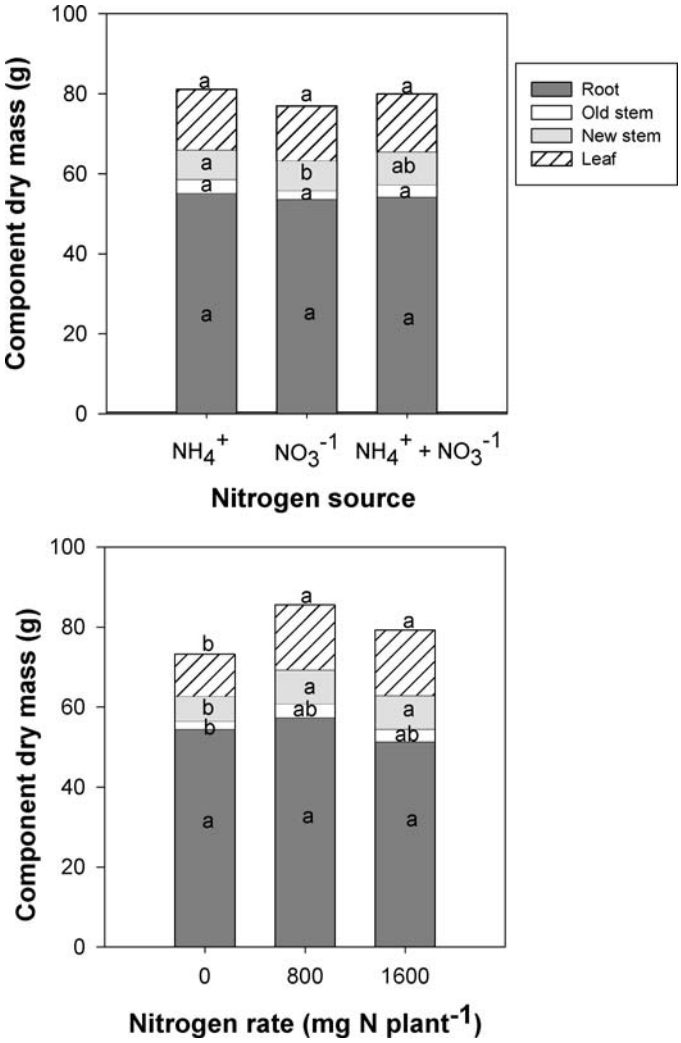

Figure 1. Black walnut component dry mass by N source (top) and N rate (bottom). Lower case letters represent significantly different means using Tukey's test of significant difference ($\alpha = 0.10$). There were no significant differences at the whole plant level.

$= 0.0020$) net photosynthesis by 96.2% in the 800 level and by 96.1% in the 1600 mg N level. As expected, chlorophyll *a*, chlorophyll *b*, and total chlorophyll increased with N addition ($P < 0.0001$; Table 3). Chlorophyll *a*:*b* ranged from 2.25 to 2.76 (Figure 2).

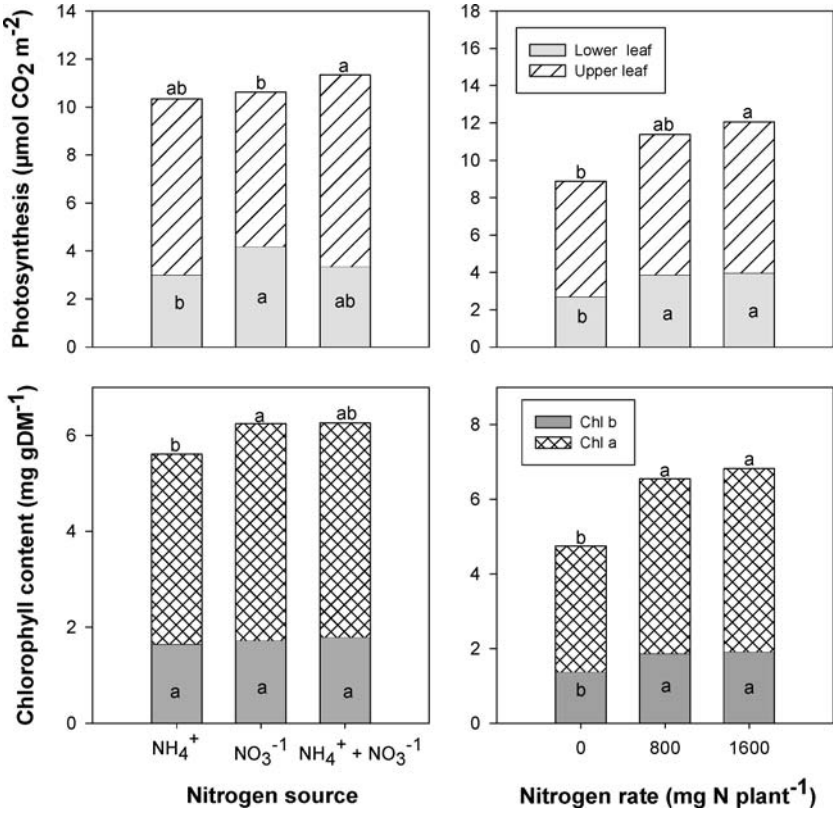

Figure 2. Black walnut photosynthesis by N source (upper left) and N rate (upper right) and chlorophyll content by N source (lower left) and N rate (lower right). Lower case letters represent significantly different means using Tukey's test of significant difference ($\alpha = 0.10$).

Nitrogen Source Effects on Growth and Physiology

Nitrogen source had a significant effect on total N ($P = 0.0715$) in the leaves, with the NH_4NO_3 source having 23.3% more N than the $NaNO_3$ source (Table 2). Nitrogen concentration was not significantly affected by N source in the old stem ($P = 0.4931$) or in the new stem ($P = 0.9751$). Leaf N concentration ranged from 2.2 to 2.4% and differed significantly (Tables 2 and 3). The significant effect of N source on the leaves suggests that these tissues are the most sensitive indicators of plant response to fertilization. The only significant effect of N source on C:N was in the roots ($P = 0.0535$), with a greater response associated with NH_4NO_3, where more C was allocated to roots (Table 2).

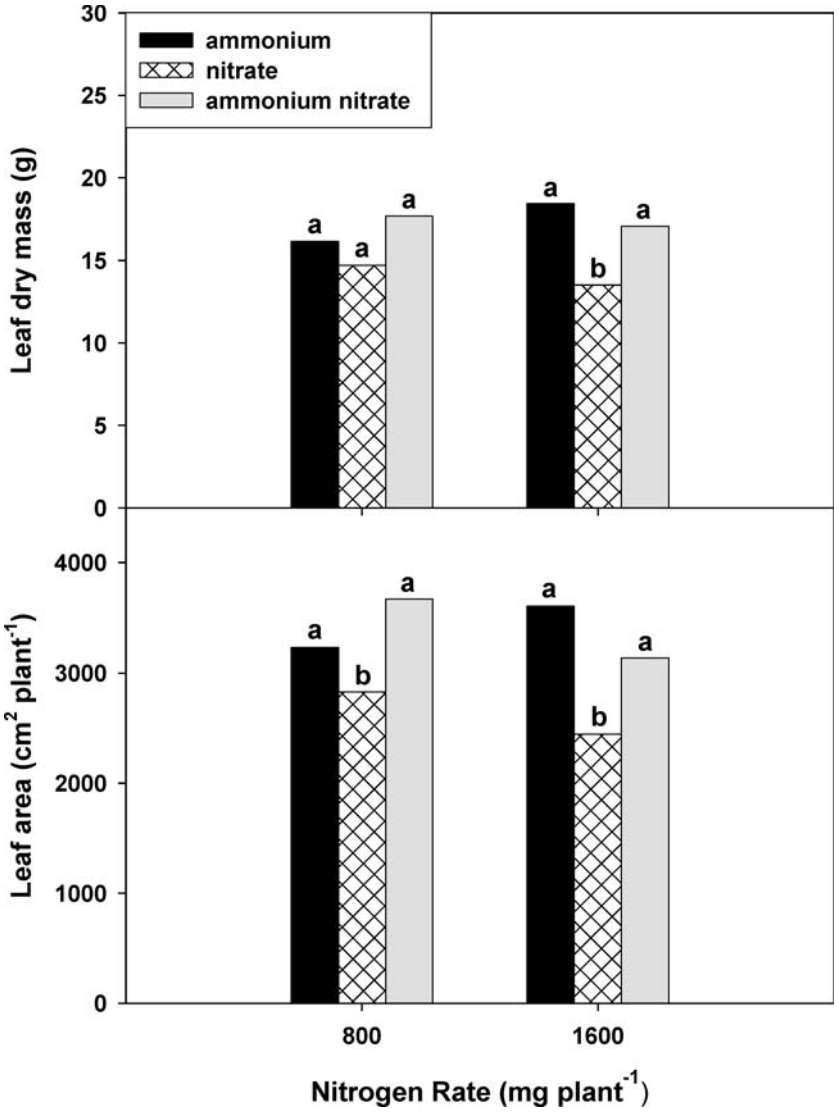

Figure 3. Black walnut leaf dry mass (top) and leaf area (bottom) interaction effects between N source and level. Lower case letters represent significantly different means using Tukey's test of significant difference ($\alpha = 0.10$).

There was a source × level interaction effect on leaf biomass ($P = 0.0089$; Table 3), with the NH_4NO_3 and NH_4^+ sources having 26.4 and 36.5% greater leaf dry mass than $NaNO_3$ (Figure 3). In the new stem, there was also a

significant effect (P = 0.0848) of N source on dry mass. For example, new stem dry mass was greater with NH_4^+ than with NO_3^- (Figure 1).

Nitrogen source affected photosynthesis in the upper (P = 0.0569) and lower leaves (P = 0.0383) (Table 3). For the lower leaves, the NO_3^- source had the highest rate of net photosynthesis, which was 39.1% greater than the NH_4^+ source (Figure 2). For upper leaves, the $NaNO_3$-fertilized seedlings had the lowest level of photosynthesis. The observed differences in photosynthesis between upper and lower leaves may signify different N allocation strategies between different sinks in relation to N source. However, observed differences in N source effects on photosynthesis by unit leaf area did not translate into an increase in total net photosynthesis at the whole plant level (P = 0.5317; Table 3). This could be due to absence of a source effect on leaf area (Table 3), even though there was a source × treatment effect in leaf area (Table 3; Figure 3).

Only chlorophyll *a* showed a significant effect of N source on chlorophyll content (P = 0.0582), with NO_3^- having 13.8% more chlorophyll *a* than NH_4^+, and NH_4NO_3 being statistically similar to both (Figure 2). There were also significant source × level interaction effects on chlorophyll *a* (P = 0.0205) and total chlorophyll (P = 0.0428) (Table 3). Interestingly, at 800 mg N, NH_4NO_3 and NO_3^- had more chlorophyll *a* than NH_4^+, but at 1600 mg, NH_4NO_3 and NH_4^+ had more chlorophyll *a* than NO_3^- (data not shown).

DISCUSSION

Nitrogen Level Effects

Prior to discussing N level and source effects, it is important to examine the impact of native fertility of the media on plant response and potential implications on experimental treatments. Foliar N concentration below 2.0% is considered to be in the deficiency range for black walnut seedlings (Phares and Finn, 1971; Ponder, 2004). Foliar N concentration of unfertilized seedlings was 1.7% (Table 2), which clearly signifies deficiency. This suggests that native supply was inadequate to meet N demand for growth of cultured plants. Thus, the choice of medium appears adequate for testing N fertility and source effects on plant growth and physiology. Furthermore, the seedlings were grown for 1 month prior to initiating fertility treatments to enable exploitation of native resources to minimize potential impacts on treatment response. The greater net N transfer to support new growth in unfertilized plants (Table 1) is consistent with the hypothesis that retranslocation evolved as a mechanism to increase nutrient use efficiency at low N availability (Salifu and Timmer, 2003). This further supports the use in this experiment of a medium with low native fertility. Diminished net N transfer with increased fertility suggests that plants relied

less on internal cycling, which may be regulated by increased uptake (Salifu and Timmer, 2003; Salifu et al., 2008).

The increased chlorophyll content with N addition (Figure 2) indicates that more N is allocated to the light-harvesting complex. By contrast, the increase in photosynthesis with fertility indicates that more N may be allocated to the enzymes of the carbon reactions. Thus, more N was presumably allocated to RUBISCO and the other enzymes of the carbon reactions, so more CO_2 could be used by the plant. The mean values for photosynthesis in the lower leaves (range from 2.45 to 4.84 μmol m^{-2} s^{-1}) correlate well with results found (range 2.96 to 5.21 μmol m^{-2} s^{-1}) for black walnut seedlings (Parker and Pallardy, 1991). Net photosynthesis for upper leaves in this study (range 5.95 to 9.06 μmol m^{-2} s^{-1}) were similar to results (5 to 11 μmol m^{-2} s^{-1}) for a *Juglans* hybrid (Frak et al., 2002). Higher net photosynthesis in upper than in lower leaves (Figure 2) suggests channeling of more N to upper growing points, which act as sinks for nutrients (Nambiar and Fife, 1987).

Similar to results noted in our study, greater N availability increased the amount of N allocated to leaves of 2-year-old *Juglans* hybrid seedlings (Frak et al., 2005). Also, greater fertility increased growth and N concentration in all tissues of 3-year-old *Juglans major* × *regia* seedlings (Simorte et al., 2001). Apparently, N was invested in the proteins and chlorophyll of the photosynthetic apparatus (Foyer et al., 2001). In other hardwood species, chlorophyll content (El Kohen and Mousseau, 1994), net photosynthesis (Ibrahim et al., 1998; Kubiske et al., 1998), or both (Bondada and Syvertsen, 2003) increased with N fertilization consistent with the results of our study.

Nitrogen Source Effects

The preference for different N sources varies with plant species (Haynes, 1986). In many cases, a mixed NH_4^+ and NO_3^- source is preferred, but in some cases, one form is favored over another (Hageman, 1980; Haynes, 1986). Nitrate uptake is coupled with H^+ uptake, whereas NH_4^+ is coupled with a change in membrane polarization caused by releasing H^+ into the external medium. Thus, a mixed source, where both N sources are taken up evenly, should result in no net change in cellular or external pH. This might explain why a mixed source may be preferred in many species, particularly if the species is sensitive to pH. The pH changes and the location of assimilation of NO_3^- affect plant physiology under different N sources.

In the current study, total chlorophyll was higher with NO_3^- and NH_4NO_3 at the 800 mg N level than NH_4^+ alone, but at the 1600 mg level, NH_4NO_3 and NH_4^+ outperformed NO_3^- alone; a similar effect was observed for chlorophyll *a* (data not shown). Nitrogen concentration in leaves was higher with the NH_4NO_3 source than with either NH_4^+ or NO_3^- alone, and in the roots it was higher than with the NH_4^+ alone. The C:N of the roots was lower with the

NH_4NO_3. Apparently, walnut seedlings responded more favorably to the mixed NH_4NO_3 source than NH_4^+ or NO_3^- alone in this study. However, the lack of significant growth response by N source, except for new stems, suggests that the favorable physiological and nutritional responses associated with NH_4NO_3 did not translate to improved growth at the whole plant level in the short-term. This has important implications in situations where increased growth may not be as desirable as increased N content. Presumably, the N and products of the light reactions of photosynthesis will be retranslocated to the roots and stem of the plant, resulting in a seedling that is not larger in size but better prepared for growth in the following season.

Results of our study are similar to those found for other woody tree seedlings and shrub species. For example, Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] had a higher concentration of N, greater growth, and higher rates of photosynthesis with NH_4NO_3 than with urea (which is similar to NH_4^+) (Brix, 1981). For pecan (*Carya illinoinensis* Wangenh. K. Koch.), a tree species in the same family as walnut (Juglandaceae), NH_4^+ was shown to be taken up preferentially to NO_3^-, but best growth and N accumulation occurred with a mixed N source (Kim et al., 2002).

If the results from this study, as well as studies with other woody species that show a preference for NH_4NO_3, are to be used in a field setting management of nitrification must be considered. In many forested systems, NH_4^+ is almost immediately converted to NO_3^- by microbial oxidation by nitrifying bacteria (Arp et al., 2002; Fillery, 2007). Therefore, to retain NH_4^+ in NH_4NO_3 N source, nitrification inhibitors must be used to prevent microbial oxidation of NH_4^+ to NO_3^- (Hageman, 1984). We suggest that if NH_4NO_3 is to be used as the preferred N source for black walnut seedlings, a nitrification inhibitor such as N-Serve$^{(tm)}$ [2-chloro-6-(trichloromethyl) pyridine] (Dow Agrosciences LLC, Indianapolis, IN, USA) should be mixed with the fertilizer to prevent microbial oxidation of NH_4^+ to NO_3^- (Prasad et al., 1971; Dinnes et al., 2002).

CONCLUSIONS

We have shown that N fertilization can improve walnut growth and photosynthetic responses under controlled environments without competition. A mixed source of N (NH_4NO_3) showed better physiological responses over NO_3^- (as $NaNO_3$) or NH_4^+ (as $(NH_4)_2SO_4$) alone. However, additional factors such as the use of nitrification inhibitors to sustain NH_4^+ in mixed N based fertilizers should be considered in a field setting. In order to be of practical importance, additional studies should be conducted to test if the results of this study are valid with vegetation control in the field. The approach adapted in this study is being proposed as an alternative technique that can be used to nutrient load 1+0 seedlings in pots prior to outplanting in the field, which would direct applied

nutrients to the seedlings rather than inadvertently stimulating growth of non-target vegetation under field scenarios. The suggested fertilization approach has been successfully applied to condition grafted black walnut plants to improve field performance (Salifu et al., 2006). Our results suggest retranslocation could serve as an important source of N to support new growth in walnut seedlings, but rigorous studies are needed to directly quantify retranslocation with ^{15}N labeling techniques to help improve our understanding of such processes in newly established walnut seedlings.

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