Nitrate reductase activity in 1+0 *Juglans nigra* L. seedlings with N fertilization

Michael Nicodemus and Douglass F. Jacobs
The Hardwood Tree Improvement and Regeneration Center (HTIRC)
Department of Forestry and Natural Resources
Purdue University, West Lafayette, IN, USA

ABSTRACT

Nitrate reductase activity (NRA) is a measure of how quickly plant organs convert nitrate (NO_3^-) into nitrite (NO_2^-) as the beginning of the process that converts inorganic nitrogen (N) into forms of N that are useful to the plant. In the leaves, the energy required for NRA is provided by the direct products of photosynthesis, whereas, in the roots, NRA requires respiratory energy to function. NRA is also known to be NO_3^--induceable.

In this study, 2 forms of N (NH_4NO_3 and $NaNO_3$) were applied to half-sib 1+0 black walnut (*Juglans nigra* L.) seedlings at three different rates (0, 400, and 800 mg N per seedling). Plants were grown for 2 months after fertilization and NRA was measured using the *in vivo* technique. It was found that there were no significant differences between N forms, or N form by level, but that there were higher total NRA values for the highest treatment level. These values were higher but not significantly so in the middle treatment compared to the low and significantly higher in the high treatment. On average, the majority of NRA occurred in the roots (67.8%).

Future studies will be designed adding more replications, an additional form of N, and higher levels of fertilization. It is hoped that refinements and changes to the design will yield more significant results. From these findings, it seems that a significant amount of respiratory energy is required for NRA with NO_3^- treatment, so other forms of N fertilizer may make a more efficient use of energy.

Figure 1. A black walnut seedling used in the study.

INTRODUCTION

The location of NO_3^- reduction has a critical influence on the energy use of the plant. If NO_3^- is reduced in the roots, there is an increase in root respiration as more energy would be required to assimilate NO_3^- and subsequently NH_4^+ (Zogg et al. 1996). Conversely, if NO_3^- is assimilated in the leaves, there would be little energy needed in the roots to transport it, since NO_3^- transport into the xylem is a passive process (Sivasankar and Oaks 1996). It would involve less energy overall since reduction in the leaves is powered mostly from excess photosynthetic energy (Schrader 1984). In the case of a plant that transports NO_3^- to the leaves, NH_4^+ fertilization would require greater respiratory energy since NH_4^+ is not transported in the xylem and must be converted into organic N in the roots (Atkins 1988).

Woody species are thought to reduce NO_3^- mainly in the roots (Smirnoff and Stewart 1985; Faure et al. 2001). This is true for most coniferous species (Yandow and Klein 1986; Sarjala et al. 1987; Sarjala 1991; Seith et al. 1994). Conversely, in the majority of studies on hardwood species, NO_3^- is found to be reduced in the leaves (Adams and Attiwill 1982b; Reilly and Edwards 1986; Stadler and Gebauer 1992; Downs et al. 1993; Gebauer and Schulze 1997; Krywult and Bytnerowicz 1997; Min et al. 1998; Black et al. 2002) but in some cases, NO_3^- was primarily reduced in the roots (Adams and Attiwill 1982a; Rothstein et al. 1996; Toselli et al. 1999). Many of these differences are species-specific, but within species, differences are associated with age of trees and environmental conditions.

The objective of this study was to determine the location of NO_3^- reduction in black walnut (*Juglans nigra* L.) seedlings. Different levels of fertilizer based on NO_3^- or half NO_3^- and half NH_4^+ were used to find the effect of fertilizer type on NO_3^-. It was hypothesized that NO_3^- would be reduced mainly in the roots, since other studies have detected NO_3^- in the xylem sap of other *Juglans* species (Prima-Putra and Botton 1998; Frak et al. 2002).

METHODS

18 half-sib (Purdue #1) 1+0 walnut seedlings were grown at the Indiana Department of Natural Resources Division of Forestry Vallonia Nursery (38° 85´ N, 86° 10´ W) and lifted and stored in a cooler at the Purdue Horticulture Farm. These were planted in 10.65 l Treepot™ containers (Stuewe and Sons, Inc., Corvallis, OR) with Scotts Metromix® 560 (Scotts Company, Marysville, OH, USA) potting media. These were kept well-watered and nutrients water provided (200 mg N l^{-1}) every two weeks. After 2 months, plants were fertilized with 0, 400, or 800 mg N as $NaNO_3$ or NH_4NO_3. The

Figure 2. Equipment used in nitrate reductase activity measurements: Upper left: Plants used in study, Upper right: Hole puncher, Lower left: NRA standards, Lower right: Spectrophotometer.

experimental design was a 3×3 factorial completely randomized design (CRD) with 3 replications. After 2 more months, NRA was assessed using methodology in Truax et al. (1994).

Roots and leaves were rinsed and then dried of surface water before sampling to minimize contamination and ensure that weights were not skewed by water on the samples. Roots were sampled that were <1 mm diameter and alive (white or light brown in color and broken into <1 cm sections by hand or using a knife. Leaf disks were cut using a hole punch and taken from the youngest fully expanded leaf. A 0.2 g fresh tissue sample was placed in a test tube containing 2 mL incubating solution (100 mM phosphate buffer [pH 7.5], 40 mM KNO_3, and 1.2% 1-propanol) and sealed and placed in the dark for 1 hr at room temperature. The enzymatic reaction was stopped by removing the plant tissue. A 1 mL aliquot was taken from the tube with a pipette, mixed with 1 mL NED (0.02%) and 1 mL sulfanilic acid, and the initial absorbance at 540 nm was read on a Perkin-Elmer LC-95 UV/Visible spectrophotometer (Perkin-Elmer Inc., Norwalk, CT, USA). After 30 minutes, the samples were again measured for absorbance at 540 nm (Truax et al. 1994).

SAS software (SAS Institute, Cary, NC, USA) was used to compare treatment effects using ANOVA (α=0.10) and significant differences between means were calculated using the method of least significant difference (LSD) at the α=0.10 level.

RESULTS and DISCUSSION

The NRA for the different components and total NRA are shown in figure 3. There was significantly more NRA in the roots compared to the shoot (p=0.0250). This is to be expected from reports of NRA in the roots of other *Juglans* spp. (Prima-Putra and Botton 1998, Frak et al. 2002). In roots, the highest level of fertilizer showed the greatest amount of NRA, but not significantly (p=0.2107). In leaves, with increasing levels of NH_4NO_3, we see a general trend of increase in NRA, which is significantly greater than seedlings supplied with $NaNO_3$ (p=0.0629), which is the opposite of what we would expect. It could be that the presence of the NH_4^+-N in the roots causes the roots to assimilate NH_4^+ preferentially, causing the NO_3^- to be transported to the leaves. The total NRA is greater in the highest treatment but not significantly (p=0.1340).

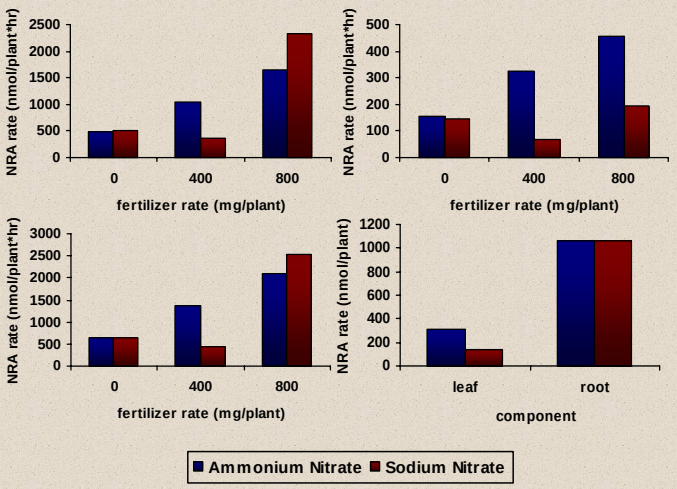

Figure 3. Tables of differences in nitrate reduction: Upper left: total NRA for roots. Upper right: Total NRA for leaves. Lower left: Total NRA for the entire plant. Lower right: Comparison of root and leaf NRA.

In this experiment, NRA occurred preferentially in the roots of black walnut seedlings. This signifies that more energy is likely expelled in these seedlings to use the NO_3^- form of N than NH_4^+, since NO_3^- requires more energy-requiring steps to be assimilated into organic N that can be used by the plant. Our data also suggests that the level of NRA is increased with N addition. This makes sense since NRA is known to be induced *de novo* by NO_3^- addition (Ting 1982; Hoff et al. 1992).

Future studies will be formulated that incorporate a larger sample size as well as N in the form of $(NH_4)_2SO_4$ to determine if the non-significant trends indicated in this paper truly represent significant differences and to test if there is an affect due to a different N source. Also, measurements of photosynthesis and respiration will be taken to evaluate more quantitatively the relative energy costs associated with assimilating the different forms of N.

REFERENCES

Adams, M.A. and P.M. Attiwill 1982a. Plant Soil 66:373-382. **Adams, M.A. and P.M. Attiwill** 1982b. Soil Biol. Biochem. 14:197-202. **Atkins, C.A.** 1988. *In* The International Congress of Plant Physiology, New Delhi, India, pp. 1023-1026. **Black, B.L., et al.** 2002. Tree Phys. 22:717-724. **Downs, M.R., et al.** 1993. Tree Struct. Func. 7:233-236. **Faure, J.-D., et al.** 2001. *In* Nitrogen assimilation by plants. Enfeldt: Science Publishers, Plymouth, UK, pp. 33-52. **Frak, E., et al.** 2002. Plant Phys.. 130:1043-1053. **Gebauer, G. and E.-D. Schulze** 1997. *In* Trees - Contributions to Modern Tree Physiology. Backhuys Publishers, Leyden, Germany, pp. 273-291. **Hoff, T., et al.** 1992. Phys. Plant. 84:616-624. **Krywult, M. and A. Bytnerowicz** 1997. Can. J. For. Res. 27:2101-2104. **Min, X., et al.** 1998. Plant Cell Env. 21:1039-1046. **Prima-Putra, D. and B. Botton** 1998. J. Plant Phys. 153:670-676. **Reilly, C.C. and J.H. Edwards** 1986. J. Plant Nut. 9:1323-1334. **Rothstein, D.E., et al.** 1996. Oecologia. 108:338-344. **Sarjala, T.** 1991. Phys. Plant. 81:89-94. **Sarjala, T., et al.** 1987. Tree Phys. 3:285-293. **Schrader, L.E.** 1984. *In* Nitrogen in crop production. ASA-CSSA-SSSA, Madison, WI, pp. 55-65. **Seith, B., et al.** 1994. Phys. Plant. 91:419-426. **Sivasankar, S. and A. Oaks** 1996. Plant Phys. Biochem. 34:609-620. **Smirnoff, N. and G.R. Stewart** 1985. Phys. Plant. 64:133-140. **Stadler, J. and G. Gebauer** 1992. Trees Struct. Func. 6:236-240.**Ting, I.P.** 1982. Plant physiology. Addison-Wesley, Reading, MA. **Toselli, M., et al.** 1999. J. Hort. Sci. Biotech. 74:118-124. **Truax et al.** 1994. Trees Struct. Func. 9:12-18. **Yandow, T.S. and R.M. Klein** 1986. Plant Phys. 81:723-725. **Zogg, G.P., et al.** 1996. Tree Phys. 16:719-725.