

Influence of the form of nitrogen nutrition on foliar nitrate reductase activity in young black locust (*Robinia pseudoacacia* L.)

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Introduction

Robinia pseudoacacia L. fixes dinitrogen in symbiosis with a specific rhizobium and, as a nitrogen-fixing tree, gives a litter with a low C/N (Moiroud and Capellano, 1981), which favors nitrification (Montagnini *et al.*, 1986).

In the field, black locust can therefore use both symbiotic and nitrate nitrogen. Data on the nitrate reduction in *R. pseudoacacia* are still very limited (Aznadi, 1984). The present work examines the effects of the symbiotic and nitrate nitrogen nutrition on the leaf nitrate reductase activity and the distribution of this enzyme activity in young black locusts.

Materials and Methods

Young black locusts inoculated with a suspension of crushed nodules were grown on a nitrogen-free nutrient solution in a growth chamber. Nitrate nutrition of the nodulated plants was assured by the addition of 4 or 10 mM NaNO₃ to the nitrogen-free solution.

Nitrogenase (N₂ase) activity was measured on intact plantlets by the acetylene reduction method. Nitrate reductase (NR) activity was measured *in vivo* on leaf disks (diameter: 12 mm). Two disks were vacuum infiltrated in 3 ml of 0.1 M NaK phosphate buffer, pH 7.5, containing 0.05 M KNO₃ and Triton X-100 (0.1%, v/v). After incubation for 1 h at 30°C in the dark, NO₂⁻ was determined spectrometrically at 540 nm in the incubation medium.

Results

Presence of a constitutive leaf NR activity affected by the N₂ase activity

The leaves of nitrogen-deficient plants had a notable NR activity considered as constitutive, since it was not induced by nitrate (Fig. 1). This enzyme activity decreased to a minimum value (≤ 1 nmol NO₂⁻·mg⁻¹ DW·h⁻¹) when the N₂ase increased after nodulation. When symbiotic nitrogen nutrition was eliminated by excision of root nodules, a notable NR activity was progressively recovered in the leaves (results not shown).

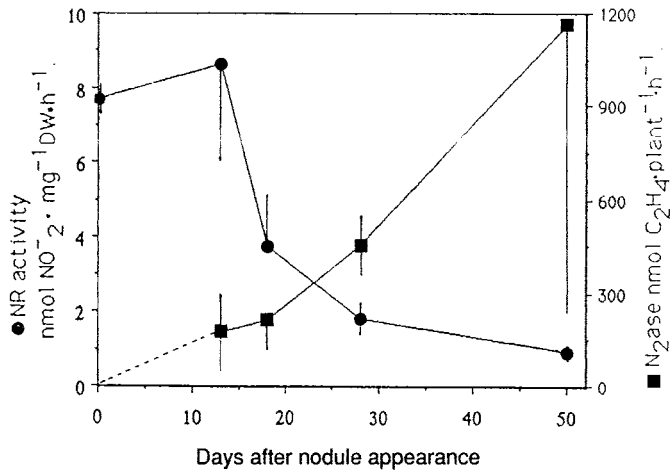


Fig. 1. Variation of foliar NR activity and nitrogenase activity after nodulation in young black locusts inoculated with crushed nodules at 1.5 mo old. Means of 4 replicates \pm SE.

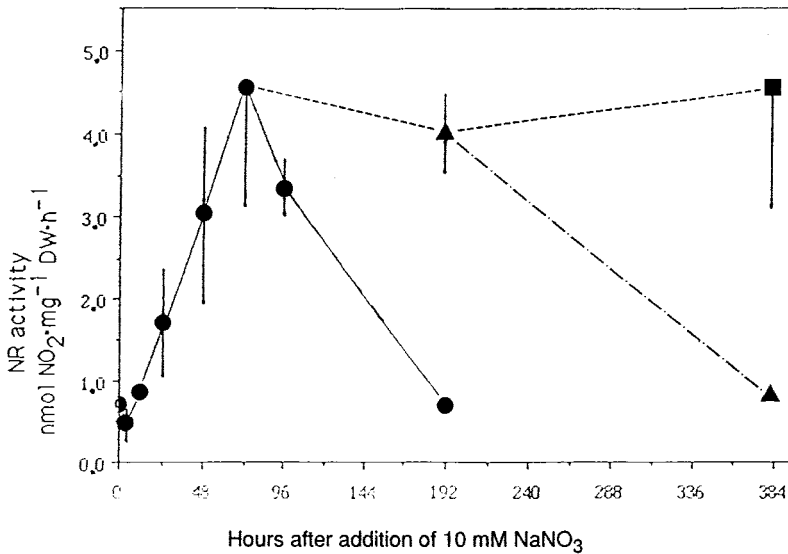


Fig. 2. Induction of foliar NR activity by nitrate (10 mM NaNO₃) in 11 mo old black locusts. The assays were made on the youngest fully expanded leaves successively (●, ▲, then ■) developed during the experiment. Means of 3 replicates \pm SE (when size of SE > size of symbol).

Effect of nitrate of leaf NR activity of nodulated plants

Administration of 4 mM NaNO₃ to 11 mo old nodulated plants did not increase leaf NR activity, whereas the 10 mM NaNO₃ dose induced high enzyme activity (Fig. 2). After 72 h of induction, the highest NR activity was found in the apical fully expanded leaf and corresponded with the highest nitrate content (Table I). When a

new leaf expanded, the NR activity decreased in the previous leaf and the highest enzyme activity was found again in the new leaf (Fig. 2).

When the nitrate supply was withdrawn, the enzyme activity recovered its minimum value after 2 d (Fig. 3).

Conclusion

Table I. Nitrate content (N-NO₃%, DW) in the apical, median and basal leaves of nodulated 11 mo old plants supplied with 10 mM NaNO₃ for 96 h.

Leaf position	Symbiotic N-nutrition	
	control plants	+ NaNO ₃ (10 mM)
Apical	0	1.560
Median	0	0.128
Basal	0	0.073

One measurement.

Notable NR activity (6–9 nmol NO₂⁻·mg⁻¹ DW·h⁻¹) was measured *in vivo* in the leaves of young nitrogen-deficient *R. pseudoacacia*; its role is unknown, but its decrease, concomitant with the advent of the N₂ase activity, indicates a relationship between both enzyme activities. The low NR activity (≤1 nmol NO₂⁻·mg⁻¹ DW·h⁻¹) of nodulated plants could be greatly increased by nitrate supplied *via* the roots. This inducible NR activity was consistently

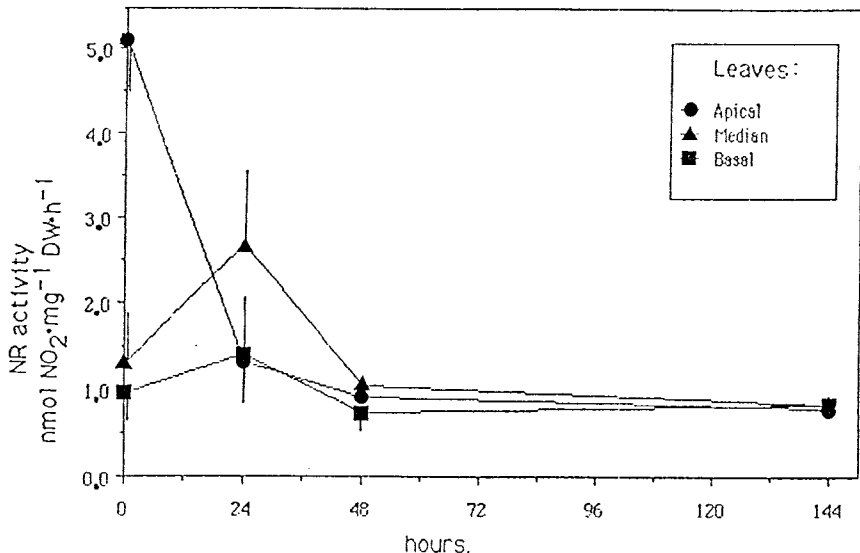


Fig. 3. Effect of the elimination of nitrate supply (at 0 h) on the NR activity in apical, median and basal leaves of 11 mo old black locusts. Means of 3 replicates ± SE (when size of SE > size of symbol).

highest in the younger expanded leaves that showed the highest nitrate content. Studies are in progress to compare these results with those obtained on leaves from field-grown *R. pseudoacacia*.

References

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