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Influence of Nitrogen Form and Concentration on the Nitrogen Fixation of *Acacia auriculiformis*

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Abstract

An experiment was conducted to study the effect of different sources of nitrogen on nitrogen fixation of *Acacia auriculiformis* seedlings. The plants were grown in pots with sand-vermiculite substrate in a greenhouse. All plants were inoculated at germination with a pre-selected *Rhizobium* strain. Low levels of nitrate depressed nodule production more than ammonium. Ammonium increased the total dry weight of plants whereas nitrate had no effect when compared with plants growing without mineral nitrogen. The early growth of seedlings in the nursery can be increased by the application of starter mineral nitrogen. In these experimental conditions, *Acacia auriculiformis* plants clearly preferred ammonium ions as a nitrogen source.

Keywords: *Acacia auriculiformis*, nitrate, ammonium, nodulation

1. Introduction

Acacia auriculiformis is a legume tree native to Australia with root nodules that can fix high levels of nitrogen. The tree grows in widely differing soils and has economic potential for paper pulp, fuel, as a shade tree and a soil cover crop (NAS, 1983). It can survive on land with a lower level of nitrogen and organic matter than most eucalypts and other trees (NAS, 1983). Under

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these conditions nitrogen fixation should be maximised. Although there are reports in the literature about the effect of sources of combined nitrogen on trees (Keltjens and Loenen, 1989; Arnold and van Viest, 1991) there are few specifically on legume trees, either young or old, or on how these sources interact with nodulation and nitrogen fixation. Different sources of nitrogen can change the electrical-balance of cells, inducing the release of H^+/HCO_3^- (Raven and Smith, 1976). Plants grown in NH_4^+ or fixing nitrogen, produce 1.22 and 0.22 mol of H^+ per mol of nitrogen assimilated respectively, and plants grown in NO_3^- produce 0.78 mol of OH^- per mol of nitrogen, taking S and P into account (Raven, 1988). The release of acids or bases in the rhizosphere can alter not only the availability of elements such as Mo, Fe, and Al (Raven et al., 1990; Jacob-Neto et al., 1991) but also the initial process of infection by *Rhizobium* (Munns, 1968).

Preliminary experiments have shown that *Acacia auriculiformis* takes at least 20 days after germination to produce its first nodules; the plants at this stage were smaller than nitrogen-fed plants. As reported for beans (Hungria et al., 1991; Jones et al., 1981), a lack of synchronization between the depletion of nitrogen in the cotyledons and the export products of nitrogen fixation from nodules can cause a period of N stress in the plant during the nursery stage. In some species, for example, *Phaseolus vulgaris* and *Glycine max*, the first fixed N is used for nodule growth (Sprent and Thomas, 1984). Early growth, until nitrogen fixation begins, can be increased by the application of starter mineral nitrogen. In this experiment, we report the effect of different sources and amounts of nitrogen on nodule formation and plant growth of *A. auriculiformis.*

2. Materials and Methods

Seeds of *Acacia auriculiformis* were scarified with concentrated sulphuric acid for 5 min, then washed 10 times with tap water. The seeds were surface sterilized with sodium hypochlorite (5%), placed in petri dishes with sterile agar/water medium (1%) and kept in a dark incubator at 28°C. After 1 week the seedlings were transferred to 400 cm^3 pots (one per pot) containing a washed vermiculite:sand mixture (2:1). After transfer, all plants were inoculated with rhizobial strain DUS 088.

Plants were grown in a glasshouse under natural summer daylight with supplemental light. The photoperiod was approximately 13 hr and day/night temperatures were 35°C/20°C.

The experiment was arranged in a factorial randomized block design with: plants without nitrogen, 3 levels of $NaNO_3$ (7,14,21 mg N $plant^{-1}$ $week^{-1}$), 3 levels of $(NH_4)_2SO_4$ (7,14,21 mg N plant^{-1} week^{-1}) with four replicates for each harvest.

The plants received a balanced nutrient medium containing $CaSO_4 \cdot 2H_2O$ 2.9 mM, $MgSO_4 \cdot 7H_2O$ 3.25 mM, KH_2PO_4 3.97 mM, K_2KPO_4 0.51 mM, $C_6H_5O_7Fe \cdot 5H_2O$ 62 μM, $ZnSO_4 \cdot 7H_2O$ 0.1 μM, H_3BO_3 57 μM, NaCl 10 μM, $Na_2MoO_4 \cdot 2H_2O$ 0.05 μM, $MnSO_4 \cdot 2H_2O$ 1.2 μM, $CuSO_4 \cdot 5H_2O$ 0.1 μM and $CoSO_4 \cdot 7H_2O$ 0.02 μM with pH 6.8. To try to maintain relatively constant N concentrations around the root systems, the rooting medium of all plants was flushed every week with tap water for half an hour and then 100 cm^3 of fresh nutrient solution was added. Plants were harvested at 40, 80 and 110 days after germination and then divided into roots, nodules and shoot. After drying at 60°C, plant materials were ground in a ball mill to a fine and uniform consistency such that it could pass through a 0,5 mm sieve. The percentage of carbon and nitrogen in the ground material was determined using a Carlo Erba elemental analyzer (model 1106).

The statistical analyses of nodule weight and nodule number were performed on transformed data ($\sqrt{x}+1$) assuming a Poisson distribution. All results were analyzed by standard statistical procedures using the SAS programme.

3. Results

At low levels, nitrate depressed nodule production more than ammonium (Fig. 1). However, at the highest level (21 mg N plant^{-1} week^{-1}), both ammonium and nitrate inhibited nodule formation in the last harvest. Ammonium at 14 mg N plant^{-1} week^{-1}, gave an increase in nodule dry weight at 110 days; at this stage there was no statistical difference between this treatment and when the plants received no mineral nitrogen.

Infectivity (measured by the number of nodules per plant), differed between the treatments (Fig. 2). The number of nodules was generally higher when no mineral nitrogen was given. For the NH_4^+-14 treatment there is a clear increase in nodule number with time, suggesting delayed nodulation, while with the other N treatments there is a trend for decreased nodule number with time, suggesting nodule loss.

Ammonium significantly increased the total dry weight of plants at 80 and 110 days (Fig. 3), whereas nitrate had no effect when compared with plants growing without mineral nitrogen. Similarly, ammonium, but not nitrate, increased total nitrogen per plant at 80 and 110 days (Fig. 4).

C:N ratio of shoots are given (Fig. 5) to compare the relative effects of treatments on carbon and nitrogen fixation. Highest values were found in the

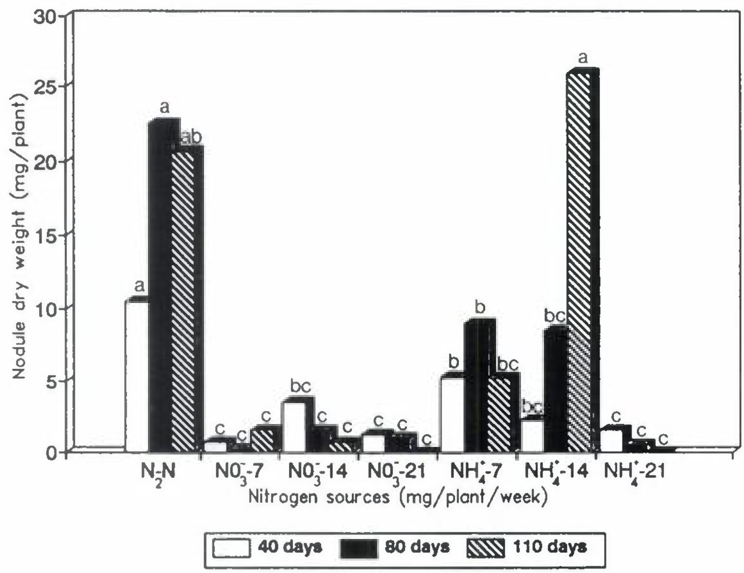

Figure 1. Effect of different sources of nitrogen on nodule dry weight of *Acacia auriculiformis*. Means with the same letter are not statistically different by Duncan's test at 5% level in each harvest.

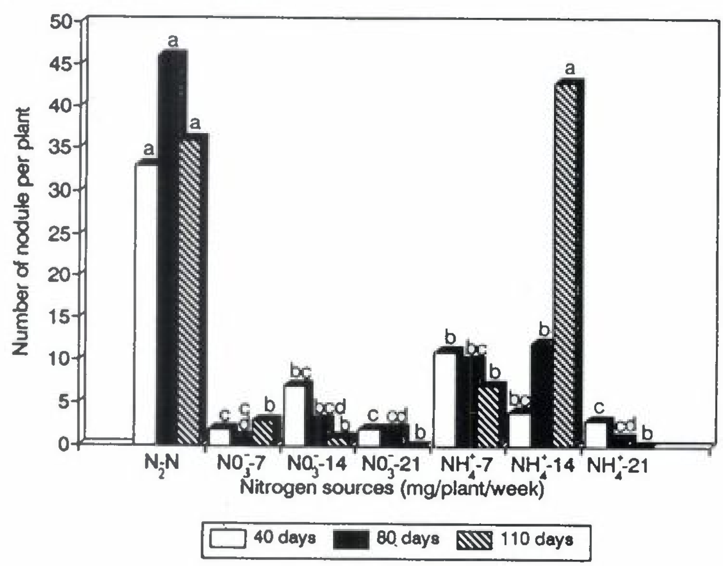

Figure 2. Effect of different sources of nitrogen on number of nodules on *Acacia auriculiformis*. Means with the same letter are not statistically different by Duncan's test at 5% level in each harvest.

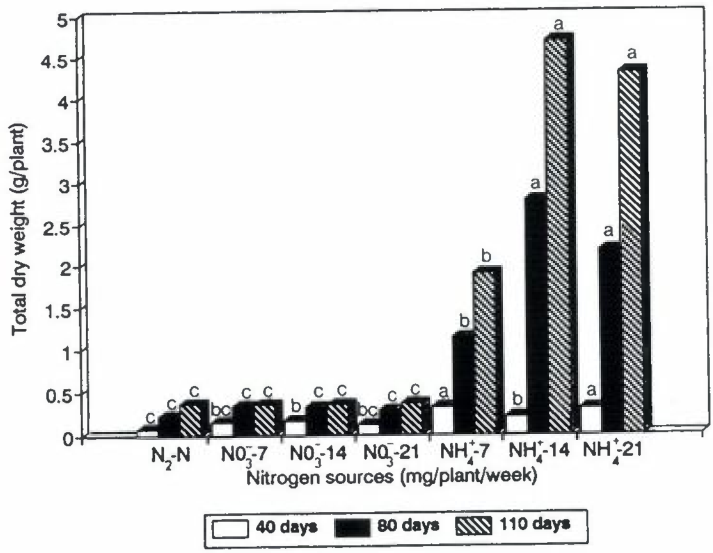

Figure 3. Effect of different sources of nitrogen on total dry weight of *Acacia auriculiformis*. Means with the same letter are not statistically different by Duncans' test at 5% level in each harvest.

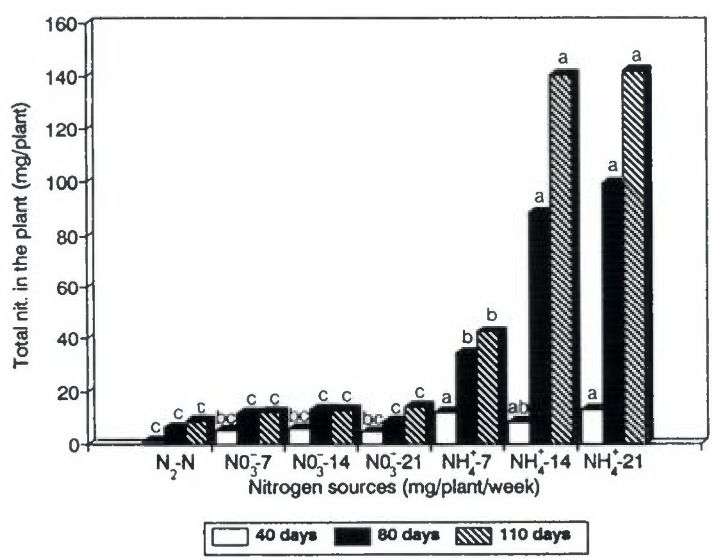

Figure 4. Effect of different sources of nitrogen on total nitrogen content of *Acacia auriculiformis*. Means with the same letter are not statistically different by Duncan's test at 5% level in each harvest.

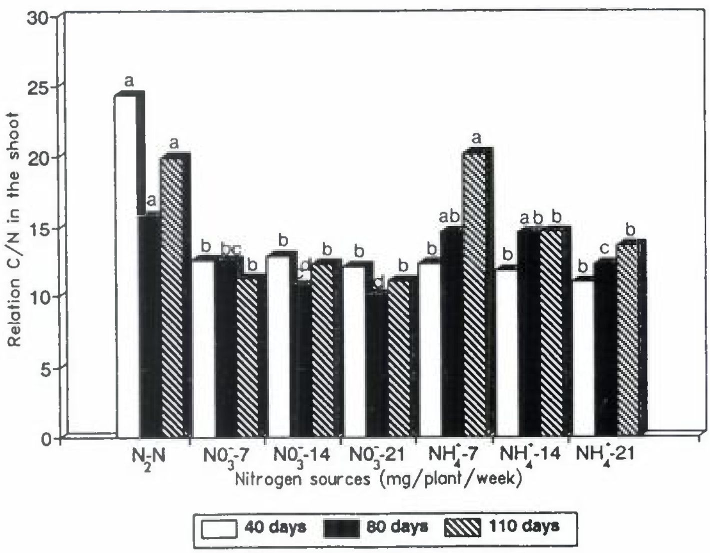

Figure 5. Effect of different sources of nitrogen on C/N ratio in the shoot of *Acacia auriculiformis*. Means with the same letter are not statistically different by Duncan's test at 5% level in each harvest.

plants reliant on nitrogen fixation at 40 days; values for nitrate treatment were much smaller.

4. Discussion

The mechanisms by which N_2 fixation is inhibited by combined N are not well understood, although a number of hypotheses have been put forward for direct and indirect effect of NO_3^- on crop species (Becana and Sprent, 1987).

Although there are few studies on nodulated woody species, there is some evidence of adverse effects of fertilizer N on nodulation. Umali-Garcia et al. (1988) found that nitrogen as urea, reduced nodulation of *Albizia falcataria* and *Acacia mangium*. Goi et al. (1986) found a negative effect of NO_3^- on nodulation of *Albizia lebbeck* at the nursery stage. Urea-N at low concentration did not affect root nodulation but increased stem nodulation and nitrogenase activity in *Sesbania rostrata*; however at higher levels of applied N, nodule number was decreased (Becher et al., 1991).

Although for energetic reasons (Arnold and van Diest, 1991) all plants should prefer NH_4^+, many non-fixing crop species prefer NO_3^-. However a number of recent studies with trees suggests that some species prefer ammonium (Keltjens and Loenen, 1989; Arnold and Van Diest, 1991).

In the present study NO_3^- inhibited nodulation more than NH_4^+ (Fig. 1). Results for total dry weight and total nitrogen (Figs. 3 and 4) at 80 and 110 days in plants given 14 or 21 mg N per week as NH_4^+, showed no significant differences. Since the NH_4^+-treated plants (14 mg N per week) had many nodules, this suggests that *A. auriculiformis* can (a) fix substantial amounts of nitrogen in the presence of NH_4^+, and (b) that N_2 and NH_4^+ are used equally effectively. The C:N ratio of shoots (Fig. 5) in nitrate-fed plants and the generally chlorotic appearance of these plants (not shown), support the suggestion that NO_3^- may be taken up but not assimilated into functional leaf tissue, thus restricting carbon fixation.

Taken together, our data are consistent with (a) a more deleterious effect of NO_3^- on nodulation, compared with NH_4^+, and (b) a preference of some wood species for NH_4^+ as a source of combined N.

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