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## Effect of ammonium nitrate on nodulation and nitrogen fixation (acetylene reduction) of the tropical legume *Sesbania rostrata*

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### Introduction

The effect of combined nitrogen on the physiology of the *Rhizobium* legume symbiosis has been the subject of many studies (reviewed by Gibson 1976; Munns 1977; Rigaud 1981). Addition of mineral nitrogen in soil or synthetic medium affects both nodulation and nitrogen fixation. The degree of effects observed depends on the concentration and the form of nitrogen compounds, the time of application, the cultivation conditions, and finally the type of plant and bacteria used.

*Sesbania rostrata* is a tropical legume which displays the unusual feature of both stem and root nodulation when associated symbiotically with the specific rhizobia ORS 571 (Dreyfus & Dommergues 1981) or WE 7 (Olsson *et al.* 1984). When the difference method, the balance method or the isotope-dilution method are used, nitrogen fixation by *S. rostrata* is about 200 kg N fixed/ha in 50 days (Rinaudo *et al.* 1983; Rinaudo & Moudiongui 1986). *Sesbania rostrata* shows a typical root-hair infection in which a meristematic zone is initiated prior to nodulation (Olsson & Rolfe 1985). Stem nodulation occurs by an alternative infection procedure referred to as 'crack entry' in which a pre-existing meristematic zone is taken over for nodule development (Duhoux 1984; Olsson & Rolfe 1985).

In a previous study (Dreyfus & Dommergues 1980), *S. rostrata* was grown hydroponically in the presence of 3 mmol/l  $\text{NH}_4\text{NO}_3$ . Root nodulation was inhibited, while stem nodulation and related nitrogen fixation (acetylene reduction) were not affected. Similar effects were then observed with other stem-nodulated legumes: *Aeschynomene scabra* (Eaglesham & Szalay 1983) and *A. afraspera* (Becker *et al.* 1986).

The present study was initiated to determine the effect of various concentrations of  $\text{NH}_4\text{NO}_3$  on both stem and root nodulation and nitrogen fixation (acetylene reduction activity) of *S. rostrata*.

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### Materials and methods

#### Glasshouse procedure

The experiment was done under natural daylight in a glasshouse with a temperature of 32°C by day and 25°C by night.

*Sesbania rostrata* seeds were treated with conc. H<sub>2</sub>SO<sub>4</sub> for 30 min, then washed thoroughly with sterile water and germinated on moist filter paper in Petri dishes. Seedlings were transferred to 15-cm plastic pots containing N-free autoclaved silica sand and gravel (one seedling per pot). The basic nutrient solution consisted of the following (mg/l): CaSO<sub>4</sub>·2H<sub>2</sub>O, 138; K<sub>2</sub>CO<sub>3</sub>, 55.2; K<sub>3</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 99; MgSO<sub>4</sub>·7H<sub>2</sub>O, 49.3; NaCl, 23.4; Fe (as NaFe-EDTA), 4; Gibson trace elements, 1 ml/l (Vincent 1970); pH 6.8. For one month before beginning experimental treatments, the plants were watered on alternate days with 50 ml sterile solution of the above nutrients containing in addition 1 mM NH<sub>4</sub>NO<sub>3</sub>.

*Sesbania rostrata* was inoculated 30 days after sowing; a 2-day-old ORS 571

**Table 1** Effect of NH<sub>4</sub>NO<sub>3</sub> concentration on plant height, plant dry weight and nitrogen content

NH <sub>4</sub> NO <sub>3</sub> applied (mM)	Plant height (cm)	Plant dry weight (g/plant)	Plant nitrogen content (mg/plant)
0	990 a	6.45 a	192.0 a
1.5	1058 a	7.45 ab	242.0 b
3.0	1226 b	8.31 bc	291.0 c
6.0	1270 b	9.38 c	420.0 d

Numbers followed by the same letter in a column are not significantly different at 5% level by Duncan's multiple range test (5 replicates).



system were inoculated with *Rhizobium*, but only one side received nitrate: inhibition of nodulation was localised, occurring only on roots in direct contact with nitrate, but nitrogenase activity of nodules formed on the nitrate-free root portion was affected.

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### Summary

The effect of ammonium nitrate on nodulation and nitrogen fixation (acetylene reduction activity) was investigated using *Sesbania rostrata*, a tropical legume with the ability to nodulate both roots and stems. Plants were grown in plastic pots with silica sand and gravel, inoculated one month after sowing and then continuously irrigated with an automatic irrigation system. Four

### Résumé

L'effet du nitrate d'ammonium sur la nodulation et la fixation d'azote (réduction de l'acétylène) a été étudié avec la légumineuse tropicale *Sesbania rostrata*, qui présente la particularité de noduler à la fois sur la tige et sur les racines. Les plantes ont été cultivées dans des pots en plastique sur un mélange constitué de sable siliceux et de gravier. Un mois après le semis, elles ont été inoculées et placées sous irrigation continue au moyen d'un système automatique permettant l'apport du milieu nutritif. Quatre concentrations en azote ont été utilisées: 0, 1.5, 3.0 et 6.0 mmol/l  $NH_4NO_3$ . Les paramètres symbiotiques ont été analysés 20 jours plus tard. La nodulation racinaire est totalement inhibée en présence de 3.0 mmol/l  $NH_4NO_3$ . Cette concentration en azote combiné n'a pas d'effet apparent sur la formation des nodules de tige, mais leur activité nitrogénase diminue de 85%: leur activité est totalement inhibée en présence de 6.0 mmol/l  $NH_4NO_3$ . L'augmentation de la concentration en nitrate d'ammonium, se traduit par une diminution de la taille des nodules de tige. Les fortes concentrations en azote combiné ont un effet inhibiteur très marqué sur les activités réductrices d'acétylène racinaire ou caulinaire de *S. rostrata*; toutefois cet effet est plus sensible au niveau racinaire qu'au niveau caulinaire.

### Resumen

Efecto del nitrato amónico en la nodulación y la fijación de nitrógeno (reducción de acetileno) de la leguminosa tropical *Sesbania rostrata*

Se estudió el efecto del nitrato amónico en la nodulación y la fijación de nitrógeno, medida mediante la reducción de acetileno, utilizando *Sesbania rostrata*, una leguminosa tropical que forma nódulos tanto en raíz como en tallo. Las plantas se cultivaron en arena de sílice y grava, en macetas de plástico, se inocularon un mes después de la siembra y a partir de este momento se regaron continua y automáticamente. Se aplicaron cuatro tratamientos de N; 0, 1.5, 3.0, y 6.0 mm  $NH_4NO_3$ . Los parámetros relativos a la simbiosis se evaluaron 20 días después. La nodulación en raíz se inhibió con 3.0 mm  $NH_4NO_3$ , sin embargo a esta concentración la nodulación en tallo no estaba inhibida aunque la actividad nitrogenasa disminuyó en un 85%, quedando totalmente inhibida a 6 mm  $NH_4NO_3$ . El incremento en la concentración de nitrato amónico produjo una disminución del tamaño de los nódulos del tallo. Elevadas concentraciones de nitrógeno mineral inhibieron fuertemente la reducción de acetileno tanto a nivel de raíz como de tallo, aunque la fijación de nitrógeno resultó más afectada en la raíz.

