# EFFECTS OF NITROGEN SOURCE ON THE GROWTH AND NODULATION OF *ACACIA MANGIUM* IN AEROPONIC CULTURE

**J. Weber [1, 2, 3, *], F. Y. Tham[3], A. Galiana[3], Y. Prin[1], M. Ducousso[1] & S. K. Lee[3]**

[1]*Laboratoire des Symbioses Tropicales et Méditerranéennes, UMR 113 Agro-M / CIRAD / INRA / IRD / Université Montpellier II, TA 10/J Campus International de Baillarguet, 34398 Montpellier Cedex 5, France*
[2]*UMR INRA-University H. Poincaré Nancy, Interactions Arbres / Microorganismes, Faculté des Sciences, BP239, 54506 Vandoeuvre-lès-Nancy Cedex, France*
[3]*National Institute of Education, Nanyang Technological University, Natural Sciences and Science Education, 1 Nanyang Walk, Singapore 637616*

*Received June 2006*

**WEBER, J., THAM, F. Y., GALIANA, A., PRIN, Y., DUCOUSSO, M. & LEE, S. K. 2007. Effects of nitrogen source on the growth and nodulation of *Acacia mangium* in aeroponic culture.** Effects of ammonium and nitrate on growth and nodulation rates of *Acacia mangium* inoculated with *Bradyrhizobium* and grown in aeroponic culture were studied. At concentrations of 13.6 and 4.9 mM, nitrate stimulated plant growth, nitrogen uptake and total chlorophyll content compared with corresponding concentrations of ammonium, which had a deleterious effect. On the other hand, nodulation was depressed with nitrate and totally suppressed with ammonium at these two concentrations. However at 0.4 mM, ammonium actually stimulated nodulation rates and resulted in robust plant growth comparable to that obtained with higher nitrate concentrations. Ammonium nitrification was confirmed to be absent from measurements of the nutrient solutions in aeroponic culture tanks.

Keywords: nitrate, ammonium, nitrification, *Rhizobium*, biomass

**WEBER, J., THAM, F. Y., GALIANA, A., PRIN, Y., DUCOUSSO, M. & LEE, S. K. 2007. Kesan sumber nitrogen terhadap pertumbuhan dan pembintilan *Acacia mangium* dalam kultur aeroponik.** Kesan ammonium dan nitrat terhadap kadar pertumbuhan dan kadar pembintilan *Acacia mangium* yang diinokulasi dengan *Bradyrhizobium* dan ditanam dalam kultur aeroponik dikaji. Pada kepekatan 13.6 dan 4.9 mM, nitrat merangsang pertumbuhan, pengambilan nitrogen dan kandungan klorofil berbanding dengan ammonium yang membawa kesan yang merosakkan. Pada kedua-dua kepekatan ini, kadar pembintilan berkurangan dengan penggunaan nitrat tetapi pembintilan disekat sepenuhnya dengan ammonium. Namun pada 0.4 mM, ammonium sebenarnya merangsang kadar pembintilan dan mengakibatkan pertumbuhan yang cergas setanding dengan pertumbuhan pokok pada kepekatan nitrat yang tinggi. Sukatan larutan nutrien di dalam tangki kultur aeroponik mengesahkan yang penitritan ammonium tidak berlaku.

## INTRODUCTION

*Acacia mangium* is a leguminous tree species originating from Queensland Australia, Papua New Guinea, Irian Jaya, the Sulawesi, Ceram and Aru islands (Pinyopusarerk *et al.* 1993). It was introduced into South-East Asia in the 1960s where it has become a major plantation species for reforestation, mainly for the supply of pulp to the paper industry. *Acacia mangium* is known for its fast growth and, in good conditions, the harvesting size for pulp production can be reached within a period of seven to eight years (Cossalter & Pye-Smith 2003).

The achievement of high growth yields on poor soils has been attributed to the presence of associations with symbiotic partners such as arbuscular mycorrhiza (AM) fungi and nitrogen fixing bacteria (de la Cruz & Garcia 1992). However, the effectiveness of these associations depends on the microbial strains involved (de la Cruz *et al.* 1992). *Acacia mangium* is specifically associated with *Bradyrhizobium* and only a restricted range of strains has been shown to be able to produce efficient $N_2$-fixing nodules (Galiana *et al.* 1990). Several trials in Africa and

* *E-mail: jean_weber31@hotmail.com*

South-East Asia demonstrated that controlled inoculation of *A. mangium* with *Bradyrhizobium* had significantly positive effects on host growth in the field (Martin-Laurent *et al.* 1999, Galiana *et al.* 2002).

In this context, aeroponic culture appeared to be a promising method for raising high quality *A. mangium* saplings that are already inoculated with selected and effective symbiotic partners prior to field planting (Martin-Laurent *et al.* 1997, 1999). *Acacia mangium* grown in aeroponic culture and inoculated with *Bradyrhizobium*, strain AUST 13C (Galiana *et al.* 1990), achieved good nodulation and growing rates when full strength nutrient solution based on a modified Hoagland's formula at 190 ppm of N was used (Martin-Laurent *et al.* 1997, Lee *et al.* 1994).

Good response of *A. mangium* to high levels of fertilization had also been observed in nurseries where doses of up to 20 g NPK/$m^2$/week still increased seedling growth without raising mortality rates (Adjers 1987). However, as reported for many annual legumes as well as *Acacia* spp. (Hansen & Pate 1987), high nitrogen fertilizations had a negative effect on nodulation intensity. On the other hand, low levels of nitrogen nutrition may initially depress early growth and nodule development until rhizobial nitrogen fixation becomes effective (Goi *et al.* 1993). For *Acacia auriculiformis* in pot culture, the nitrogen source rather than its concentration has been shown to exert the main effect in promoting nodulation and plant growth. Ammonium was clearly preferred to nitrate as it increased the total dry weight and nodulation rates at low concentrations (Goi *et al.* 1993).

The optimal nitrogen source and concentrations promoting both growth and nodulation of *A. mangium* in aeroponic culture, however, have not been determined. In this study we aim to improve the nitrogen nutrition of *A. mangium* in aeroponic culture by determining the effects of both nitrogen source and nitrogen levels on its growth rates and nodulation intensities.

## MATERIALS AND METHODS

### Aeroponic culture system

Aeroponic culture was carried out in 120 (L) × 75 (W) × 60 cm(H) troughs equipped with individual nutrient tanks of 100 l capacity each. Nutrients were renewed weekly and prepared as follows: reverse-osmosis purified water, $NH_4Cl$ or $KNO_3$ was added in the appropriate concentrations according to the treatments described below, 1.44 mmol $l^{-1}$ $K_2SO_4$, 1 mmol $l^{-1}$ $K_2HPO_4$, 2.46 mmol $l^{-1}$ $MgSO_4$ $7H_2O$, 0.9 mmol $l^{-1}$ $CaCl_2$ $2H_2O$, 0.6 µmol $l^{-1}$ $(NH_4)_6$ $Mo_7O_{24}$ $4H_2O$, 4.32 µmol $l^{-1}$ $MnSO_4H_2O$, 9.54 µmol $l^{-1}$ $H_3BO_3$, 0.24 µmol $l^{-1}$ $CuSO_4$ $5H_2O$, 0.2 µmol $l^{-1}$ $ZnSO_4$ $7H_2O$, 0.1 µmol $l^{-1}$ $CoSO_4$ $7H_2O$, 56 µmol $l^{-1}$ Fe EDTA. The volumes of the nutrient solutions in the tanks were maintained daily by replenishing any water loss through evaporation with reverse-osmosis purified water and the pH adjusted to 6 ± 0.1 using a normal solution of $H_2SO_4$ or NaOH. The nutrients were put into continuous circulation using individual centrifugal pumps. The nutrient solution was supplied to the roots in the form of a fine mist spray for 40 s at intervals of 30 s. When not supplied to the roots, the nutrients were redirected into the 100 l tanks. The aeroponic troughs were exposed outside with temperatures ranging from 25 to 34 °C, relative humidity ranging from 80 to 85% and high illuminance (daylight with a maximum of 800 to 1000 µmol $m^{-2}$ $s^{-1}$). Small individual transparent plastic roofs protected the plants of each system from direct rainfall.

### Seed germination and inoculation

Seeds of *Acacia mangium* (Seedlot No. 19297, Australian Tree Seed Centre, CSIRO, Australia) were germinated for seven days on moist tissue paper at room temperature (26 ± 2°C). The seedlings were then carefully transferred into sponge plugs which had previously been soaked in water and grown for another week in the greenhouse at 28 ± 4 °C. Twelve-day old seedlings were then transferred into aeroponic troughs with their root systems suspended in air (Martin-Laurent *et al.* 1997). The plants were inoculated by soaking each root system in a four-day-old yeast mannitol culture (0.5 g $l^{-1}$ $K_2HPO_4$, 0.2 g $l^{-1}$ $MgSO_4$ $7H_2O$, 0.1g $l^{-1}$ NaCl, 1 g $l^{-1}$ yeast extract and 10 g $l^{-1}$ mannitol; Vincent 1970) of *Bradyrhizobium* Aust 13C (Galiana *et al.* 1990).

### Experimental design

A first experiment was designed to determine the effects of different forms of nitrogen fertilizer on plant development. It consisted of a factorial

design of two controlled factors. The first factor was nitrogen concentration with three levels: 0.4, 4.9, and 13.6 mmol $l^{-1}$ of nitrogen. The second factor was nitrogen form with two levels: $NH_4Cl$ and $KNO_3$ as nitrogen sources. There were six treatments corresponding to the interaction between both factors studied and one additional treatment without N. Seventy-five plants were used for each treatment. The plants for each treatment were grown in identical aeroponic tanks placed next to each other. The plants were inoculated 2 weeks after their transfer to aeroponics. Twenty-five plants from each treatment were randomly harvested 7, 17 and 37 days after their inoculation with *Bradyrhizobium*.

A second experiment was designed to test the nitrification process in the nutrients. It consisted of four treatments: 0.4 mmol $l^{-1}$ $NH_4Cl$, 0.4 mmol $l^{-1}$ $NH_4Cl$ with 5 mg $l^{-1}$ 2-chloro-6-(trichloromethyl) pyridine (Nitrapyrin), 0.4 mmol $l^{-1}$ $KNO_3$, no nitrogen added. *Acacia mangium* seedlings for the treatments, 75 individuals per treatment, were planted in strictly identical aeroponic systems. The plants were inoculated with *Bradyrhizobium* 12 days after their transfer to the aeroponic system. Ammonium and nitrate concentrations for each nutrient tank were measured daily over a period of seven days, eight weeks after plants were transferred to aeroponics.

Due to limited availability of time and number of strictly identical culture systems, the experiments have not been replicated. Variances of factors have thus not been addressed.

## Measurement of plant development

For each plant, root length and number of nodules were recorded. Plants were then oven dried for four days at 70 °C for the measurement of shoot and root weights and dry nodule weight.

## Measurement of leaf chlorophyll content

Total chlorophyll content was measured using six replicates per treatment. From each plant, 0.05 g of the third phyllode was sampled. The fresh samples were cut into thin slices and soaked in 5 ml of N,N-dimethylformamide in the dark for 48 h at 4 °C. The solvent absorbance was measured at 647 and 664 nm using a UV-visible spectrometer. Chlorophyll content was calculated according to Wellburn (1994) and expressed in micrograms per mg of fresh weight.

## Plant nitrogen and carbon content

Plant total leaves were dried for one week at 60 °C and ground into a fine powder. Ten samples were analysed for each treatment. Total carbon and nitrogen contents were determined by flash combustion followed by gas chromatographic separation in a CHNS element analyser Eurovector EA 300.

## Ammonium and nitrate in the nutrients

Ammonium was determined by its reaction with alkaline phenol and hypochlorite which forms indophenol blue in proportion to the ammonia concentration (US Environmental Protection Agency 1983). Nitrate was quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) was determined by its reaction with sulphanilamide coupled with N-(1-napphtyl) ethylenediamine dihydrochloride which forms a magenta dye in proportion to the nitrite concentration (US Environmental Protection Agency 1983). Measurements were performed using an automated ion analyser. Measurements were repeated three times for each sample and expressed as a mean value in ppm of N.

## Statistical analysis

Results were examined by two-factor analysis of variance (ANOVA). Assumptions of normal distributions were assessed using the Kolmogorov-Smirnoff test and the Levenes's analysis of variance test. For data that did not satisfy the conditions of normality and equal variance at $\alpha = 0.05$ level, ANOVA and post hoc tests were performed on ranks (Yandell 1997). There was 28% mortality of plants in the treatment with 13.6 mM of $NH_4Cl$. As such, for this treatment, measurements and calculations were only from the surviving plants. Nodule weight was considered for nodulated plants only. Student Newman Keul's (SNK) post-hoc tests were used for comparing groups of equal numbers and Tukey's extended Honestly Significant Difference (HSD) test (Spjøtvoll & Stoline 1973) for comparisons with groups of unequal numbers. All statistics were examined at $\alpha = 0.05$ level. Test procedures were carried out using the software Statistica version 6 (Statsoft Inc.).

## RESULTS

### Effects of nitrogen source and concentration on growth and development of *Acacia mangium*

The absence of nitrogen supply led to very poor plant development and a high C/N ratio (Figure 1). Thirty-seven days after inoculation with *Bradyrhizobium*, plants without nitrogen supplied had a height of only 7.0 ± 0.4 cm compared with plants in aeroponics supplemented with for example, 0.4 mM $NH_4Cl$, 13.6 mM $KNO_3$ and 4.9 mM $KNO_3$ that had heights of 36.4 ± 1.4 cm, 36.5 ± 1.4 cm and 36.4 ± 1.1 cm respectively. This was, thus, between five and six times shorter than average despite the fact they were nodulated. The phyllodes were too small in these plants growing without nitrogen supply and measurement of total chlorophyll could not be made.

Thirty-seven days after inoculation, higher nitrate concentration of 4.9 and 13.6 mM stimulated plant development in terms of shoot height but not in terms of shoot weight and root length (Figure 1, Table 1). For 0.4 mM of nitrate, a lower shoot height, chlorophyll content and a

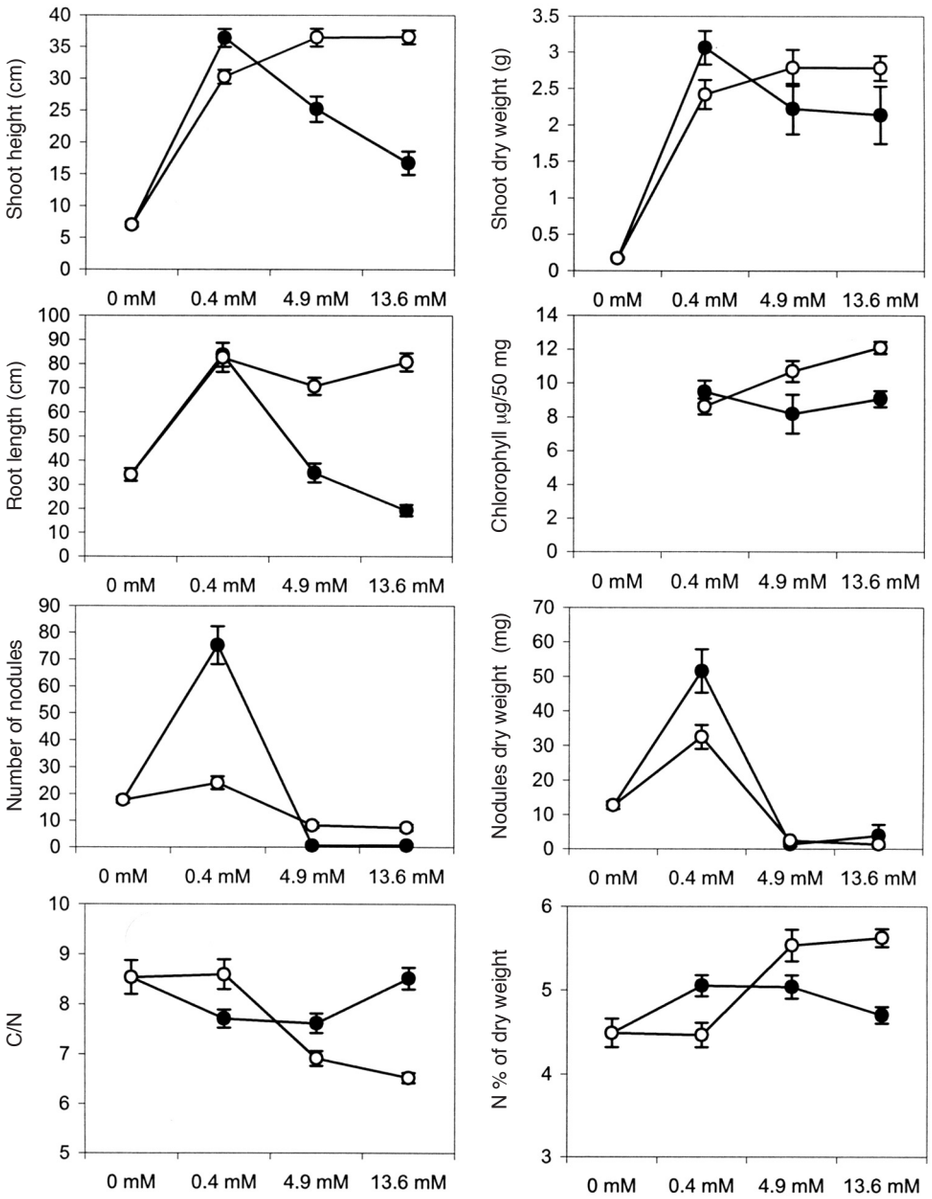


**Figure 1** Effects of two different sources of nitrogen $NH_4Cl$ and $KNO_3$ at four concentrations 0, 0.4, 4.9 and 13.6 mM on the development and nodulation of *A. mangium* 37 days after inoculation with *Bradyrhizobium* AUST 13C. Mean responses for $NH_4Cl$ are represented by solid circles and for $KNO_3$ by open circles. Bars indicate standard errors. Chlorophyll content was not measured at 0 mM of nitrogen.

**Table 1** Experimental factors tested alone or in combination for their effects on the development and nodulation of *A. mangium* in aeroponic culture 37 days after inoculation with *Bradyrhizobium* AUST 13C. Significant variations for $\alpha = 0.05$ level are indicated in bold.

| Test | P values | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Shoot height | Shoot weight | Root length | C/N | %N | Total Chl. | Nodules number | Nodules weight |
| Data | ranked | ranked | raw | raw | raw | ranked | ranked | ranked |
| Two-way ANOVA | | | | | | | | |
| N source | **<0.000** | 0.393 | **<0.000** | **<0.000** | **0.018** | **0.005** | **<0.000** | 0.462 |
| N concentration | **0.012** | 0.5187 | **<0.000** | **<0.000** | **0.001** | **0.028** | **<0.000** | **<0.000** |
| N source x N concentration | **<0.000** | **0.0136** | **<0.000** | **<0.000** | **<0.000** | **0.002** | **<0.000** | **<0.000** |
| Post-hoc pair wise comparisons | HSD | HSD | HSD | SNK | SNK | SNK | HSD | HSD |
| $NH_4^+$ vs. $NO_3^-$ within | | | | | | | | |
| 0.4 mM | **0.013** | 0.358 | 0.999 | **0.008** | **0.021** | 0.587 | **<0.000** | **0.007** |
| 4.9 mM | **<0.000** | 0.473 | **<0.000** | **0.016** | **0.038** | 0.072 | **<0.000** | 0.446 |
| 13.9 mM | **<0.000** | 0.672 | **<0.000** | **<0.000** | **<0.000** | **0.001** | **<0.000** | 0.987 |
| Within $NH_4^+$ | | | | | | | | |
| 0.4 mM vs. 4.9 mM | **<0.000** | 0.107 | **<0.000** | 0.751 | 0.943 | 0.649 | **<0.000** | **<0.000** |
| 4.9 mM vs. 13.6 mM | 0.074 | 1 | 0.241 | **0.007** | 0.091 | 0.79 | 0.902 | 0.941 |
| 0.4 mM vs. 13.6 mM | **<0.000** | 0.223 | **<0.000** | **0.006** | 0.182 | 0.536 | **<0.000** | **0.007** |
| Within $NO_3^-$ | | | | | | | | |
| 0.4 mM vs. 4.9 mM | **0.005** | 0.845 | 0.366 | **<0.000** | **<0.000** | **0.045** | **<0.000** | **<0.000** |
| 4.9 mM vs. 13.6 mM | 0.999 | 1 | 0.56 | 0.177 | 0.647 | 0.102 | 0.926 | 0.152 |
| 0.4 mM vs. 13.6 mM | **0.002** | 0.875 | 0.999 | **<0.000** | **<0.000** | **<0.000** | **<0.000** | **<0.000** |
| $NH_4^+$ vs. $NO_3^-$ between | | | | | | | | |
| 0.4 mM and 4.9 mM | **<0.000** | 0.107 | **<0.000** | 0.751 | 0.943 | 0.649 | **<0.000** | **<0.000** |
| 4.9 mM and 0.4 mM | 0.542 | 0.988 | **<0.000** | **0.006** | **0.014** | 0.707 | **<0.000** | **<0.000** |
| 4.9 mM and 13.6 mM | **<0.000** | 0.517 | **<0.000** | **0.001** | 0.022 | **0.001** | **<0.000** | 0.995 |
| 13.6 mM and 4.9 mM | **<0.000** | 0.633 | **<0.000** | **<0.000** | **<0.000** | 0.074 | **<0.000** | 1 |
| 0.4 mM and 13.6 mM | 0.997 | 0.956 | 0.996 | **<0.000** | **0.014** | **0.005** | **<0.000** | **<0.000** |
| 13.6 mM and 0.4 mM | **<0.000** | 0.995 | **<0.000** | 0.779 | 0.229 | 0.795 | **<0.000** | 0.123 |

higher C/N ratio suggested an effect of nitrogen shortage. No effect of nitrate concentration on shoot height, shoot weight and root length could be detected at 7 and 17 days after inoculation (Figures 2 and 3, Table 2).

Interactions between the forms of nitrogen source used and nitrogen concentrations were revealed for all growth parameters measured and at all collecting periods except for shoot height 7 days after inoculation (Tables 1 and 2). In contrast to nitrate, ammonium at 0.4 mM produced greater shoot height 17 days after inoculation and greater shoot height and leaf nitrogen content 37 days after inoculation (Figures 1 and 3, Tables 1 and 2). Seventeen days after inoculation, the shoot height of plants grown with 0.4 mM ammonium was significantly higher than those grown with 13.6, 4.9 or 0.4 mM nitrate.

Comparing nitrate as a source of nitrogen, ammonium had a deleterious effect at higher concentrations. With 13.6 mM of ammonium, 28% of the plants died. At concentrations of 13.6 and 4.9 mM, ammonium had a significantly negative effect on root length and shoot height except at 7 days after inoculation where only 13.6 mM of ammonium had a negative effect (Figures 1, 2 and 3, Tables 1 and 2).

However, this effect was less pronounced when shoot weights were compared (Figure 2). Shoot weight of plants supplied with ammonium at these levels were found not to be significantly different from those grown in similar concentrations of nitrates 17 and 37 days after inoculation (Tables 1 and 2). Plants growing in nutrient solutions supplied with 13.6 mM of ammonium had a higher C/N ratio and a lower N content in their phyllodes than those growing in solutions with lower concentrations of ammonium, i.e. ammonium at 4.9 mM and 0.4 mM (Figure 1, Table 1).

## Effects of nitrogen source and concentration on nodulation in *Acacia mangium*

For both ammonium and nitrate as nitrogen sources, the number of nodules remained constant or even diminished slightly between 17 and 37 days after inoculation while the root continued to grow extensively during this period (Figures 1 and 3). The consequences were that nearly all the nodules were distributed along a

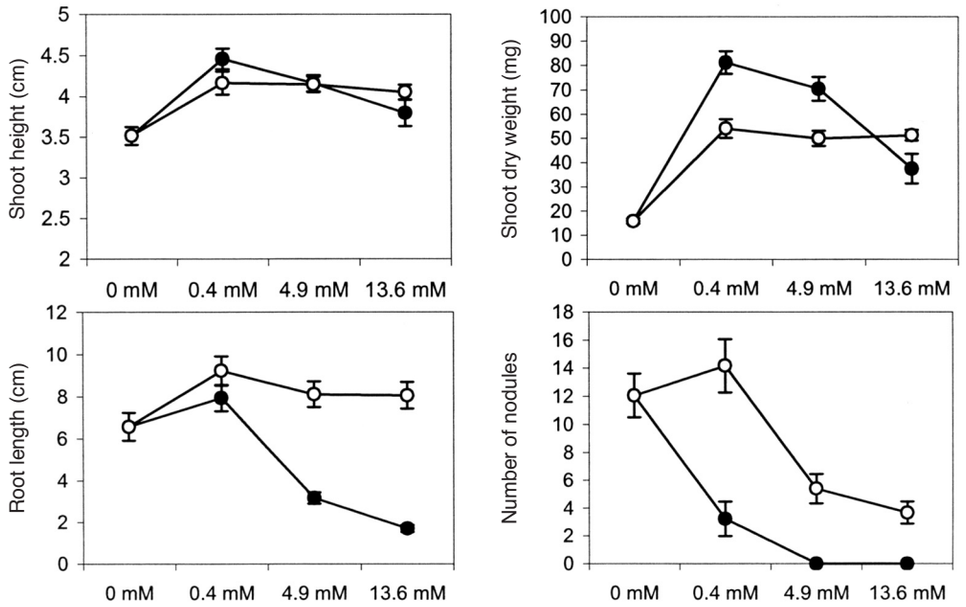


**Figure 2** Effects of two different sources of nitrogen $NH_4Cl$ and $KNO_3$ at four concentrations 0, 0.4, 4.9 and 13.6 mM on the development and nodulation of *A. mangium* 7 days after inoculation with *Bradyrhizobium* AUST 13C. Mean responses for $NH_4Cl$ are represented by solid circles and for $KNO_3$ by open circles. Bars indicate standard errors.

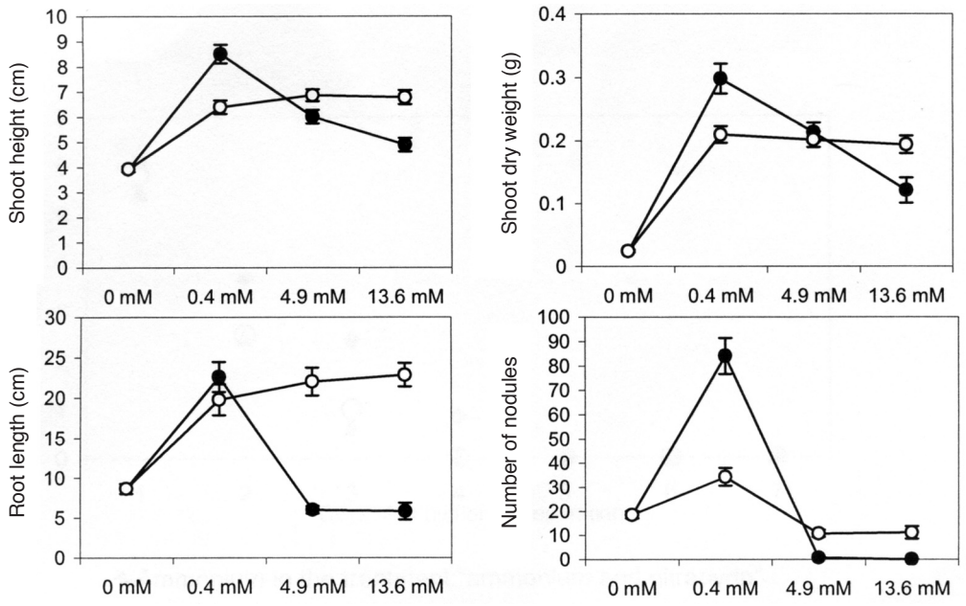


**Figure 3** Effects of two different sources of nitrogen $NH_4Cl$ and $KNO_3$ at four concentrations 0, 0.4, 4.9 and 13.6 mM on the development and nodulation of *A. mangium* 17 days after inoculation with *Bradyrhizobium* AUST 13C. Mean responses for $NH_4Cl$ are represented by solid circles and for $KNO_3$ by open circles. Bars indicate standard errors.

**Table 2** Experimental factors tested alone or in combination for their effects on the development and nodulation of *A. mangium* in aeroponic culture 7 and 17 days after inoculation with *Bradythizobium* AUST 13C. Significant variations for $\alpha = 0.05$ level are indicated in bold.

| Test | P values | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 7 days | | | | 17 days | | | |
| | Shoot height | Shoot weight | Root length | Nodule number | Shoot height | Shoot weight | Root length | Nodule number |
| Two-way ANOVA | raw | rank | rank | rank | raw | rank | rank | rank |
| N source | 0.877 | **<0.000** | **<0.000** | **<0.000** | 0.385 | 0.783 | **<0.000** | **<0.000** |
| N concentration | **0.008** | **<0.000** | **<0.000** | **<0.000** | **<0.000** | **<0.000** | **<0.000** | **<0.000** |
| N source x N concentration | 0.084 | **<0.000** | **<0.000** | 0.448 | **<0.000** | **<0.000** | **<0.000** | **<0.000** |
| Post-hoc pairwise comparisons | | | | | | | | |
| $NH_4^+$ vs. $NO_3^-$ within | | | | | | | | |
| 0.4 mM | 0.47 | **<0.000** | 0.61 | **<0.000** | **<0.000** | 0.057 | 0.658 | **<0.000** |
| 4.9 mM | 1 | **0.002** | **<0.000** | **<0.000** | 0.273 | 0.995 | **<0.000** | **<0.000** |
| 13.9 mM | 0.802 | 0.716 | **<0.000** | **<0.000** | **<0.000** | 0.134 | **<0.000** | **<0.000** |
| Within $NH_4^+$ | | | | | | | | |
| 0.4 mM vs. 4.9 mM | 0.454 | 0.545 | **<0.000** | **0.018** | **<0.000** | 0.052 | **<0.000** | **<0.000** |
| 4.9 mM vs. 13.6 mM | 0.466 | **<0.000** | 0.412 | 1 | 0.126 | **0.011** | 0.999 | 0.675 |
| 0.4 mM vs. 13.6 mM | **0.014** | **<0.000** | **<0.000** | 0.135 | **<0.000** | **<0.000** | **<0.000** | **<0.000** |
| Within $NO_3^-$ | | | | | | | | |
| 0.4 mM vs. 4.9 mM | 0.999 | 0.934 | 0.812 | **0.001** | 0.847 | 0.993 | 0.818 | **<0.000** |
| 4.9 mM vs. 13.6 mM | 0.991 | 0.999 | 0.999 | 0.846 | 0.999 | 0.997 | 0.979 | 0.943 |
| 0.4 mM vs. 13.6 mM | 0.983 | 0.989 | 0.705 | **<0.000** | 0.93 | 0.909 | 0.364 | **<0.000** |
| $NH_4^+$ vs. $NO_3^-$ between | | | | | | | | |
| 0.4 mM and 4.9 mM | 0.407 | **<0.000** | 0.999 | **0.002** | **<0.000** | **0.01** | 0.999 | **<0.000** |
| 4.9 mM and 0.4 mM | 1 | 0.051 | **<0.000** | **<0.000** | 0.933 | 1 | **<0.000** | **<0.000** |
| 4.9 mM and 13.6 mM | 0.985 | **0.007** | **<0.000** | **<0.000** | 0.397 | 0.92 | **<0.000** | **<0.000** |
| 13.6 mM and 4.9 mM | 0.505 | 0.855 | **<0.000** | **<0.000** | **<0.000** | **0.046** | **<0.000** | **<0.000** |
| 0.4 mM and 13.6 mM | 0.13 | **<0.000** | 0.999 | 0.104 | **<0.000** | **0.001** | 0.997 | **<0.000** |
| 13.6 mM and 0.4 mM | 0.453 | 0.39 | <0.000 | **<0.000** | **<0.01** | **0.01** | **<0.000** | **<0.000** |

Unequa-N Honestly Significant Difference (HSD) test was used.

20 cm basal root segment. The effect of nitrogen source on nodulation changed with time. Seven days after inoculation and at corresponding nitrogen concentrations, nodules were always more numerous with nitrate than ammonia (Figure 2, Table 2). However, at 17 and 37 days after inoculation, 0.4 mM ammonium stimulated the greatest nodulation. At high levels of nitrogen, 13.6 and 4.9 mM, nodulation was significantly reduced with the use of nitrate and nearly totally inhibited with the use of ammonium (Figures 1 and 3, Tables 1 and 2).

### Estimation of possible nitrification in aeroponic culture

Eight weeks after the beginning of culture in aeroponics and one week after solution renewal, no nitrate formation was detected in any of the nutrient tanks (Figure 4). Four days after the change of nutrients, all the supplied nitrogen had been used. The decrease in nitrogen concentration in the nutrients was similar for the treatments supplied with both ammonium and nitrate. The decrease in ammonium concentration was slightly less rapid when nitrapyrin, a nitrification inhibitor, was added (Figure 4). The amplitude of pH variations increased proportionally with the concentration of nitrogen. The pH increased with the use of nitrate and decreased with the use of ammonium (Figure 5).

## DISCUSSION

In aeroponic culture, plant roots are subjected to a highly aerated environment, which, together with addition of ammonium, is favourable for the establishment of nitrifying bacteria that may oxidize a part of the ammonium into nitrate. However, there was no evidence for nitrification. The observed delay in the decrease of ammonium in the tank supplied with nitrapyrin was probably not linked to the repression of a hidden nitrification process because, in this case, uptake of nitrate should

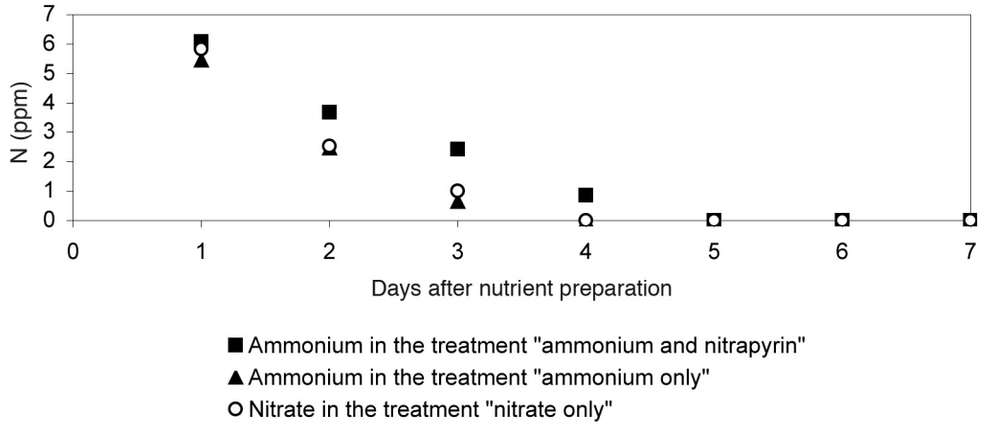


**Figure 4** Evolution of nitrate and ammonium concentrations in the nutrient tanks eight weeks after the transfer of plants to the aeroponics. Only treatments for which ammonium and nitrate could be measured are represented.

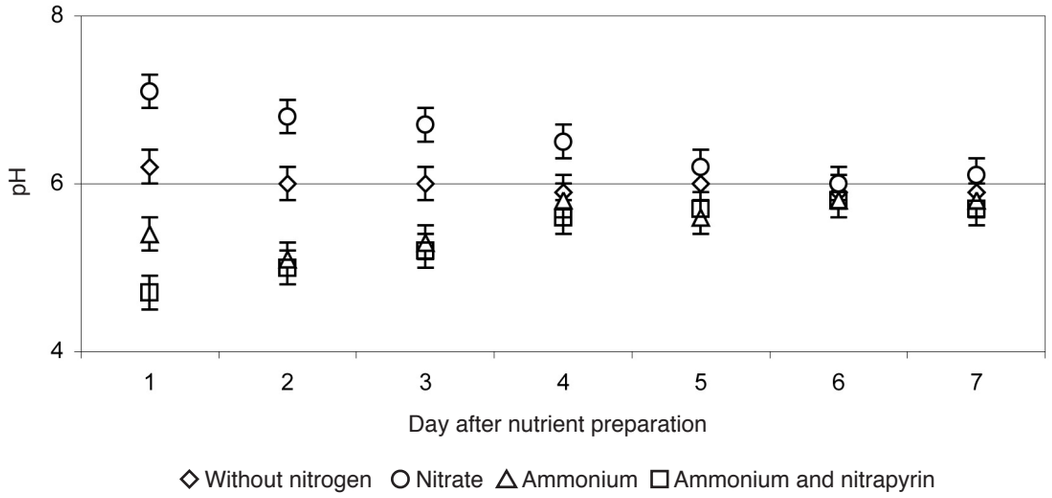


**Figure 5** pH values measured in the nutrient tanks 24 hours after daily adjustments to pH 6 ± 0.1. Values measured eight weeks after the transfer of plants to the aeroponics. Bars represent an arbitral imprecision range of ± 0.2 pH units.

have been faster than the uptake of ammonium. Moreover, the opposite and distinct effect of the supplied nitrogen source on pH variations confirmed indirectly that no significant amount of ammonium had been nitrified.

The absence of nitrification in the aeroponics tanks supplied with 0.4 mM ammonium might be explained by the relative acidity of the nutrients in which pH values varied between 4.5 and 6. For these values, the development of nitrifying bacteria is known to decrease drastically (Hagiopan & Ridley 1998). A constant aeration of the nutrient solution that tends to release dissolved $CO_2$ and the use of reverse-osmosis purified water might also have consequently decreased the available carbonate alkalinity required for the development of the ammonia oxidizing bacteria (Biesterfeld *et al.* 2001). The nutrient solutions were observed to have low buffering capacities and the pH variations were in correlation with the acidification and alkalization induced by the uptake of ammonium and nitrate respectively. The consumption of a single source of nitrogen could be indirectly monitored by the variations of the pH. In the case of an application on an industrial scale, it would be possible to use the data from the pH control for automatically adjusting the nitrogen supply

to the exact needs of the plants. It would, thus, also be feasible to adjust the pH of the nutrients with an appropriate control of the dosage of combined ammonium and nitrate solution, saving the use of NaOH and $H_2SO_4$ for the pH corrections.

The saplings that were not supplied with nitrogen had very poor development even though they were nodulated with an effective nitrogen-fixing *Bradyrhizobium* strain (Galiana *et al.* 1990). This stunted development had not been observed with the same treatment for the experiment designed for the evaluation of nitrification (data not shown). For this experiment, the inoculation of the seedlings with *Bradyrhizobium* had proceeded nine days earlier, suggesting that a stress period between the moment of depletion of nitrogen in the cotyledons and the export of fixed nitrogen from the nodules was responsible for the stunted development. At 0.4 mM of nitrogen, *A. mangium* showed a preference for ammonium as a nitrogen source for its growth. At 17 days, the 0.4 mM ammonium treatment gave significantly greater plant height and shoot weight than those obtained with 4.9 and 13.6 mM of nitrate. This difference was not observed at 37 days, probably because nitrogen becoming a limiting factor for older and bigger plants. This could have been the cause of a reduced expression of the growth stimulation observed when using ammonium at 0.4 mM.

The comparatively taller shoots and higher nitrogen content observed with ammonium at 0.4 mM could be explained by higher nodulation rates suggesting improved $N_2$ fixation. With 0.4 mM nitrate, the plants had a higher C/N ratio and lower nitrogen and leaf chlorophyll content suggesting a lack of nitrogen. A similar diminishing effect of nitrate on nodulation was obtained with *A. auriculiformis* in pot culture (Goi *et al.* 1993). Experiments on pea (Gulden & Vessey 1997, Fey & Vessey 2003, Bollman & Vessey 2006), white clover (Fey & Vessey 2004) and soybean (Gan *et al.* 2004) showed that, in contrast to nitrate, ammonium stimulated nodulation and plant mass when supplied at low concentrations. The mechanisms of this stimulation remain unknown. It has been argued that a low concentration of ammonium stimulates nodulation in pea via an increase of the cytokinin/auxin ratio (Fey & Vessey 2003, 2004). Bollman and Vessey (2006) discussed a possible link between the nitrogen source and the root soluble carbon availability as a regulator of nodulation (Tricot *et al.* 1997).

Nitrogen fixation, even with the use of selected and efficient rhizobial strains, was not sufficient to meet the nitrogen needs for maximal growth. This might be linked to the very fast growth rates of *A. mangium* that are achieved in aeroponic culture (Martin-Laurent *et al.* 1997). The use of low concentrations of ammonium as a nitrogen source permitted both an optimal vegetative development and high nodulation rates to be achieved.

## AKNOWLEDGEMENTS

The authors thank Nanyang Technological University, National Institute of Education, Singapore and the French Ministry of Foreign Affairs for financial support.

## REFERENCES

Adjers, G. 1987. Tropical peat in Southeast Asia and its use as growth substrate in a forest tree nursery in South Kalimantan, Indonesia. MSc thesis, University of Helsinki, Finland.

Bollman, M. I. & Vessey, J. K. 2006. Differential effects of nitrate and ammonium supply on nodule initiation, development, and distribution on roots of pea (*Pisum sativum* L.) *Canadian Journal of Botany* 84: 893–903.

Biesterfeld, S., Farmer G, Russell, P. & Figueroa, L. 2001. Effect of alkalinity type and concentration on nitrifying biofilm activity. *WEFTEC 2001 Conference Proceedings*. 13–17 October 2001, Atlanta.

Cossalter, C. & Pye-Smith, C. 2003. *Fast-Wood Forestry. Myth and Realities*. Center for International Forestry Research, Bogor.

De La Cruz, R. E. & Garcia M. U. 1992. Nitrogen fixation and mycorrhizae in acacias on degraded grasslands. Pp. 58–71 in Kamis, A. & Taylor, D. A. (Eds.) in *Tropical Acacias in East Asia and Pacific*. Winrock International, Bangkok.

De La Cruz, R. E., Lorilla, B. E. & Zarate, J. 1992. Application of mycorrhiza in barerooting and direct seeding technologies for reforestation. Paper presented at the BIO-REFOR Workshop. 19–21 May 1992, Tsukuba.

Fey, H. & Vessey, J. K. 2003. Involvement of cytokinin in the stimulation of nodulation by low concentration of ammonium in *Pisum sativum* L. *Physiologia Plantarum* 118: 447–455.

Fey, H. & Vessey, J. K. 2004. Further investigation on the role of auxin and cytokinin in the $NH4^+$-induced stimulation of nodulation using white clover transformed with the auxin-sensitive reporter *GH3: gusA. Physiologa Plantarum* 121: 674–681.

Galiana, A., Chaumont, J., Diem, H. G. & Dommergues, Y. R. 1990. Nitrogen-fixing potential of *Acacia mangium* and *Acacia auriculiformis* seedlings inoculated with *Bradyrhizobium* and *Rhizobium* spp. *Biology and Fertility of Soils* 9: 261–267.

Galiana, A., Balle, P., N'Guessan Kanga, A. & Domenach, A. M. 2002. Nitrogen fixation estimated by the $^{15}N$ natural abundance method in *Acacia mangium* Willd. inoculated with *Bradyrhizobium* sp. and grown in silvicultural conditions. *Soil Biology and Biochemistry* 34: 251–262.

Gan, Y. B., Stulen, I., Van Keulen, H. & Kuiper, P. J. C. 2004. Low concentrations of nitrate and ammonium stimulate nodulation and $N_2$ fixation while inhibiting specific nodulation (nodule DW $g^{-1}$ root dry weight) and specific $N_2$ fixation ($N_2$ fixed $g^{-1}$ root dry mass) in soybean. *Plant and Soil* 258: 281–292.

Goi, S. R., Sprent, J. I., James, E. K. & Jacob-Neto, J. 1993. Influence of nitrogen form and concentration on the nitrogen fixation of *Acacia auriculiformis*. *Symbiosis* 14: 115–122.

Gulden, R. H. & Vessey, J. K. 1997. The stimulating effect of ammonium on nodulation in *Pisum sativum* L. is not long lived once ammonium supply is discontinued. *Plant and Soil* 195: 195–205.

Hagiopan, D. S. & Ridley, J. G. 1998. A closer look at the bacteriology of nitrification. *Aquacultural Engineering* 18: 223–244.

Hansen, A. P. & Pate, J. S. 1987. Evaluation of the $^{15}N$ natural abundance method and xylem sap analysis for assessing N fixation of understorey legumes in Jarrah (*Eucalyptus marginata* Donn ex Sm.) forest in S.W. Australia. *Journal of Experimental Botany* 38: 1446–1458.

Lee, S. K., Liu, C. Y., Wong, Y. W. & Sim, H. B. 1994. Effect of the nozzle designs on the growth of some tropical vegetables in aeroponic systems in the tropics. Pp. 49–54 in Loh, S. C. *et al.* (Eds.) *Proceedings of the International Conference on Agrotechnology in the Commonwealth Singapore*. Republic of Singapore.

Martin-Laurent, F., Fremont, M., Lee, S. K., Tham, F. Y., Prin, Y., Tan, T. K. & Diem, H. G. 1999. Effect of inoculation with selected *Bradyrhizobium* spp. on the survival and growth of *Acacia mangium* saplings after 20 months in the field. *Journal of Tropical Forest Science* 11: 470–483.

Martin-Laurent, F., Lee, S. K., Tham, F. Y., He, J., Diem, H. G. & Durand, P. 1997. A new approach to enhance growth and nodulation of *Acacia mangium* through aeroponic culture. *Biology and Fertility of Soils* 25: 7–12.

Pinyopusarerk, K., Liang, S. B. & Gunn B. V. 1993. Taxonomy, distribution, biology, and use as an exotic. Pp. 1–9 in Awang, K. & Taylor, D. (Eds.) Acacia mangium *Growing and Utilization*. MPTS Monograph Series No. 3. Winrock International and FAO, Bangkok.

Spjøtvoll, E. & Stoline M. R. 1973. An extension of the T-method of multiple comparison to include the case with unequal sample size. *Journal of the American Statistical Association* 68(344): 975–978.

Tricot, F., Crozat, Y. & Pellerin, S. 1997. Root system growth and nodule establishment on pea (*Pisum sativum* L.). *Journal of Experimental Botany* 48: 1935–1941.

US Environmental Protection Agency. 1983. *Methods for Chemical Analysis of Water and Wastes*. EPA-6000/4-79–020 Methods 365.4 and 350.1.

Vincent, J. M. 1970. *A Manual for the Practical Study of the Root-Nodule Bacteria*. Blackwell Scientific Publication, Oxford.

Wellburn, A. R. 1994. The spectral determination of chlorophylls a and b as well as total carotenoids using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* 144: 307–313.

Yandell, B. S. 1997. *Practical Data Analysis for Designed Experiments*. Chapmann & Hall, New York.