EUCALYPTUS-ACACIA MIXED PLANTING EFFECTS ON THE NODULAR NITROGENASE AND NITRATE REDUCTASE ACTIVITY IN *ACACIA NILOTICA* SEEDLINGS

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POKHRIYAL, T. C., CHAUKIYAL, S. P. & SINGH, U. 2001. *Eucalyptus-Acacia* mixed planting effects on the nodular nitrogenase and nitrate reductase activity in *Acacia nilotica* seedlings. Effects of *Eucalyptus* mixed planting on the seasonal patterns of nitrogenase and nitrate reductase activites and nodule biomass were reported in the roots of *Acacia nilotica* seedlings. Nitrogenase and *in vivo* nitrate reductase activities and nodule biomass per plant followed almost similar trends in *Acacia* single and *Eucalyptus-Acacia* mixed planting treatments. No significant effects were observed in nitrogenase and nitrate reductase activities in acacia nodules in the mixed planting compared to single. However, nitrogenase activity and nodule biomass varied seasonally in both the planting treatments.

Key words: Nitrogenase (N_2ase) - nitrate reductase (NR) - *Eucalyptus* - *Acacia nilotica* - mixed planting

POKHRIYAL, T. C., CHAUKIYAL, S. P. & SINGH, U. 2001. Kesan penanaman campur *Eucalyptus-Acacia* ke atas aktiviti nitrogenase dan nitrat reduktase nodul di dalam anak benih *Acacia nilotica*. Kesan penanaman campur *Eucalyptus* ke atas corak bermusim aktiviti nitrogenase dan nitrat reduktase serta biojisim nodul dilaporkan untuk akar anak benih *Acacia nilotica*. Aktiviti nitrogenase dan nitrat reduktase *in vivo* serta biojisim nodul setiap pokok mengikut trend yang hampir sama dengan rawatan *Acacia* tunggal dan rawatan penanaman campur *Eucalyptus-Acacia*. Tiada kesan bererti dicerap dalam aktiviti nitrogenase dan nitrat reduktase di dalam nodul akasia dalam penanaman campur berbanding dengan penanaman tunggal. Bagaimanapun, aktiviti nitrogenase dan biojisim nodul berubah-ubah mengikut musim dalam kedua-dua rawatan penanaman.

Introduction

Nitrogen (N) deficiency is a major cause of low productivity in forest plantation crops, which are typically subjected to repeated harvests. Intercropping of legumes with non-legumes has been used as one of the most popular cropping systems for balancing soil N status. Intercropping is increasingly practiced under various afforestation programmes for energy conservation with the escalating demand for fuel, fodder and commercial timber in relation to the increasing population and decreasing resources of the developing countries.

Increased soil N availability and tree growth have been reported when non-nitrogen fixing trees are intercropped with nitrogen fixing trees (Tarrant & Trappe 1971, Haines & De Bell 1979, Du Cros *et al.* 1984, De Bell *et al.* 1989). While selecting a nitrogen fixing species for intercropping, the possibility of competition between the associated species, which may involve allelopathy as well as demand for light, water and nutrients, should be given due consideration. Rice (1979) observed that the chemicals released by plants influence the growth of their neighbouring plants. In

Eucalyptus hybrids higher yields are obtained in short rotations due to its rapid growth (Walter 1973, Whitesell 1975). Therefore, there is a need to explore the possibilities of such combinations of non-nitrogen fixing and nitrogen fixing tree species, which can supplement N requirement under various plantation programmes. In the recent past, *Eucalyptus* and *Acacia* species have been widely grown in single and mixed rows on the field boundaries mostly under agroforestry plantation programmes. However, the allelopathic effects of certain *Eucalyptus* species due to toxic chemicals released by the leaves and litter have been reported (Al Mousawi & Al Naib 1975, May & Ash 1990). These effects may prevent the successful intercropping of *Eucalyptus* species with nitrogen fixing species.

Recently, considerable controversy has arisen about the establishment of *Eucalyptus* plantations. Large-scale plantations have been associated with various adverse effects on soil and underground water levels (Poore & Fries 1985). However, De Bell *et al.* (1985) observed beneficial changes when *Eucalyptus* was grown in mixed plantings with nitrogen fixing *Albizia* trees in Hawaii. In contrast, a decrease in nitrate reductase (NR) activity and biomass production in the leaf, stem and root were reported by Pokhriyal *et al.* (1995) in *Eucalyptus-Acacia* mixed compared to single planting treatments.

Nitrogenase (N_2ase) and NR co-exist in nodules of leguminous plants (Evans & Russel 1979) and their activities have been correlated in symbiotic nitrogen fixation (Cheniae & Evans 1960). However, not many studies emphasise on whether *Eucalyptus* inhibits nitrogen fixation and nitrate assimilation in the nodules of associated nitrogen fixing tree species. Our objective was to study the effect of *Eucalyptus* in a mixed (*Eucalyptus* and *Acacia*) planting on N_2ase and NR activities in *Acacia nilotica* root nodules under nursery conditions.

Materials and methods

One-month-old seedlings of *Eucalyptus tereticornis* and *Acacia nilotica* were obtained from the Seed Testing Laboratory of Forest Research Institute, Dehra Dun, India. The seedlings were transplanted into 30 cm diameter earthen pots filled with soil and farmyard manure, mixed in 3:1 ratio. Two planting treatments, namely, single and mixed, were tested for a one-year duration. In the single planting treatment, *Eucalyptus* and *Acacia* seedlings were planted separately in individual pots, whereas in the mixed (*Acacia* + *Eucalyptus*) treatment, both species were grown in the same pot. The details about the experimental procedures are discussed elsewhere by Pokhriyal *et al.* (1995).

Seedlings were allowed to grow for the period of three months to establish and develop symbiotic association with *Rhizobium*. Nodules were excavated, washed, weighed and compared for N_2ase (nmoles C_2H_2 reduced) and *in vivo* NR (nmoles NO_3^- reduced) activities. Biomass production in *Acacia* grown in single and in *Eucalyptus-Acacia* mixed planting treatments were determined at monthly intervals. N_2ase activity was determined by acetylene-reduction method as described by Hardy *et al.* (1968). Approximately 1 g nodule each was put into the rubber serum-stoppered incubation vials containing 10% acetylene in shaking water bath for 1 h at 25 °C. The ethylene produced by the reduction of acetylene was measured by injecting 1 ml of the gas mixture into Porapack-N column (1.8 m × 3.12 mm OD and 80–100 mesh) of M/S CIC Gas Chromatograph. Nitrogen was used as the carrier gas. Ethylene produced was quantified with reference to the standard ethylene gas supplied by EDT Research, London. The method used for the estimation of *in vivo* NR activity was similar to that described by Hageman and Hucklesby (1971) and modified by Pokhriyal *et al.* (1988). Overall, annual activities were divided into three seasons, namely, winter (November, December, January, February), summer (March, April, May, June) and rainy (July, August, September, October) respectively by calculating the average readings of four months. The observations were based on three replications from which means, standard errors and levels of significance were calculated (Snedecor & Cochran 1967).

Results

Nodule N_2ase, NR activities and biomass produced per plant followed similar trends in both *Acacia* single and *Eucalyptus* mixed plantings. Maximum and minimum N_2ase activities (nmoles acetylene reduced g^{-1} fresh wt. h^{-1}) in July and February respectively and two peaks in the months of November and June for total N_2ase activity (nmoles acetylene reduced pl^{-1} h^{-1}) were observed under both planting treatments in *Acacia* root nodules (Figure 1). The N_2ase activity tend to be higher in *Eucalyptus-Acacia* mixed plantings but it did not differ significantly compared to *Acacia* alone, whereas values for the monthly observations differed significantly ($p < 0.05$) among themselves.

In both planting treatments, maximum and minimum NR activities (nmoles NO_3^- reduced g^{-1} fresh wt. h^{-1}) were observed in the months of July and February respectively. Total NR activity (nmoles NO_3^- reduced pl^{-1} h^{-1}) exhibited three distinct peaks in November, April and July in *Acacia* alone and *Eucalyptus* mixed plantings (Figure 2). The differences in the monthly observations were significant ($p < 0.05$) among themselves in both planting treatments. Maximum nodule fresh weights were recorded during January and December in both single and mixed grown plants respectively, and minimum in March (Figure 3). However, the differences in the nodule biomass were observed to be higher in the single compared to the mixed plantings.

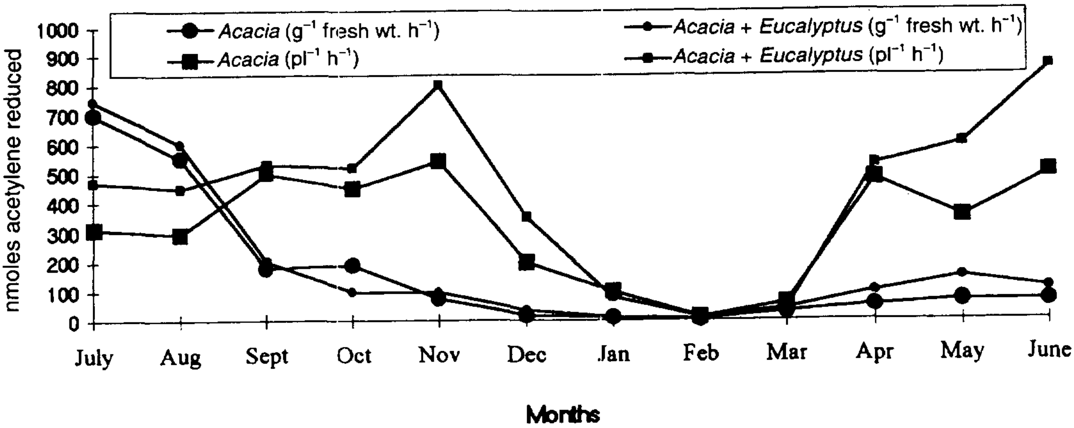

Figure 1. *Eucalyptus* mixed planting effects on nitrogenase activity in *Acacia nilotica* seedlings

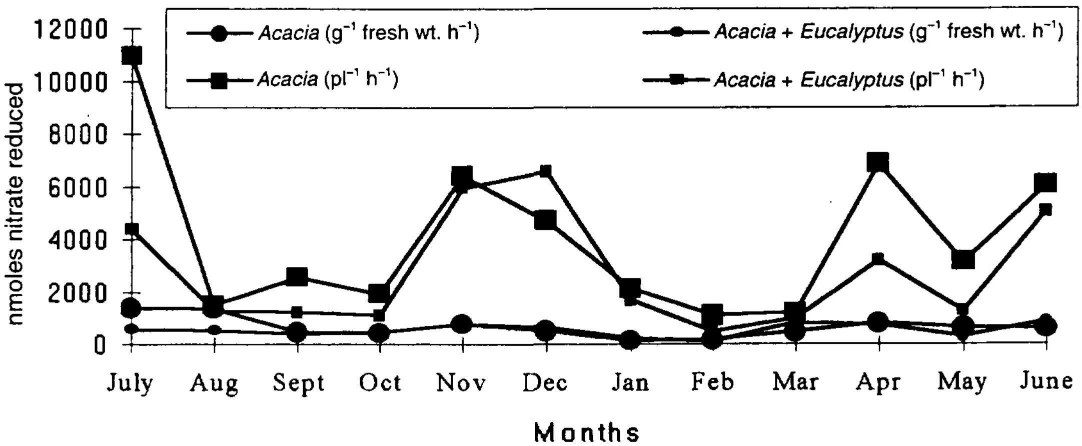

Figure 2. *Eucalyptus* mixed planting effects on nodular nitrate reductase activity in *Acacia nilotica* seedlings

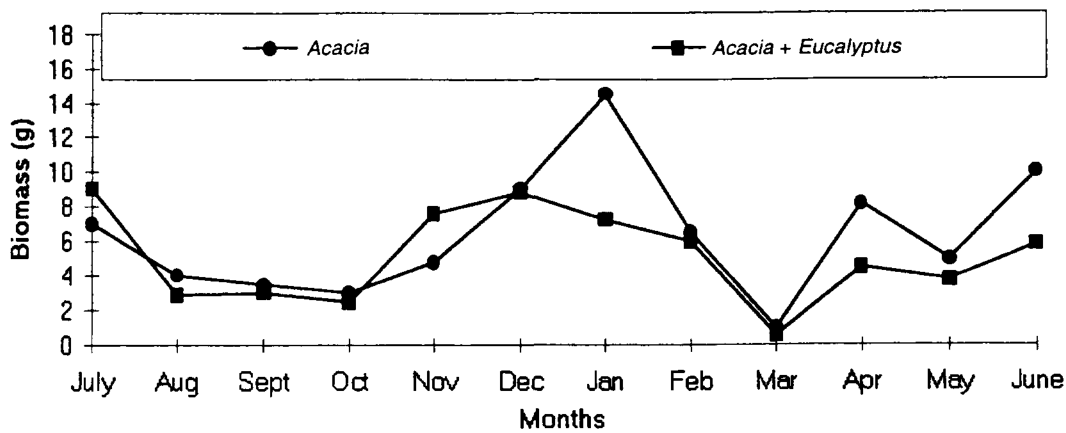

Figure 3. *Eucalyptus* mixed planting effects on nodule fresh weight biomass per plant in *Acacia nilotica* seedlings

On the seasonal average basis, maximum nodule biomass was recorded during the winter and minimum in the rainy season in both single and mixed plantings. Maximum and minimum N_2ase activities were recorded in the rainy and winter seasons in both planting treatments. Maximum and minimum NR activities occurred respectively in summer and winter in *Acacia* alone and winter and rainy seasons in *Eucalyptus* mixed plantings (Table 1).

Discussion

Nitrogen fixing tree species increase the growth and nitrogen content of non-nitrogen fixing species (Agboola & Fayemi 1972, Remison 1978, Eaglesham *et al.* 1981). However, available quantitative data are insufficient to establish whether there is

Table 1. Effect of seasonal changes on nodule biomass, its nitrogenase and *in vivo* nitrate reductase activity per plant in *Acacia* single and *Acacia* + *Eucalyptus* mixed seedlings

Variable	Planting treatment	Season			Significant/ insignificant differences
		Winter	Summer	Rainy	
Nodule biomass (g) pl^{-1}	*Acacia*	8.9	6.8	4.3	Season – *
	Acacia + *Eucalyptus*	7.6	4.1	4.1	Planting treatment – NS
Nitrogenase activity (nmoles C_2H_2 reduced pl^{-1} h^{-1})	*Acacia*	220.5	340.1	683.6	Season – **
	Acacia + *Eucalyptus*	319.8	546.9	732.6	Planting treatment – NS
Nitrate reductase activity (nmoles NO_3^- reduced pl^{-1} h^{-1})	*Acacia*	3579.9	4261.4	4208.5	Season – NS
	Acacia + *Eucalyptus*	3669.9	2583.5	1976.4	Planting treatment – NS

NS-Non-significant, *Significant at 1% level of probability, **Significant at 5% level of probability

competition between the associated nitrogen fixing and non-fixing species for the various physiological and biochemical activities in the nodules.

In the present study, both *Acacia* single as well as *Acacia-Eucalyptus* mixed plantings, displayed no significant differences in the N_2ase and NR activities in the root nodules of *Acacia*. Sheel *et al.* (1992) reported that *Eucalyptus* was more sensitive towards growth and NR activity compared to *Acacia* seedlings in the mixed plantings. Pokhriyal *et al.* (1995) reported a suppression in the NR activity and biomass of different plant parts in *Eucalyptus-Acacia* mixed plantings compared to *Eucalyptus* and *Acacia* alone. No reports are available on the adverse effects of *Eucalyptus* close planting on the nodular nitrogen metabolism. There is substantive evidence to show that *Eucalyptus* leaf litter leachates have allelopathic effects on associated vegetation (Al Mousawi & Al Naib 1975, Basu *et al.* 1987). Conversely, reports have also shown these vegetation benefit from interplantation with nitrogen fixing trees such as *Eucalyptus* (De Bell *et al.* 1985, Schubert 1985). Jobidon and Thibault (1982) reported an inhibition of nodulation in *Alnus crispa* due to *Populus balsamifera* leaf leachates. N_2ase and NR activities as well as nodule biomass in *Eucalyptus-Acacia* mixed and *Acacia* alone plantings have followed almost similar trend as the seasonal variations. Almost similar results for leaf, stem and root biomass and NR activity in both planting treatments under different seasons were reported by Pokhriyal *et al.* (1995).

The results in the present study confirm that *Eucalyptus* root leachates have no adverse effects on nitrogen fixation, assimilation and nodulation in the *Eucalyptus* mixed *Acacia* seedling roots compared to *Acacia* alone. Significant seasonal differences were recorded in N_2ase activity and nodule biomass production in both plantings (Table 1). However, an increase and decrease in the seasonal N_2ase and NR activities in *Eucalyptus-Acacia* mixed and single plantings might be compensating the nitrogen requirement by manipulating these enzymatic activities with the seasonal variations (Pokhriyal *et al.* 1991).

In the silvicultural management system, nitrogen deficiency of forest soils can be properly managed by the selection of suitable intercropping practices. Although, there is substantial experimental evidence to show that inclusion of nitrogen fixing plants in a managed forest system could prove beneficial, the same has, however, not been put to widespread use. There is a need for a careful study on the behaviour of different nitrogen fixing and non-fixing tree species before such intercropping combinations can be safely recommended for practical adoption on a large scale basis.

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