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EUCALYTUS AND *ACACIA* MIXED PLANTING EFFECTS ON *IN-VIVO* NITRATE REDUCTASE ACTIVITY AND BIOMASS PRODUCTION

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POKHRIYAL, T.C., CHAUKIYAL, S.P. & SINGH, U. 1995. *Eucalyptus* and *Acacia* mixed planting effects on *in-vivo* nitrate reductase activity and biomass production. *Eucalyptus tereticornis* and *Acacia nilotica* mixed planting effects on the seasonal nitrate reductase (NR) activity and biomass production were studied under pot culture conditions. A progressive increase in NR activity and biomass production in different plant parts, i.e. leaf, stem and root was observed in *Eucalyptus* and *Acacia* species in both, single and mixed planting treatments. Maximum *in-vivo* NR activity was observed during the summer followed by winter and rainy seasons in both the species and planting treatments. Mixed planting treatment suppressed NR activity and biomass of different plant parts as compared to single planting but the decrease was only significant between *Eucalyptus* and mixed *Eucalyptus* - *Acacia* plantings.

Key words: *In-vivo* nitrate reductase (NR) activity - biomass - *Eucalyptus* - *Acacia*

POKHRIYAL, T.C., CHAUKIYAL, S.P. & SINGH, U. 1995. Kesan-kesan penanaman campuran *Eucalyptus* dan *Acacia* pada keaktifan nitrat reduktase *in-vivo* dan pengeluaran biojisim. Kesan-kesan penanaman campuran *Eucalyptus tereticornis* dan *Acacia nilotica* pada keaktifan nitrat reduktase (NR) bermusim dan pengeluaran biojisim dikaji di bawah keadaan-keadaan kultur pasir. Kenaikan progresif dalam keaktifan NR dan pengeluaran biojisim pada bahagian tumbuhan yang berbeza iaitu daun, batang dan akar dilihat pada spesies *Eucalyptus* dan *Acacia* dalam kedua-dua rawatan penanaman tunggal dan bercampur. Keaktifan NR *in-vivo* yang maksimum dilihat semasa musim panas diikuti dengan musim sejuk dan musim hujan pada kedua-dua spesies dan rawatan penanaman. Rawatan penanaman bercampur menyekat keaktifan NR dan biojisim pelbagai bahagian tumbuhan berbanding dengan penanaman tunggal tetapi penurunan ini hanya ketara antara penanaman *Eucalyptus* dan campuran *Eucalyptus Acacia.*

Introduction

Inter-planting of nitrogen-fixing with non-fixing tree species involves sharing of site and resources during the process of growth and development. The only source of nitrogen that is generally available in the acceptable form in the soil is the nitrate and ammonium. The predominant form of nitrogen available to the plant is nitrate, since in most parts of the soil ammonium-nitrogen is rapidly nitrified into nitrate-nitrogen. It has been reported that NR activity could be correlated with actual accumulation of reduced nitrogen (Dalling *et al.* 1975, Brunetti & Hageman 1976). The regulation of NR activity in higher plants is very complex and not fully understood because of many inter-relationships existing among regulatory factors.

Its regulation appears to differ from species to species as well as different plant parts (Hewitt *et al.* 1978, Lee 1980, Schrader & Thomas 1981). Lee and Stewart (1978) and Pate (1983) observed that most of the woody species reduce nitrate only in the roots, except at very high nitrate supply, whereas Al Gharbi and Hipkin (1984) and Smironoff *et al.* (1984) reported that a number of woody species possess considerable *in-vivo* NR activity in the leaves.

In the recent past, *Eucalyptus* and *Acacia* species have been widely grown in single and mixed rows on field boundaries mostly under agro-forestry plantation programmes. Rice (1979) observed that the chemicals released by the plants variously influence the growth behaviour of neighbouring and under vegetation. There are evidences that *Eucalyptus* leaf litter leachates have an allelopathic effect on the associated vegetation (Lerner & Evenari 1961, Del Moral & Muller 1970, Basu *et al.* 1987). *Eucalyptus* trees show significant changes in their morphological characters when grown in mixed planting with nitrogen- fixing trees (De Bell *et al.* 1985). However, effects of mixed planting on nitrate assimilation pattern and biomass production in different plant parts of *Acacia nilotica* (nitrogen-fixing) and *Eucalyptus tereticornis* (non-fixing) species are not well known. Here, our objective was to study the inter-planting effects of *Eucalyptus* and *Acacia* on the seasonal nitrate reductase activity and biomass production in different plant parts under nursery conditions, so that combination of these species can be recommended for various afforestation programmes.

Materials and methods

One-month-old seedlings of *Eucalyptus tereticornis* and *Acacia nilotica* were procured from the Seed Testing Laboratory of the Forest Research Institute, Dehra Dun, India. The seedlings were transplanted to 30 cm diameter earthen pots filled with soil and farm yard manure, mixed in 3:1 ratio. Two planting treatments, i.e. single and mixed, were tested for one year duration. In single planting treatment *Eucalyptus* and *Acacia* seedlings were planted separately in each individual pots, whereas in the mixed planting treatment, both the species were grown together in the same pot. *Eucalyptus*, single (*Eucalyptus*) and mixed (*Eucalyptus* + *Acacia*), similarly, *Acacia*, single (*Acacia*) and mixed (*Acacia* + *Eucalyptus*) planting treatments were compared respectively for the seasonal nitrate reductase activity and biomass among themselves.

The seedlings were allowed to establish for four weeks. Three plants were harvested at monthly intervals in each treatment. The roots were separated from the soil with the help of regulated water pressure to avoid root damage. After thorough washing, these plants were wrapped in moist muslin cloth and brought to the laboratory. Different plant parts, i.e. leaf, stem and roots, were separated, and after excess of moisture was removed with filter papers, they were weighed and finally kept for the determination of *in-vivo* NR activity, with the remaining plant materials kept in the oven at 80 °C to obtain their dry weights. The method used for the estimation of *in-vivo* NR activity was similar to that described by Hageman and Hucklesby (1971) and earlier adopted for *Eucalyptus* (Pokhriyal & Raturi

1984) and *Acacia* (Pokhriyal *et al.* 1988). The nitrite produced during reaction was determined by the method described by Evans and Nason (1953). All the estimates were carried out in duplicate. Total NR activity (n moles NO_3^- reduced per plant part h^{-1}) was calculated by multiplying the *in-vivo* NR activity (n moles NO_3^- reduced g^{-1} fresh wt h^{-1}) by fresh weight of plant parts at each sample. Means and standard errors were calculated in the usual manner (Snedecor & Cochran 1975). Analysis of variance was done and least significant differences were calculated wherever possible.

Results

NR activity of different plant parts, i.e. leaf, stem and root, increased progressively with growth in *Eucalyptus* and *Acacia* species and planting (single and mixed) treatments. NR activity in different plant parts followed a similar pattern in both the species and planting treatments (Figure 1). Maximum NR (g^{-1} fresh wt. h^{-1}) activity was observed in the leaves followed by the root and stem, whereas, the total NR (per plant part h^{-1}) activity was maximum in roots followed by stem and leaves in both the species and planting treatments (Figure 2). NR activity was maximum during summer followed by winter and the rainy season in the both species and planting treatments (Table 1).

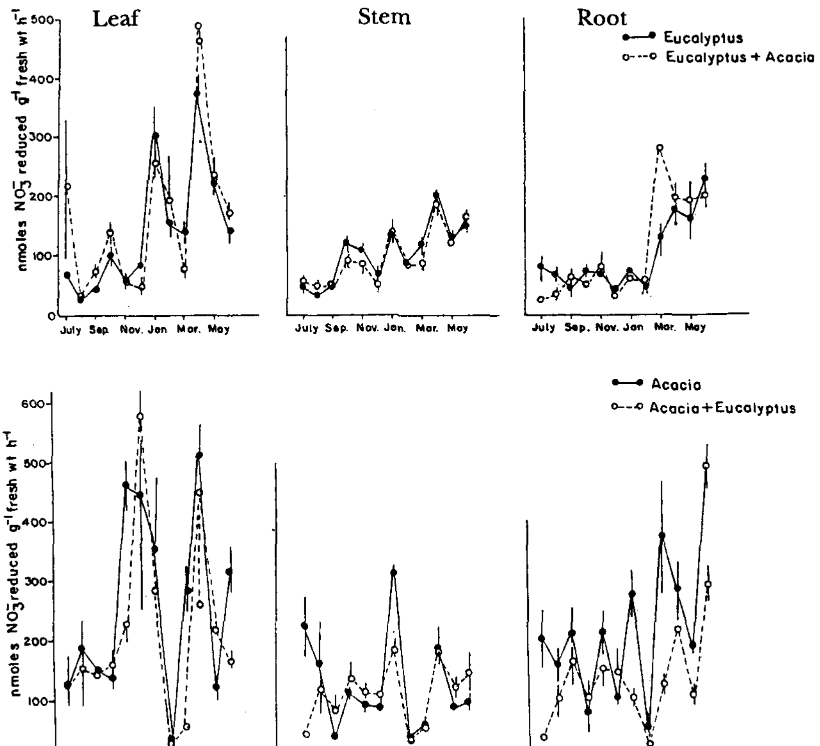

Figure 1. Mixed planting effects on NR (n moles NO_3^- g^{-1} fresh wt. h^{-1}) activity in leaf, stem and root of *Eucalyptus* and *Acacia* seedlings

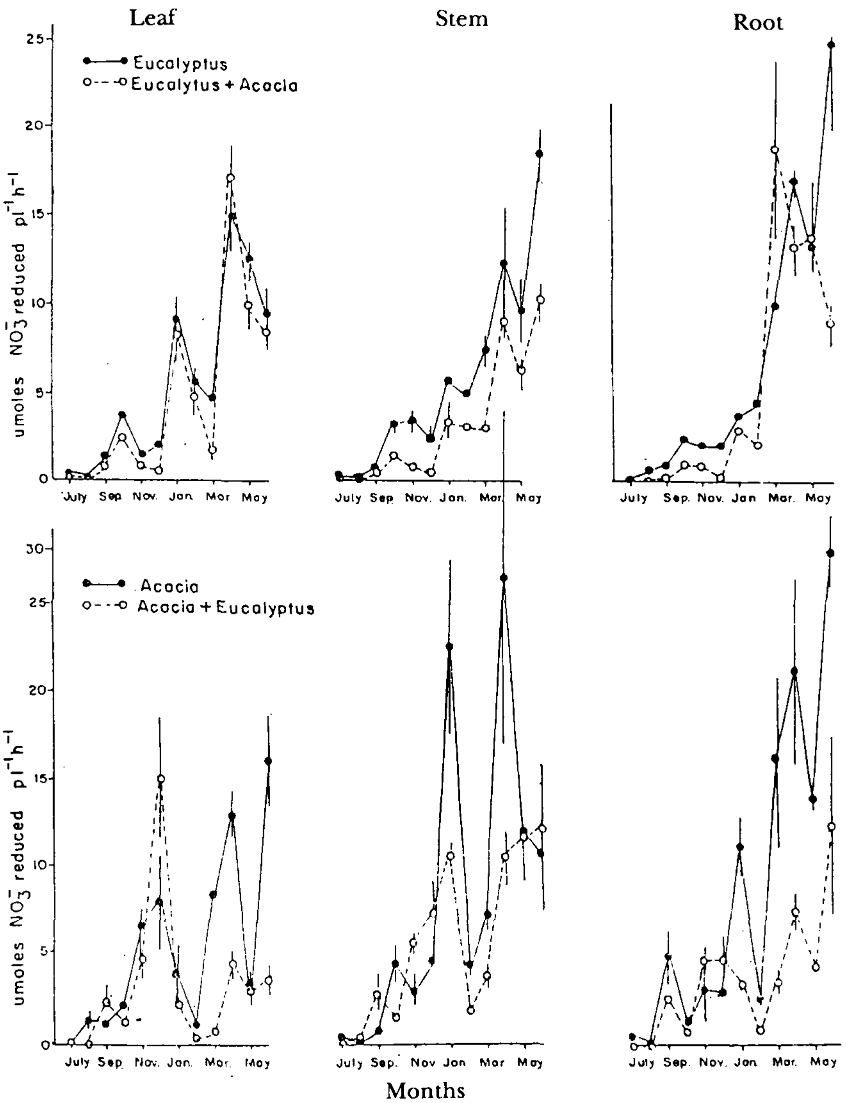

Figure 2. Mixed planting effects on total NR (n moles NO_3^- per plant part h^{-1}) activity in leaf, stem and root of *Eucalyptus* and *Acacia* seedlings

Fresh and dry weight values of different plant parts were always lower in the mixed planting as compared to single treatments in both species (Figure 3). The mixed planting treatment significantly suppressed the fresh and dry weights of different plant parts of *Eucalyptus* in all the three seasons, whereas no significant differences in the biomass were observed between *Acacia* single and mixed planting treatments except for fresh weight values during the summer season (Table 2).

Table 1. Effects of seasonal changes on NR [n moles NO_3^- reduced g^{-1} fresh wt. h^{-1} and per plant part (Pl^{-1}) h^{-1}] activity in single and mixed planting treatments in *Eucalyptus* and *Acacia* seedlings

Season	Plant part	n moles NO_3^- reduced	*Eucalyptus* Single	*Eucalyptus* Mixed	Level of significance	*Acacia* Single	*Acacia* Mixed	Level of significance
Rainy	Leaf	g^{-1} h^{-1}	56.23	66.07	ns	144.54	102.33	ns
		Pl^{-1} h^{-1}	833.29	323.89	2.29*	745.59	535.67	ns
	Stem	g^{-1} h^{-1}	47.86	52.48	ns	89.13	79.43	ns
		Pl^{-1} h^{-1}	629.22	201.56	2.91**	861.79	643.58	ns
	Root	g^{-1} h^{-1}	60.53	38.19	3.14**	201.37	77.86	3.86**
		Pl^{-1} h^{-1}	692.15	187.33	3.89**	853.10	330.75	2.38*
Winter	Leaf	g^{-1} h^{-1}	120.22	97.72	ns	194.98	177.83	ns
		Pl^{-1} h^{-1}	3224.04	1851.83	ns	2566.26	2358.31	ns
	Stem	g^{-1} h^{-1}	93.11	79.98	ns	104.23	97.50	ns
		Pl^{-1} h^{-1}	3641.67	1287.66	3.6**	6531.31	5317.41	ns
	Root	g^{-1} h^{-1}	55.21	47.97	ns	128.23	83.56	ns
		Pl^{-1} h^{-1}	2792.54	892.07	4.28**	3380.64	2606.15	ns
Summer	Leaf	g^{-1} h^{-1}	194.98	199.53	ns	263.03	162.18	2.13*
		Pl^{-1} h^{-1}	9212.98	6795.16	ns	8519.22	2219.73	5.66**
	Stem	g^{-1} h^{-1}	144.21	131.83	ns	104.23	113.76	ns
		Pl^{-1} h^{-1}	10322.86	6300.86	2.9***	10176.54	6547.87	2.27**
	Root	g^{-1} h^{-1}	169.07	204.17	ns	283.14	169.04	3.13**
		Pl^{-1} h^{-1}	14467.72	12291.36	ns	19377.6	5742.49	5.56**

* = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level, ns = not significant.

Table 2. Effects of seasonal changes on the biomass of different plant parts in single and mixed planting treatments in *Eucalyptus* and *Acacia* seedlings

Season	Plant part	Bio mass (g)	*Eucalyptus* Single	*Eucalyptus* Mixed	Level of significance	*Acacia* Single	*Acacia* Mixed	Level of Significance
Rainy	leaf	fresh	17.54	6.36	3.11**	5.97	5.60	ns
		dry	6.39	2.98	2.52*	4.56	2.74	ns
	stem	fresh	10.81	4.39	2.42*	7.96	7.14	ns
		dry	5.79	2.12	2.46*	6.64	6.01	ns
	root	fresh	12.74	5.02	2.52*	4.24	3.94	ns
		dry	6.33	2.59	2 42*	2.63	2.20	ns
Winter	leaf	fresh	28.96	17.71	2 39**	12.22	11.92	ns
		dry	11.69	7.60	2 58*	5.88	5.20	ns
	stem	fresh	37.74	13.41	3 41**	61.48	53.49	ns
		dry	17.29	8.32	2 99**	36.63	29.92	ns
	root	fresh	48.39	19.40	3 20**	27.08	32.79	ns
		dry	23.08	10.71	2.45*	15.45	16.12	ns
Summer	leaf	fresh	45.42	32.97	2.13*	31.04	12.41	5.28**
		dry	21.66	16.10	2.93*	14.48	8.20	ns
	stem	fresh	85.80	49.60	4.24**	141.71	68.82	5.56**
		dry	46.68	23.52	4.23***	78.25	58.25	ns
	root	fresh	93.22	55.02	3.52**	67.33	32.90	4.07**
		dry	47.29	29.03	3.25**	35.48	26.87	ns

* = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level, ns = not significant.

Discussion

It is a well established fact that in any intercropping system, nitrogen fixing species increases growth and nitrogen contents of non-fixing species (Agboola & Fayemi 1972, Remison 1978, Eaglesham *et al.* 1981, Reddy & Willy 1981). However, little quantitive data are available on the competition between the associated nitrogen fixing and non-fixing species for the various constraints, i.e. nutrients, moisture, etc.

NR activity is known to fluctuate in response to the changes in environmental conditions, and such fluctuation usually also influences the capacity of different plant parts to assimilate nitrate. NR activity observed in this study has not followed any trend with the growth in *Eucalyptus* and *Acacia* species in the single and mixed planting treatments, although both the species behaved in almost similar manner under both planting treatments. In a similar study of *Alnus incana*, a higher NR activity was observed in the shoot and root tips compared to the leaf (Sellstedt 1986), while a reversed trend was reported in *Robinia pseudoacacia* (Johnson *et al.* 1991).

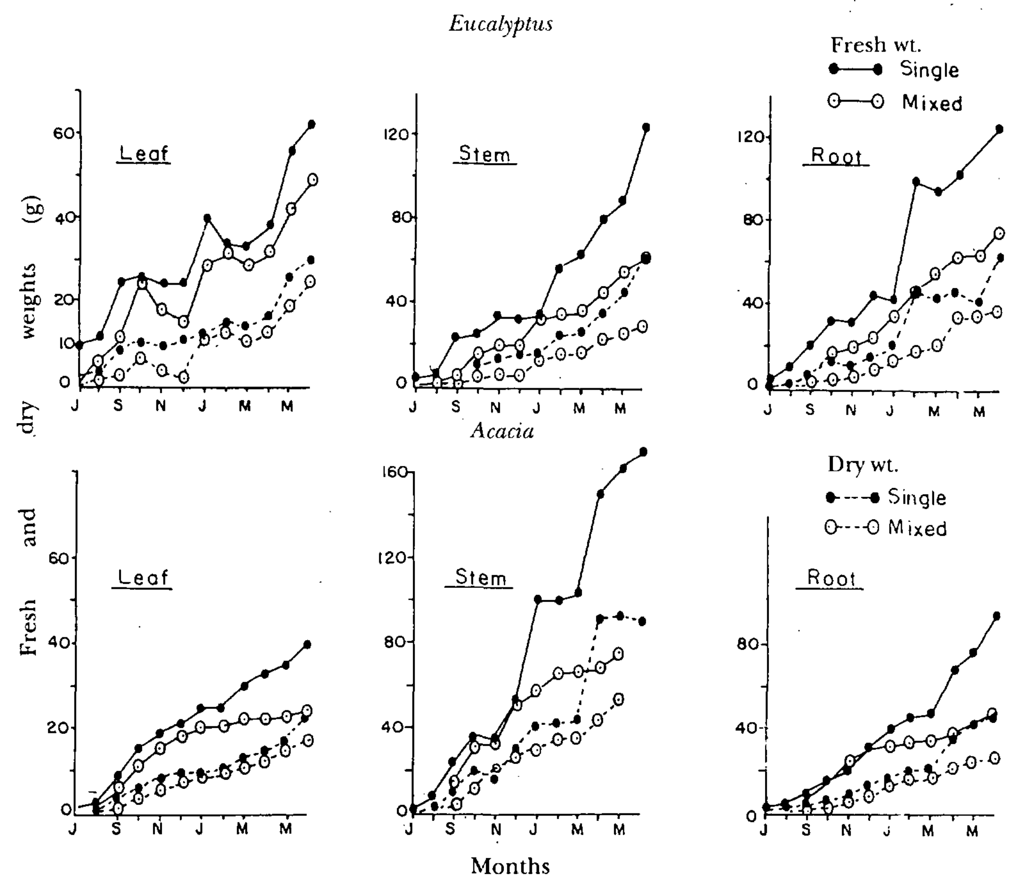

Figure 3. Mixed planting effects on fresh and dry weights of different plant parts of *Eucalyptus* and *Acacia* seedlings

Seasonal variations have also significantly reduced NR activity and biomass in *Eucalyptus* grown under the mixed planting as compared to the single treatment, whereas the differences between the single and mixed *Acacia* plants were non-significant. The reason for this suppression may be attributed to the competition for the available soil moisture and nutrients along with the carrying capacity in respect to the limitation of space for the root volume in the mixed planting treatments. That both the species have followed almost a similar trend for NR activity and biomass in both planting treatments indicates that the mixed planting treatment in this study had no detrimental effects.

Table 3. Effects of *Eucalyptus* and *Acacia* mixed planting on the fresh weight and total NR ($pl^{-1} h^{-1}$) activity

Character	Species	Plant part	Planting treatment		Significant level
			Single	Mixed	
Fresh wt. Pl^{-1}	*Eucalyptus*	leaf	28.85	15.46	**
		stem	32.72	14.30	***
		root	28.72	17.43	***
	Acacia	leaf	13.10	9.39	ns
		stem	41.06	29.74	ns
		root	19.82	16.20	ns
NR $Pl^{-1} h^{-1}$	*Eucalyptus*	leaf	2914	1561	***
		stem	2854	1141	***
		root	3035	1271	***
	Acacia	leaf	2537	1334	ns
		stem	3997	2819	ns
		root	3823	2843	ns

** = significant at 1% level, *** = significant at 0.1% level, ns = not significant.

However, on the basis of logarithmic transformations of values, a significant decrease in the NR activity and biomass of different plant parts was observed in the mixed *Eucalyptus - Acacia* as compared to *Eucalyptus* single planting, whereas no such effects were observed in the case of *Acacia* (Table 3). *Eucalyptus* plants suffered significant depression when grown in close proximity with *Acacia* , contrary to the findings of De Bell *et al.* (1985), who reported an increase in the morphological parameters of *Eucalyptus saligna* planted with *Acacia melanoxylon* and *Albizia falcatoria* separately. *Acacia* being xerophytic in character, with additional nitrogen-fixing advantage, proved to be a more dominant and stronger competitor as compared to *Eucalyptus* when grown in the inter-cropping system at the early stages of growth. In silvicultural management systems, the nitrogen deficiency of forest soils can properly be amended by the selection of suitable intercropping practices. Therefore, there is a need to screen the behaviour of different nitrogen-fixing and non-fixing tree species thoroughly before recommending any inter-cropping plantation programme.

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