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SEEDLING GROWTH AND NITROGENASE ACTIVITY OF *ALNUS* AND *ALBIZIA* IN SIKKIM

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Abstract: Seedling growth of N_2-fixing *Alnus nepalensis* D. Don and *Albizia stipulata* Boiv. was measured in nursery conditions. The nitrogenase activities of these tree species were estimated in agroforestry systems under *in situ* conditions by using acetylene reduction technique. Comparisons show that the root nodule biomass was three times and the leaf area about two times more in one year old seedlings of *Alnus* than *Albizia*. The belowground to aboveground dry matter ratio increased with the seedling age in *Alnus,* while it decreased in *Albizia*. *Alnus* showed weaker proportion of belowground parts in the early stages of seedling and then slowly developed with age. In contrast, *Albizia* showed well-established belowground part right from one month old seedling stage. Leaf area in both *Alnus* and *Albizia* increased with seedling age. *Alnus* is a semideciduous while *Albizia* is a deciduous. However, leaf litterfall in both the species was not recorded in the seedling stage in spite of measurements also made during the peak litterfall months of these species. Leaf area ratio of *Alnus* was higher than *Albizia* at 180 days seedlings stage and continued to remain higher supporting the fact that *Alnus* performed more efficiently than *Albizia*. Net assimilation rate when compared in these species between 90-360 days of seedling age clearly showed higher values for *Alnus* indicating better growth performance than *Albizia*. Nitrogenase activity in the growing season was 1.28 times higher in case of *Alnus* compared to *Albizia*. Mean tree N_2-fixation was 126 g/tree/year by *Alnus* and 55 g/tree/year by *Albizia*.

INTRODUCTION

Many angiospermic plants other than leguminous plants also possess N_2-fixing root nodules (Becking 1977, Akkermans and Van Dijk 1981). The actinomycetous endophyte of such nodules belongs to the genus *Frankia* (Becking 1970). *Frankia* symbioses have been recorded in more than 140 plant species, belonging to 17 genera of 8 different families within the angiospermeae. The genus *Alnus* belonging to the family Betulaceae of order Fagales is the most prominent of all the actinorhizals. This genus is extensively studied for N_2-fixation and its biology (Trappe *et al.* 1968, Briggs *et al.* 1978, Gordon *et al.* 1979, Hibbs *et al.* 1994). All the 35 species of *Alnus* are distributed in temperate belts and high latitude countries. Only two species are known to exist in the Himalaya viz., *Alnus nepalensis* D. Don (Himalayan alder) in the eastern Himalaya and *Alnus nitida* Endl. in the north western Himalaya. In Sikkim, only *A. nepalensis* is found and it grows most predominently in the region between 1000 m and 2500 m elevation (Fig. 1). In the eastern Himalaya it is distributed widely in the hills of Darjeeling, eastern Nepal, Sikkim, Bhutan, Arunachal Pradesh, Nagaland and the Khasi

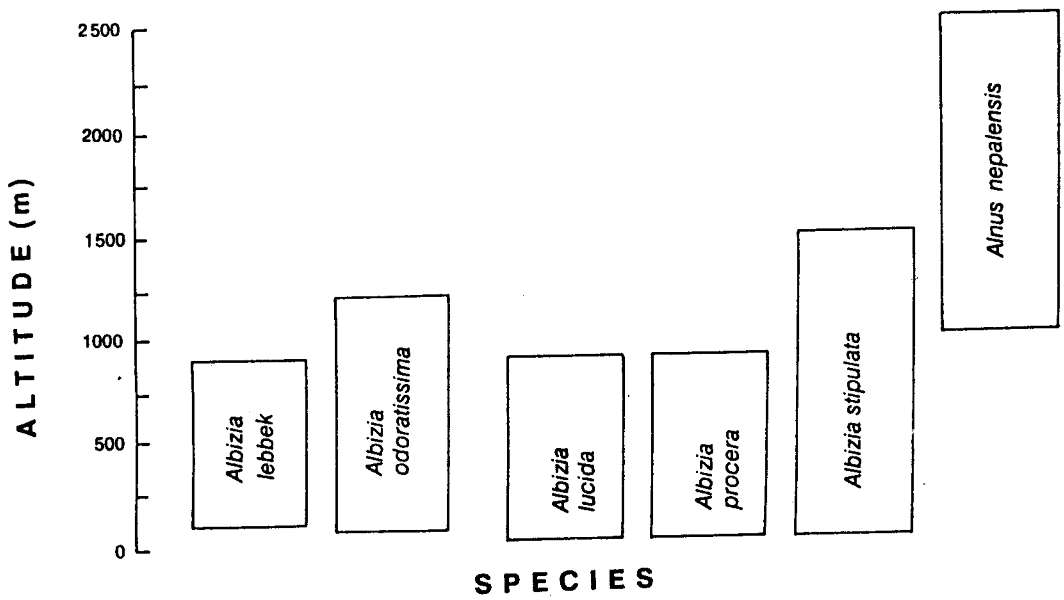

Fig. 1. Altitudinal distribution of *Albizia* spp. and *Alnus nepalensis* in Sikkim.

hills of Meghalaya. *A. nepalensis* is locally called as "Uttis". The roots of *A. nepalensis* are nodulated with *Frankia* as an endophyte and are efficient in N_2-fixation (Sharma 1988). *A. nepalensis* is a common species in natural and plantation forests in the Sikkim Himalaya. It is used as a chief associate/shade/nurse tree in large cardamom (*Amomum subulatum* Roxb.) agroforestry systems in the region.

Genus *Albizia*, belonging to subfamily Mimosoideae of the family Leguminaceae is most widely distributed in tropical and subtropical belts of the world. In India, *Albizia* is distributed in tropical and subtropical climate all over the country and the genus has taken an important place in agroforestry systems (Kumar and Toky 1994). In Sikkim, five species viz., *Albizia lebbek* Benth, *A. lucida* Benth, *A. odoratissima* Benth, *A. procera* Benth and *A. stipulata* Boiv. are found cultivated as well as in natural areas. All these are tree by habit. The altitudinal distribution of these five species of *Albizia* is given in Fig. 1. *A. stipulata* is more widely distributed in the Sikkim Himalaya. It is found in natural forest, degraded areas and also cultivated in agroforestry system in the region. It grows in the foot hills and extends up to 1500 m altitude. It is locally called "rato-siris" and is a robust tree that produces timber and useful fuelwood. It regenerates naturally in landslide exposed soils and riparian habitats. It is nodulated with rhizobium as an endophyte and expected to fix nitrogen. *A. stipulata* is also grown as an associate species in the mandarin agroforestry systems in the region.

Both *Alnus* and *Albizia* are most important nitrogen fixing trees in Sikkim and these are used as major associate species in the agroforestry systems in the region. There is no information on seedling growth of these species, hence this study was conducted. Nitrogen fixation is the most important functional attribute of these trees and in this study rates of its fixation was estimated under *in situ* conditions in *Alnus*-cardamom and *Albizia*-mandarin agroforestry systems.

MATERIALS AND METHODS

Seedlings of *Alnus nepalensis* and *Albizia stipulata* were raised in nursery for growth analysis. They were planted at 15 cm distance apart on raised beds. Roots of both *Alnus* and *Albizia* seedlings were self nodulated by the endophyte strains present in the nursery soil. *Alnus* seedlings were harvested in replicates of ten at 90, 180, 360 and 540 days, while *Albizia* seedlings at 30, 90, 180 and 360 days. The heights of the harvested seedlings were recorded and separated into different components viz., stem and branch, leaf, root and root nodules. Leaf area of each plant was measured using high precision digital planimeter. Dry weights of plant components were determined by drying the samples at 80°C for 48 h. Moisture content of different components was also recorded.

On the data generated from the above harvested seedlings the following growth parameters were calculated using methods described by Larcher (1983) and Causton and Venus (1981): (i) belowground: above ground dry matter ratio, (ii) specific leaf area, (iii) leaf area ratio, and (iv) net assimilation rate. Nitrogenase enzyme activity in root nodules of *Alnus* and *Albizia* was measured under *in situ* conditions by harvesting the root nodules with small intact roots followed by incubating the root nodules in vials with 10% v/v acetylene in air and then by placing them in the agroforestry floor. About 1 g fresh weight of root nodules was incubated in each vial for one hour. This was followed by estimations of ethylene using Perkin-Elmer GC 8700 model. The standard reference was prepared using research grade 99.5% pure ethylene (Aldrich Chemical Company, Inc., USA). The measurements of nitrogenase enzyme activity were carried throughout the growing season from April to October at different times of the day and mean values were arrived for a species in a growing season. The reduction of acetylene to ethylene as an assay for nitrogenase activity was first used by Stewart *et al.* (1967) and since then a number of investigators have used this assay on attach or detach nodules of both symbiotic legumes and actinorhizals (Akkermans 1971, Hardy *et al.* 1973, Huss-Danell 1978, Binkley 1981, Sharma and Ambasht 1984, 1988).

To approximate the quantities of nitrogen fixed, the growing period acetylene reduction by root nodules was converted to moles of N_2 by using assumed conversion ratio of three moles of C_2H_2 per mole N_2. The C_2H_2/N_2 conversion factor was based on the ratio of C_2H_2 reduced to moles of N_2 fixed with theoretical ratio from electron requirement being three. The ratio has been determined for a variety of N_2-fixing systems and the theoretical value of three appears to be justified for diazotrophs *in vivo* (Hardy *et al.* 1973). Annual stand N_2-fixation for each species was achieved by multiplying root nodule biomass in a tree with pooled growing season nitrogenase activity rates in terms of N_2-fixation and then by number of trees in a hectare.

RESULTS AND DISCUSSION

Seedling heights of both *Alnus* and *Albizia* increased with age. At 360 days mean height of *Albizia* was greater than *Alnus*. Leaf area of the whole plant also increased with seedling age in both the plants. However, the leaf area of *Alnus* was double of *Albizia* at 360 days. Dry weights of roots, leaf, stem and branch, and total plant increased with age in both the plants. The values of these parameters were higher in *Alnus* as compared to *Albizia* when compared at 360 days. Root nodule harbours the endophyte responsible for nitrogen fixation and its biomass increased with seedling age in both the plants. At the seedling stage of 360 days root nodule biomass was three times higher in *Alnus* compared to *Albizia*.

Alnus is a temperate species while *Albizia* is a subtropical one. Generally transplantation of seedlings from nursery to the field is carried out at about 1 year 6 months old plants of *Alnus* and 1 year old plants of *Albizia*. Therefore, looking into transplanting age, the growth estimations were carried out up to 540 days old seedlings in *Alnus* and 360 days old seedlings in *Albizia*. As expected, shoot height, leaf area and component dry weights of both *Alnus* and *Albizia* increased with seedling age.

Belowground:aboveground dry matter ratio is an important morphological character. The ratio was very low in *Alnus* up to 180 days and then increased thereafter to be more than 0.3 (Table 1). In case of *Albizia*, the ratio was fairly good right from 30 day old seedlings to 360 days (Table 1).

Specific leaf area in both *Alnus* and *Albizia* increased with age indicating thin leaves of relatively larger area at established seedling stage compared to early phase of seedlings after germination. The specific leaf area of *Albizia* remained always higher than *Alnus* when compared up to 360 days indicating thinner leaves of *Albizia* than *Alnus* (Fig. 2). The leaf area ratio of both *Alnus* and *Albizia* increased with seedling age, indicating higher efficiency at later stages of seedling growth. Leaf area ratio of *Alnus* was lower than *Albizia* at 90 days but thereafter it increased at higher rates to be greater than *Albizia* consequently showing higher efficiency of growth (Fig. 2). Net assimilation rate in both *Albizia* and *Alnus* was higher in early stages of seedling growth which sharply decreased to be nearly equal

Table 1. Growth performances at different stages of seedlings of Alnus nepalensis and Albizia stipulata. Values are mean ±SE expressed as per plant basis (n=10)

Species	Month	Seedling age (day)	Shoot height (cm)	Leaf area (cm2)	Root nodule fresh weight* (g)	Root dry weight (g)	Leaf dry weight (g)	Stem and branch dry weight (g)	Total dry matter (g)	Belowground /aboveground dry matter ratio
Alnus nepalensis	Apr '93	90	15.8 ±2.4	339 ±26	0.359 ±0.087	0.543 ±0.045	3.145 ±0.326	2.819 ±0.311	6.612 ±0.648	0.108
	Jul '93	180	44.4 ±2.6	1318 ±314	0.983 ±0.290	1.144 ±0.285	6.866 ±0.823	6.170 ±0.757	14.468 ±1.704	0.11
	Jan '94	360	86.3 ±5.2	3656 ±582	3.654 ±0.562	7.613 ±1.348	10.452 ±1.144	16.519 ±2.431	35.648 ±4.845	0.322
	Jul '94	540	127.3 ±9.7	6521 ±827	4.535 ±0.500	14.153 ±2.344	13.008 ±1.243	28.848 ±2.868	57.328 ±5.551	0.37
Albizia stipulata	Apr '93	30	8.7 ±1.3	82 ±5	0.084 ±0.011	0.727 ±0.042	0.541 ±0.038	0.483 ±0.019	1.916 ±0.102	0.423
	Jun '93	90	26.7 ±8.8	479 ±38	0.393 ±0.057	2.805 ±0.154	2.230 ±0.081	2.035 ±0.082	8.174 ±0.816	0.391
	Sep '93	180	38.5 ±6.4	835 ±71	0.527 ±0.035	4.517 ±0.311	3.842 ±0.173	4.186 ±0.321	12.872 ±1.064	0.392
	Mar '94	360	93.8 ±11.1	1846 ±158	1.214 ±0.116	7.422 ±0.316	6.524 ±0.414	9.173 ±0.846	25.314 ±2.216	0.341

*Presented on fresh weight basis as nitrogenase activity is estimated of fresh live nodule samples.
Mean moisture content: Alnus - root nodule 71%, root 68%, leaf 72% and stem & branch 68%; Albizia - root nodule 70%, root 65%, leaf 67% and stem & branch 64%.

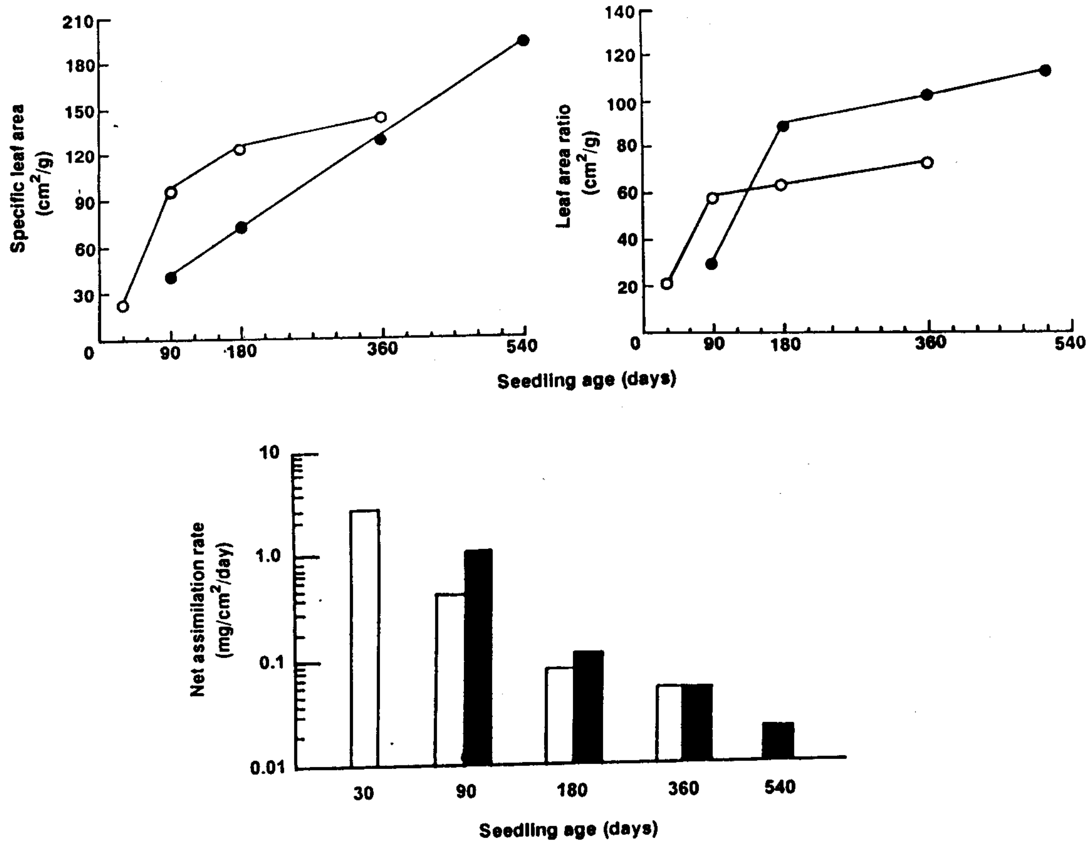

Fig 2. Performance of seedlings of *Albizia stipulata* (hollow) and *Alnus nepalensis* (shaded) at different stages of growth in nursery

in both the species at 360 days. The net assimilation rate always remained higher in *Alnus* compared to *Albizia* seedling (Fig. 2).

Alnus is a semideciduous tree while *Albizia* is deciduous. Leaf litterfall in both the species was not recorded in the seedling stage in spite of measurements also made during peak litterfall months of these species. This is a very interesting physiological behaviour that enables seedlings to grow continuously by retaining the assimilating structure. This is an energy conserving strategy at early stages of development of these species. High values of leaf area ratio indicate the plant to be highly efficient. Leaf area ratio of *Alnus* was higher than *Albizia* at 180 days seedling stage and continued to remain higher supporting the fact that *Alnus* performed more efficiently than *Albizia.* Although the biochemical reactions are occurring throughout the plant, it is only in certain parts that materials are assimilated. The uptake of mineral ions is small compared with the uptake of carbon by photosynthesis. Photosynthesis in green parts of the shoot other than the foliage is usually small and the leaf is regarded as the sole assimilatory organ (Causton and Venus 1981). Net assimilation rate quantifies the increase in dry matter with reference to the assimilating area (foliage) in a given time interval. Net assimilation rate when compared in *Alnus* and *Albizia* between 90-360 days of seedling age clearly showed higher values for *Alnus* indicating better growth performance of *Alnus* than *Albizia*. Performance of seedling of *Alnus* was better than *Albizia* with respect to most of the growth parameters.

Nitrogenase enzyme activity was estimated using acetylene reduction technique in root nodules of *Alnus* from the *Alnus*-cardamom and *Albizia* from the *Albizia*-mandarin agroforestry stands. The acetylene reduction was carried over throughout the growing season from April to October 1994 in both the stands. Acetylene reduction (µmole C_2H_2/g nodule dry weight/hour) in the growing season was 1.28 times higher in case of *Alnus* compared to *Albizia* (Table 2). The N_2-fixation rate was 55 µmole N/g nodule dry weight/day in *Alnus* and 43 µmole N/g nodule dry weight/day in *Albizia*. Nodule biomass per tree was also estimated in the peak growing period and it was 1.88 times greater in *Alnus* than *Albizia*. However,' *Alnus* density was 517 trees/ha and *Albizia* density was only 56 trees/ha. This has caused high values of root nodule biomass having 201 kg/ha of *Alnus* in the *Alnus*-cardamom stand while it was just 12 kg/ha of *Albizia* in *Albizia*-mandarin stand. Higher rate of nitrogen fixation and greater nodule biomass in the *Alnus*-cardamom stand influenced nearly 21 times higher stand N_2-fixation than *Albizia*-mandarin stand (Table 2). Mean tree N_2-fixation was 126 g/tree/year by *Alnus* and 55 g/tree/year by *Albizia*.

A few estimates have been published for nodule biomass of *Alnus* while no report on *Albizia* is available under field conditions. Zavitkovski and Newton (1968) estimated nodule biomass of 117 kg/ha/year in 7 year and 244 kg/ha in the 30 years old pure *Alnus rubra* stands. Akkermans and Van Dijk (1976) recorded an average nodule biomass of 454 kg/ha in a 20 year old *A. glutinosa* stand and Binkley (1981) reported 390 and 110 kg/ha in *A. rubra* and *A. sinuata* stands, respectively. Sharma and Ambasht (1986) estimated root nodule biomass in an age sequence of pure *A. nepalensis* plantations in the eastern Himalaya and reported 457 kg/ha in the 7 year and 149 kg/ha in the 56 year old stands. In the present study, root nodule biomass was 201 kg/ha in *Alnus*-cardamom

Table 2. Nitrogenase activity, nodule biomass and nitrogen accretion through fixation in *Alnus nepalensis* of *Alnus*-cardamom agroforestry and in *Albizia stipulata* of *Albizia*-mandarin agroforestry stands

Function	Species	
	Alnus nepalensis	*Albizia stipulata*
Nitrogenase activity		
Acetylene reduction (µmol C_2H_4/g nodule dry weight/hour)	15.08±1.78	11.83±0.68
Nitrogen fixation (µmol N_2/g nodule dry weight/day)*	55.29	43.38
Nodule biomass		
g/tree	388±42	206±27
kg/ha	201	12
Annual tree N_2-fixation (g/tree/year)+	126	55
Annual stand N_2 fixation (kg/ha/year)+	65.34	3.06

*Average active 11 hours of day and C_2H_2: N_2 ratio of 3:1 were used.

+Values are pooled for growing season, i.e. for the period April to October 1994.

and 12 kg/ha in *Albizia*-mandarin agroforestry systems. A very low root nodule biomass of *Albizia* in the stand was mainly attributed to lower density of *Albizia* tree. The nodule biomass when compared on per tree basis also showed that *Alnus* tree (388 g/tree) had higher values than *Albizia* tree (206 g/tree).

Average annual accretion of nitrogen through atmospheric fixation in *Alnus* ecosystems based on acetylene reduction assay was highest (130 kg/ha/year) in *A. rubra* as reported by Binkley (1981). The lowest accretion value of 20 kg/ha/year was recorded in 15-20 year old *A. sinuata* and *A. crispa* mixed stand (Binkley 1981). Annual accretion of 43 kg/ha in 30 year old *A. incana* (Johnsrud 1978) and 58 kg/ha in 5-20 year old *A. glutinosa* stand (Akkermans and van Dijk 1976) were also reported. Most estimates of annual nitrogen fixation in mixed stands deal with *Alnus rubra* mixed with *Douglas-fir (Pseudotsuga menziesii)*. These studies have generally found N_2-fixation of about 20 to 85 kg/ha annually. Other combinations, such as *Alnus rubra* with *Populus trichocarpa*, and *A. rubra* with *A. glutinosa* also appear to have similar rates. Annual nitrogen accretion was reported to be highest (117 kg/ha/year) in 7 year stand and lowest (29 kg/ha/year) in 56 year stand when estimated in an age sequence of *Alnus nepalensis* stands in the eastern Himalaya (Sharma and Ambasht 1988). In the present study cardamom agroforestry where *Alnus* is about 7-8 years old fixed 65 kg/ha/year and this value is comparable with other reports from various parts of the world (see Binkley 1992). The nitrogen build up strategy and N_2-fixation in *Alnus* species are counter balanced to a considerable extent, irrespective of their distribution, species to species differences, nodule age, plantation age and microclimatic regulation. The nitrogen fixation of 3 kg/ha/year by *Albizia* in *Albizia*-mandarin agroforestry in the present stand was a very low contribution mainly attributed to lower tree density hence lower nodule biomass. However, the average growth period N_2-fixation rate in *Alnus nepalensis* (55.29 μmol N/g nodule dry weight/day) and *Albizia stipulata* (43.38 μmol N/g nodule dry weight/day) are comparable. About 32 μmol N/g nodule fresh weight/day in *Albizia lebbek* (Ming-Mao Ding *et al.* 1986, Roskoski *et al.* 1982); and 28 μmol N/g nodule fresh weight/day in *A. odoratissima* and in *A. procera* have been reported by Ming-Mao Ding *et al.* (1986).

Nitrogen fixing *Alnus* and *Albizia* trees have recently become an integral part of traditional agroforestry systems in Sikkim. Both *Alnus* and *Albizia* have capability of fixing nitrogen at a high rate and if these trees are planted at a reasonable density then significant amount of annual nitrogen accretion can be achieved. *Alnus* can take up a keystone role in temperate and *Albizia* in subtropical agroforestry systems.

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