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Effect of light intensity and rhizobial inoculum on the performance of two tropical legumes

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ABSTRACT

A 22-week experiment was conducted in controlled environments to investigate the effects of light and inoculum application on the growth, nodulation and nitrogen fixation of 2 tropical legumes, viz Siris tree [*Albizia lebbeck* (L. Benth] and *Subabul* (*Leucaena leucocephala* (Lam.) de Wit.]. Two light regimes–high light and low light (stimulated shade) were used. Plants were either inoculated without the addition of inorganic nitrogen or they received inorganic nitrogen without inoculum application. Control plants neither received inoculum nor inorganic nitrogen. The results showed that in both species under high light, the inoculated plants outgrew the uninoculated ones. Although there appears to have been more investment of the biomass in the leaf area under simulated shade conditions for the inoculated plants, the lower net assimilation rate resulted in lower growth rate. The controlled plants had the least growth under both light regimes, though they grew better under low light indicating that these legumes may be able to withstand nitrogen stress under shade conditions. Also more nodules were produced and more nitrogen fixed under high light than under the low light. Although nitrogen fixation was reduced under low light, nitrogen uptake was higher for *Albizia lebbeck* for the inoculated treatments than the uninoculated treatments.

Key words: *Albizia lebbeck, Leucaena leucocephala,* Light intensity, Inoculum, Leaf area, Net assimilation rate, Nodulation, Nitrogen fixation

Nitrogen fixation is increasingly becoming important in meeting crop and soil-nitrogen needs in view of the problems associated with long-term inorganic fertilizer usage. Of all the biological systems involved in nitrogen fixation, the legume – *Rhizobium* symbiotic system has proved to be the most effective and widespread, though recent literature acknowledges substantial fixation of nitrogen by non-symbiotic systems.

Siris tree *[Albizia lebbeck* (L.) Benth] and *subabul Leucaena leucocephala* (Lam) de Wit.] used for these studies, are the 2 common tropical legumes which serve as multipurpose trees in tropical farming systems. The literature is sparse on how the environment affects the growth, nodulation and nitrogen-fixing abilities of these legumes. Since mutual shading is involved in the use of these legumes in certain agroforestry systems, it is important to assess their abilities to nodulate and fix nitrogen under varying light conditions. Hence an experiment was conducted to study how the light affects growth, nodulation and nitrogen fixation in these legumes.

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MATERIALS AND METHODS

The seeds were obtained from Ibadan (7:23^{0}N and 3.56^{0}E). They were soaked in hot water and allowed to cool for 1 hr before planting. The rooting medium was perlite and vermiculite in a 50:50 (1:1) ratio. The seeds were planted in seed trays placed on the floor of 2 controlled environment rooms which simulated sun and shade tropical environments. At the appearance of the cotyledons, seedlings were transplanted into 1 litre tubes containing the rooting medium.

Inoculation

Rhizobial cultures specific to each plant species were obtained from the University of Dundee and subculturing was done using the method and broth recommended by Vinclent (1970). During transplanting the root primordia of the recipient plants were dipped in the inoculum before being put in the 1 litre tubes. A further inoculation involving the injection of 2 ml inoculum in the root zone was done a week after transplanting.

Nutrient stock solutions

Two sets of solutions were prepared and used for the

Table 1 Comparison of means under high light using a two-tail t-test

Parameter	*A. lebbeck*			*L. leucocephala*		
	T_1	T_2	*t-test*	T_1	T_2	*t-test*
Shoot height (cm)	33.2 ± 4.4	33.2 ± 4.4	**	33.2 ± 4.4	33.2 ± 4.4	***
No of leaves	67.2 ± 11.5	71.2 ± 8.3	ns	179.6 ± 19.8	151.2 ± 20.9	*
Dry-matter yield (g)	13.4 ± 2.2	16.2 ± 2.4	ns	29.2 ± 1.4	19.7 ± 1.4	***
Leaf area (cm^2)	285.6 ± 37.9	254.9 ± 46.5	ns	299.80 ± 35.29	149.79 ± 17.44	**
Relative growth rate (g/g/week)	0.18 ± 0.002	0.19 ± 0.004	ns	0.23 ± 0.004	0.21 ± 0.004	**
Net assimilation rate (g/cm^2/week)	0.006± 0.0002	0.008 ± 0.001	ns	0.015 ± 0.002	0.017 ± 0.002	ns
Leaf area ratio (cm^2/week)	21.9 ± 0.9	15.5 ± 0.9	***	10.16 ± 0.74	7.51 ± 0.40	**
Leaf weight ratio	0.18 ± 0.01	0.14 ± 0.001	***	0.13 ± 0.009	0.10 ± 0.005	**
Specific leaf area (cm^2/g)	122.7 ± 0.7	11.5 ± 4.4	ns	77.60 ± 2.25	73.2 ± 3.86	ns
	T_1	T_2	*t-test*	T_1	T_2	*t-test*
Shoot height (cm)	33.2 ± 4.4	9.9 ± 0.7	**	63.7 ± 6.8	6.7 ± 0.3	***
No. of leaves	67.2 ± 11.5	14.6 ± 1.4	**	179.4 ± 19.8	18.2 ± 1.6	***
Dry-matter yield (g)	13.4 ± 2.2	0.4 ± 0.06	**	29.2 ± 1.4	0.9 ± 0.1	***
Leaf area (cm^2)	285.6 ± 37.9	7.97 ± 1.51	**	299.80 ± 35.29	7.38 ± 0.88	***
Relative growth rate (g/g/week)	0.18 ± 0.002	0.02 ± 0.004	**	0.23 ± 0.004	0.07 ± 0.006	***
Net assimilation rate (g/cm^2/week)	0.006± 0.0002	0.001 ± 0.00005	**	0.015 ± 0.002	0.004 ± 0.001	***
Leaf area ratio (cm^2/week)	21.9 ± 0.9	18.9 ± 1.5	**	10.16 ± 0.74	8.34 ± 0.74	***
Leaf weight ratio	0.18 ± 0.08	0.12 ± 0.009	***	77.7 ± 2.25	0.09 ± 0.005	***
Specific leaf area (cm^2/g)	122.74 ± 7.89	165.55 ± 13.79	*	77.60 ± 2.25	96.39 ± 6.56	*
	T_1	T_2	*t-test*	T_1	T_2	*t-test*
Shoot height (cm)	22.3 ± 2.8	9.9 ± 0.7	**	3.3 ± 4.8	6.7 ± 0.3	**
No. of leaves	71.2 ± 8.3	14.6 ± 1.4	***	151.4 ± 20.9	18.2 ± 1.6	**
Dry-matter yield (g)	16.2 ± 2.4	0.4 ± 1.4	**	0.21 ± 0.004	0.07 ± 0.006	***
Leaf area (cm^2)	254.9 ± 46.5	7.97 ± 1.51	***	0.017 ± 0.006	0.004 ± 0.001	***
Relative growth rate (g/g/week)	0.19 ± 0.004	0.002 ± 0.004	***	0.017 ± 0.006	0.004 ± 0.001	***
Net assimilation rate (g/cm^2/week)	0.01 ± 0.001	0.001 ± 0.0001	**	149.78 ± 17.44	7.38 ± 0.88	***
Leaf area ratio (cm^2/week)	15.5 ± 0.9	18.9 ± 1.5	**	7.51 ± 0.40	8.34 ± 0.74	ns
Leaf weight ratio	0.14 ± 0.01	0.12 ± 0.01	**	0.10 ± 0.005	0.09 ± 0.005	***
Specific leaf area (cm^2/g)	111.5 ± 4.4	165.6 ± 13.8	**	73.24 ± 3.86	96.39 ± 6.56	**

*P<0.05; **P<0.01; ***P<0.001

T_1, High light plus inoculum but without inorganic nitrogen; T_2, high light minus inoculum but receiving inorganic nitgron; T_3, high light minus both inoculum and inorganic nitrogen

study. The first solution was prepared using the Ingestad (1979) recommendation, while the second solution was a modification of Ingestad formula to exclude nitrogen salts. However, the levels of the other elements in the second solution were raised to maintain the ionic balance present in the first solution. The solution containing nitrogen salts was used for the non-inoculum (–R) treatment, while the second solution excluding nitrogen salts was used for the inoculum (+R) treatment.

The treatment combinations were : T_1, high light plus inoculum but without inorganic N; T_2, high light minus inoculum but receiving inorganic N; T_3, high light minus both inoculum and inorganic N; T_4, low light plus inoculum but without inorganic N; T_5, low light minus inoculum but receiving inorganic N; and T_6, low light minus both inoculum and inorganic N. Each treatment was replicated 5 times for each species. The treatment without inoculum and without inorganic nitrogen served as the control and form the basis for testing and comparing the effects of inoculum and inorganic N application.

Mean relative growth rate, mean net assimilation rate, final leaf ratio, as well as specific leaf area were calculated using standard formula from Hunt (1978). T-test comparing means in various treatments was done using the stat view soft-ware in the Apple Mac. Samples were prepared for N natural abundance determination following the method proposed by Handley *et al.* (1991) and estimates of the per cent of the total N of the tissue that was fixed was calculated:

$$N\ (\%)\ \text{fixed} = \frac{x-y}{x-f} \times 100$$

where x, ^{15}N of presumed non-nitrogen fixing plant; y, ^{15}N of presumed nitrogen fixing plant; and f, ^{15}N of a plant grown hydroponically without added N.

RESULTS AND DISCUSSION

Height growth, leaf production and dry-matter

In both species and under both light regimes treatment, plant grown under T_1, T_2, T_4 and T_5 grew well throughout the period of experimentation. The control plants, and plants under T_3 and T_6 scarcely grew at all. Under high light and in both species plants of T_1 treatment outgrew those of T_2 treatment (Table 1). Inoculum application perhaps enhanced shoot height under high light. With regards to leaf production in *A. lebbeck*, the plants of treatment T_2 produced more leaves than those of T_1 treatment under high light, though the difference was not significant (Table 1). However, in *L. leucocephala* the plants of T_1 treatment gave more leaves than those of T_2 treatment and this difference was statistically significant (Table 1). Also in both species, the T_4 and T_2 treatments resulted in production of the least number of leaves (Table 1). The response pattern for dry-matter yield under high light was very similar to that for leaf production (Table 1). It appears that in both species the 2 factors of light and inoculum application affected growth, as revealed in the increase in height under high light and the decrease in height under low light when inoculum was applied. There is enough evidence that light and nitrogen can do indeed affect the growth of plants in a given environment.

Although there were no significant differences in relative growth rate, net assimilation rate and dry-matter yield between the T_1 and T_2 treatments in *Albizia lebbeck* grown under high light, significant differences existed between these treatments in leaf area ratio, leaf weight ratio and specific leaf area. Thus the increased shoot height observed for the T_1 treatment may have resulted from an investment of more biomass in the leaf area. There is an evidence that a compensating effect to growth do occur due to changes

Table 2 Comparison of means under low light using a two-tail t-test

Parameter	*A. lebbeck*					*L. leucocephala*				
	T_4	T_5	*t-test*	T_4	T_5	*t-test*
Shoot height (cm)	33.5 ± 2.0	48.2 ± 2.7	***	44.2 ± 2.4	51.2 ± 1.4	**
No of leaves	37.0 ± 2.4	35.6 ± 2.2	ns	54.2 ± 1.2	54.8 ± 3.3	ns
Dry-matter yield (g)	2.4 ± 0.4	3.5 ± 0.3	**	1.9 ± 0.1	3.4 ± 0.1	***
Leaf area (cm^2)	227.4 ± 2.9	219.1 ± 14.2	ns	94.1 ± 5.99	144.4 ± 3.96	***
Relative growth rate (g/g/week)	0.14 ± 0.004	0.16 ± 0.01	***	49.5 ± 0.8	42.03 ± 0.03	***
Net assimilation rate (g/cm^2/week)	0.001± 0.0001	0.002 ± 0.0002	**	0.15 ± 0.002	0.018 ± 0.004	***
Leaf area ratio (cm^2/week)	100.6 ± 6.3	62.3 ± 1.9	***	0.002 ± 0	0.003 ± 0	**
Leaf weight ratio	0.30 ± 0.02	0.20 ± 0.004	**	0.18 ± 0.01	0.15 ± 0.004	***
Specific leaf area (cm^2/g)	330.8 ± 1.16	302.9 ± 7.03	**	269.02 ± 8.9	290.2 ± 9.6	***
	T_1	T_2	*t-test*	T_1	T_2	*t-test*
Shoot height (cm)	33.5 ± 2.04	20.8 ± 2.1	***	44.2 ± 2.4	17.80 ± 2.5	***
No. of leaves	37.0 ± 2.4	14.2 ± 1.99	***	54.2 ± 1.2	16.8 ± 2.4	***
Dry-matter yield (g)	2.4 ± 0.04	0.7 ± 0.1	**	1.9 ± 0.1	0.8 ± 0.1	***
Leaf area (cm^2)	227.4 ± 2.9	18.4 ± 2.23	**	94.1 ± 5.99	24.8 ± 6.95	***
Relative growth rate (g/g/week)	0.14 ± 0.004	0.1 ± 0.01	***	49.5 ± 0.8	30.44 ± 6.1	*
Net assimilation rate (g/cm^2/week)	0.001± 0.0001	0.001 ± 0.0002	ns	0.15 ± 0.002	0.11 ± 0.002	***
Leaf area ratio (cm^2/week)	100.6 ± 6.3	26.7 ± 1.1	***	0.002 ± 0	0.002 ± 0.0002	ns
Leaf weight ratio	0.3 ± 0.02	0.1 ± 0.01	***	0.18 ± 0.01	0.09 ± 0.02	***
Specific leaf area (cm^2/g)	330.8 ± 11.6	330.1 ± 56.1	ns	269.02 ± 8.9	349.2 ± 10.6	***
	T_5	T_6	*t-test*	T_5	T_6	*t-test*
Shoot height (cm)	48.2 ± 2.7	20.8 ± 2.1	***	51.2 ± 1.4	17.8 ± 2.5	***
No. of leaves	35.6 ± 2.2	14.2 ± 1.99	***	54.8 ± 3.3	16.8 ± 2.4	***
Dry-matter yield (g)	3.53 ± 0.25	0.69 ± 0.09	***	3.4 ± 0.1	0.8 ± 0.1	***
Leaf area (cm^2)	219.1 ± 14.2	18.43 ± 2.3	***	144.4 ± 3.96	24.8 ± 6.95	***
Relative growth rate (g/g/week)	0.16 ± 0.01	0.09 ± 0.01	***	42.03 ± 0.3	30.4 ± 6.1	ns
Net assimilation rate (g/cm^2/week)	0.002± 0.0002	0.001 ± 0.0002	ns	018.0 ± 0.004	0.11 ± 0.002	***
Leaf area ratio (cm^2/week)	62.3 ± 1.8	27.7 ± 1.1	***	0.003 ± 0	0.002 ± 0.0002	**
Leaf weight ratio	0.20 ± 0.004	0.10 ± 0.01	***	0.15 ± 0.004	0.09 ± 0.02	**
Specific leaf area (cm^2/g)	302.9 ± 7.03	310.1 ± 56.1	ns	290.2 ± 9.6	349.2 ± 10.6	***

* $P<0.05$; **$P<0.01$; ***$P<0.001$

T_4, Low light plus inoculum but without inorganic nitrogen; T_5, low light minus inoculum but receiving inorganic nitgron; T_6, low light minus bothinoculum and inorganic nitrogen

Table 3 Nitrogen uptake, nodule number and nitrogen fixed for *Albizia lebbeck* and *Leucaena leucocephala* under high and low light

Parameter	T_1		T_2		T_3		T_4		T_5		T_6	
	A. lebbeck	*L. leucocephala*	*A. lebbeck*	*L. leucocephala*	*A. lebbeck*	*L. leucocephala*	*A. lebbeck*	*L. leucocephala*	*A. lebbeck*	*L. leucocephala*	*A. lebbeck*	*L. leucocephala*
N uptake	328.01	431.28	235.96	2.29	5.87	5.87	52.15	35.97	81.85	41.85	6.06	5.87
Nodule no.	88.80	0	0	0	0	0	66.20	19.80	0	0	0	0
Nodule *dry* weight (g)	0.24	0	0	0	0	0	0.05	0.04	0	0	0	0
N (%) fixed	69.05	0	0	0	0	0	80.99	85.32	0	0	0	0
Total N fixed (mg)	226.49	0	0	0	0	0	42.23	30.69	0	0	0	0

Details of T_1, T_2, T_3, T_4, T_5 and T_6 are given under materials and methods

in dry-matter allocation (Kuppers *et al.* 1988). Under high light, efficient photosynthetic translocation in this species may have resulted in the partitioning of photosynthate to the shoot, thus enhancing shoot growth in the inoculum minus inorganic nitrogen treatment T_1.

The fact that the growth rate of a plant can be a function of its internal nitrogen concentration (Hirose 1988) was manifested in this experiment. Results from this experiment show that nitrogen uptake (internal nitrogen concentration expressed as dry weight of plant) was higher for the T_1 treatment than the T_2 treatment in both species.

The control plants T_3 and T_6, ie plants receiving neither inoculum nor inorganic nitrogen, had the least growth and in this treatment under both light regimes, the control plants grew better under low light (Tables 1,2), thus indicating that these tropical legumes may be able to withstand nitrogen stress under shade conditions. Leaf area, ratio, leaf weight ratio and dry weight were significantly lower under this treatment than the other treatments, hence the poor relative growth rate. The results further confirm the usefulness of nitrogen in growth and development of plant. The N uptake was least for these control plants.

Nodulation and nitrogen fixation

In both species, more modules were under high light than under low light. The *A. lebbeck* produced more nodules than *L. leucocephala* in both light regimes (Table 3). Also in both species, quantitatively, more nitrogen was fixed under high light than under low light (Table 3). This expectedly follows the pattern of nodulation since fixation of nitrogen (other factors being equal) is linearly related to the amount of effective nodules produced. The reduction in nodulation and nitrogen fixation under low light may be due to the influence of the physical environment (Gibson 1977, Lie 1974). Legumes are particularly sensitive to spectral composition and both module formation and plant growth are affected by far-red wave lengths. Lie (1969) showed that exposure of roots to far-red light alone retarded the nodulation of *Pisum sativum* L. and *Vicia faba* L., indicating an involvement of phytochrome in nodulation. Gibson (1968), reported that nodule formation and plant growth are affected by far-red wave lengths. Gibson (1968) observed nodule formation on tube-grown plants whose roots received light, whereas such plants grown with darkened roots failed to nodulate. It appears that the rhizobuim bacteria under low light condition require more time to develop and infect the roots of these legumes. Although nitrogen fixation is reduced under low light, nitrogen uptake is higher in *A. lebbeck* for the T_4 than the T_5 treatment.

Tropical legumes such as the ones used for this study appear to be light demanders. Generally, growth was better when plants were under high light. Morphologically plants under low light had thinner, narrow leaves and stems than those grown under light. Etiolation of plant species nevertheless is usually the case under low light (shade)

conditions.

The application of biological nitrogen sufficiently enhanced growth under high light condition in both the species studied. The inoculated plants had higher relative growth rates and nitrogen uptake under high light than the uninoculated plants. In addition, more nitrogen was fixed under high light than under low light. These plants could be self sustaining in nitrogen acquisition when the necessary rhizobia exist in the soil. This may justify their inclusion in natural and cultivated fallows in both traditional and modern agroforestry systems.

Of the 2 components of relative growth rates, net assimilation rate and leaf area ratio, light appears to have had a large effect on leaf area ratio. Plants grown under low light exhibited a higher leaf area ratio in all treatments than the plants grown under high light. It appears therefore that as a compensatory mechanism, plants under low light invested more of their biomass in the leaf area.

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