A TEST OF THE NITROGEN-FIXING ABILITY OF THREE AGROFORESTRY TREE LEGUMES, Tagasaste (*Chamaecytisus palmensis*), Port Jackson (*Acacia saligna*) and *Sesbania sesban*, WHEN INOCULATED WITH THREE RHIZOBIAL STRAINS.

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ABSTRACT

The nitrogen-fixing ability of three tree legumes was tested. Tagasaste (*Chamaecytisus palmensis*), Port Jackson (*Acacia saligna*) and *Sesbania sesban* seedlings were inoculated with rhizobial strains XIA1, XIB2 and XHZ1 and grown in sterilised sand with controlled nutrient additions for twelve weeks. Nodulation was induced by strains XIA1 and XHZ1 on roots of *C. palmensis* (non-effective) and *A. saligna* (effective). Strain XIB2 did not induce nodulation in any of the three plant species. No nodulation was observed in the case of *S. sesban*. Other plant growth parameters including plant and embryo nitrogen content, plant height and dry mass, root:shoot ratio and relative growth rates, were determined. Total plant nitrogen content and relative growth rates were found to be higher for nodulated *A. saligna* plants when compared with non-nodulated controls. Possible reasons for the observed nodulation patterns and nitrogen fixation effects as reflected by growth parameters are discussed.

INTRODUCTION

With the increasing problems associated with synthetically-produced nitrogen fertilizer such as ground-water contamination (Newbould 1989) and the high energy costs involved in synthesis (1.5-2 kg fossil fuel/1 kg N-fertilizer, Von Wistinghausen 1985), more interest is now being directed toward the role of biological nitrogen-fixation, especially by members of the legume family. Barnet and Catt (1991) point out that most studies on the symbiotic relationships between legumes and bacteria have been undertaken on herbaceous species and there is consequently little information on woody legumes.

At a recent conference on agroforestry (Nelspruit 1991) and in some of the less-formal literature on the subject, I have noticed what appears to be an over-simplification of the process and an over-exaggeration of the benefits of nitrogen-fixation by trees. Trees are often advertised as being fertilizers, fixing hundreds of kilograms of nitrogen/hectare and thereby increasing soil fertility. However, the availability of this fixed-nitrogen to other plants and the specificity of the legume-bacterium symbiosis is often not considered. This lack of knowledge could lead to substantial decreases in productivity in tree-planting projects. At a large-scale agroforestry project in one of the independent homelands, nine-month old *Leucaena leucocephala* trees being grown in bags, had not been inoculated by the (qualified) forester in charge and at only 30 cm tall, had clearly not realised their full potential (pers. obs.).

Young (1989) has noted that nitrogen-fixing trees may increase soil fertility via leaf litter and, when grown in combination with crops, can reduce root competition for nitrogen. Increases in nitrogen availability to associated crops via senescence of roots and nodules following severe pruning might also be important in agroforestry. Sanginga *et al.* (1990 in Danso *et al.* 1992) reported the death of approximately half the nodules during a three week period following severe pruning in the case of *Leucaena leucocephala*.

An intercropping system involving combinations of perennial legumes has proved successful in providing perennial fodder for livestock (Snyman 1992). This particular system is controversial (Armstrong 1992) as the main intercrop used is the invasive alien, *Acacia saligna* (Port Jackson). This species is intercropped mainly with the tree lucern, tagasaste (*Chamaecytisus palmensis*). *C. palmensis* is becoming increasingly important as a high-quality fodder crop in South Africa and to date has been cultivated in the western, southern and eastern Cape (Esterhuyse 1989). Farmers have been requesting bacterial inoculants for *C. palmensis* from the Nitrogen Fixation Unit of the Plant Protection Research Institute (PPRI) in Pretoria (Staphorst pers. comm.). At the moment, three bacterial strains are recommended for inoculating *C. palmensis*, but the success of these strains on nitrogen-fixing ability in this species has not been tested previously. The bacterial strains are as follows;

XIA1 isolated from *Cytisus scoparius* (L.)
XIB2 isolated from *Chamaecytisus supinus* (L.)
XHZ1 isolated from *C. decurrens* (Possibly *Cytisus decumbens*)
(Obtained by PPRI in 1981 from Nitrogin Co. Milwaukee, Wisconsin, USA).

The aim of this study was to inoculate *C. palmensis* plants with the three bacterial symbionts (rhizobia) to establish which are the most effective in infecting *C. palmensis* roots and initiating nitrogen-fixation. In addition to testing *C. palmensis*, these rhizobia were tested on *A. saligna* and *Sesbania sesban* (another popular agroforestry species) to see if there was any nodulation and nitrogen-fixation. The reasons for testing these species was to examine the possibility that the strains recommended for one species may be effective in nodulating other species. This result is of importance when different legume species are grown in combination, in order to determine the specific rhizobial requirements of the host plants. Although it is known that there is variation in the specificity of tree legume-rhizobia symbioses (Nuti and Casella 1989), there is little information on the specific rhizobial requirements of potentially useful leguminous trees (Danso *et al.* 1992).

In Fabaceae (Leguminosae) symbiotic relationships, three genera of rhizobia have been described. These are *Rhizobium, Bradyrhizobium* and *Azorhizobium* (Sprent and Sprent 1990). These authors point out that some legume species nodulate effectively with more than one bacterial genus and that a single rhizobial strain may have a wide host specificity, nodulating hosts from different legume sub-families.

The main questions posed in this study concerned the effectiveness of rhizobial strains XIA1, XIB2 and XHZ1 on the nodulation and nitrogen-fixation of *C. palmensis* and what additional effects these rhizobial strains might have in inducing nodulation and N-fixation in *A. saligna* and *S. sesban*.

METHODS

The plant species used in this study are as follows:

Chamaecytisus palmensis (Christ) (tagasaste), which is in the legume sub-family Papilionoideae and originates from the Canary Islands (Wiersama *et al.* 1990).

Acacia saligna (Labill.) (Port Jackson Willow) is in the sub-family Mimosoideae and is indigenous to S.W. Australia (National Academy of Sciences 1981).

Sesbania sesban (L.) is indigenous to a large areas of Africa and Asia (Wiersama *et al.* 1990) and is a member of the legume sub-family Papilionoideae.

Seeds of *C. palmensis, A. saligna* and *S. sesban* were surface-sterilised using a calcium hyperchlorite solution, pre-treated with boiling water (*C. palmensis* and *S. sesban*) or concentrated H_2SO_4 (*A. saligna*) as required, and allowed to germinate in petri dishes at the beginning of June 1992. One week later, the sprouting seeds were transferred to pots containing acid-washed, autoclaved sand, and once all plants had produced cotyledons (two weeks later), they were given their first nutrient feeding and inoculants were administered.

For each of the species there were five treatments as follows:

CODE	TREATMENT
1	Inoculum XIA1, hereafter inoculum 1
2	Inoculum XIB2, hereafter inoculum 2
3	Inoculum XHZ1, hereafter inoculum 3
C	A nitrogen-free control
N	A nitrate-fed (70 ppm) control

For each treatment there were 10 replicates per species, giving a total of 10 x 3 x 5 = 150 plants.

The inocula (obtained in the form of sterile peat cultures from the Nitrogen Fixation Unit of the PPRI in Pretoria), were applied in equal quantities as a "pinch" of powder per plant, sprinkled over the surface of the sand.

All plants were watered weekly with a Long-Ashton (nitrogen-free) solution (Hewitt and Smith 1975) except for the nitrate-fed plants which received a 70 ppm KNO_3 solution, added to the Long-Ashton solution. The Long-Ashton solution contained all the major macronutrients as well as micronutrients and trace elements required for plant growth.

The pots in which the plants were grown were placed into smaller pots which created a drainage area (air layer) through which the chance of contamination of rhizobia via water uptake was eliminated. A layer of black PVC beads was placed around each plant to prevent algal growth on the sand surface.

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Plants were grown in a glass house for a further nine weeks, giving a total of 12 weeks since seed treatment. Plants were rotated each week in the glasshouse to minimize light and temperature differences during the growing period. Besides the weekly nutrient feeding, plants were watered with deionised water as required. The glasshouse did not have a permanent heating system, but was periodically heated during the day when temperatures dropped substantially.

At the beginning of September, height and number of leaves were measured, and indications of damage by insects or yellowing noted. Plants were then harvested, separated at the cotyledon scar and the presence of nodules noted. Plant sections were oven-dried for 48 hours at 60° C. Dry mass (mg) for below and above the cotyledon (also termed root and shoot for convenience) was measured on a balance and the dry mass and number of nodules recorded. All masses were measured at four decimal places of a gram and converted to milligrams.

Total nitrogen analyses using four replicates from each treatment for each species were performed using the Kjeldahl digestion method. Seed total nitrogen analyses were also performed, using 10 seed embryos per species.

The mass of 100 seeds for each species was measured and converted to embryo mass using the ratio of testa mass to embryo mass from a sample of 10 oven-dried seeds. This mean embryo mass, together with the mean total biomass of plants at the end of 12 weeks was used to calculate the relative growth rates (R.G.R.) for the three species, using the formula:

$$\mathrm{R.G.R.} = (\ln W_{t=2} - \ln W_{t=1})/t2-t1 \qquad \text{(Hunt 1978)}$$

where $W_{t=2}$ is the mass of the plant

This assumes exponential growth

$W_{t=1}$ is the embryo mass

and $t2-t1$ is 12 weeks.

In the analysis of the roots for total nitrogen, the nodules were included where present.

A one way analysis of variance was performed using the total nitrogen content values for the seeds of each species. Two way analyses of variance (species and treatment) were performed using total plant nitrogen content, plant height and biomass of the three species. All statistical analyses were performed using the Statgraphics software package (Graphic Software Systems Inc., U.S.A. 1989).

RESULTS

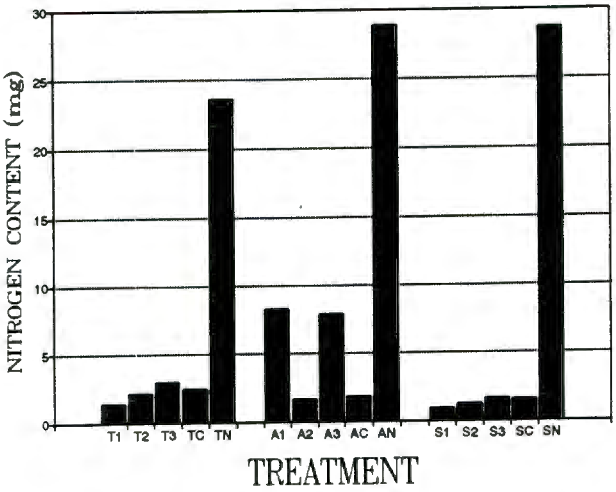
Table 1. The occurrence of nodulation in *C. palmensis*, *A. saligna* and *S. sesban* under three inoculum treatments and two control treatments following 12 weeks growth. Embryo nitrogen contents, seed mass, seed N concentration (percentage N per seed), embryo mass and embryo N concentration (percentage N per embryo) are also shown for the three species.

SPECIES	TREATMENT	NODULATION	EMBRYO N (mg)	SEED MASS (mg)	%N	EMBRYO MASS(mg)	%N
			$\overline{X}$ S.E.	$\overline{X}$ S.E.		$\overline{X}$ S.E.	
C.palmensis	Inoc 1	+	1.29±0.03	18.36±0.40	7.1	8.81±0.41	14.7
C.palmensis	Inoc 2	-					
C.palmensis	Inoc 3	+					
C.palmensis	Control	-					
C.palmensis	Nitrate	-					
A.saligna	Inoc 1	+	0.77±0.02	12.42±0.24	6.2	7.97±0.19	9.7
A.saligna	Inoc 2	-					
A.saligna	Inoc 3	+					
A.saligna	Control	-					
A.saligna	Nitrate	-					
S.sesban	Inoc 1	-	0.46±0.01	7.58±0.14	6.1	3.33±0.09	13.7
S.sesban	Inoc 2	-					
S.sesban	Inoc 3	-					
S.sesban	Control	-					
S.sesban	Nitrate	-					

Nitrogen data

What stands out from Table 1 is the fact that only two of the inoculants (1 and 3) effected nodulation, and only in *C. palmensis* and *A. saligna*.

Seed (~~embryo~~) nitrogen contents (Table 1) show the highest value for *C. palmensis*, followed by *A. saligna* and *S. sesban*. The concentration of N per seed (%), however, was found to be similar for all species (Table 1).
Embryo N concentration (%), was found to be highest for *C. palmensis*, followed closely by *S. sesban* and thirdly, *A. saligna*.



what of N concentration?
mg g^{-1} dry wt?

Figure 1. Total plant nitrogen contents (mg N plant $^{-1}$) calculated for *C. palmensis, A. saligna* and *S. sesban* for the five treatments following 12 weeks growth. Details of the treatments are given below.

Key to the above and to subsequent figures:
T = tagasaste (*C. palmensis*), A = *A. saligna*
S = *S. sesban;*
1, 2 and 3 = rhizobial strains XIA1, XIB2 and XHZ1.
C = nitrogen-free control, N = nitrate-fed control.

From Figure 1 it can be seen that there was a substantially higher N content in the nitrate-fed plants for each species and that the *A. saligna* plants in which nodulation occurred, also had relatively high nitrogen contents.

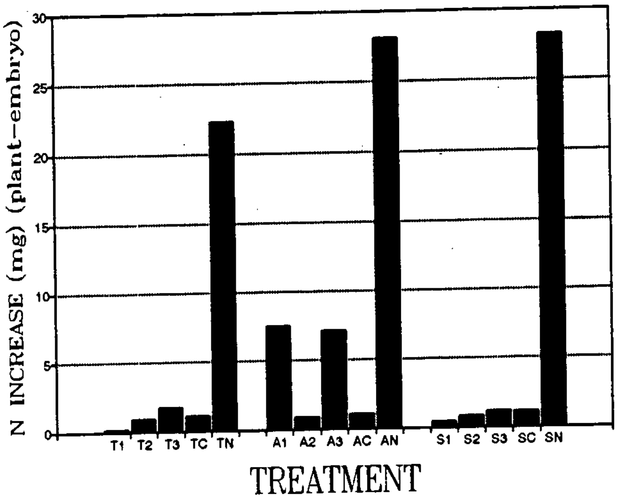


Figure 2. Nitrogen increment values (mg N plant $^{-1}$) after 12 weeks growth for *C. palmensis, A. saligna* and *S. sesban,* calculated by subtracting seed (embryo) N from total plant N.

Figure 2, reflects the trends represented in figure 1. Experimental error (eg. minute amounts of N in nutrient solution) may account for the small increases in nitrogen in the non-fed and non-fixing plants.

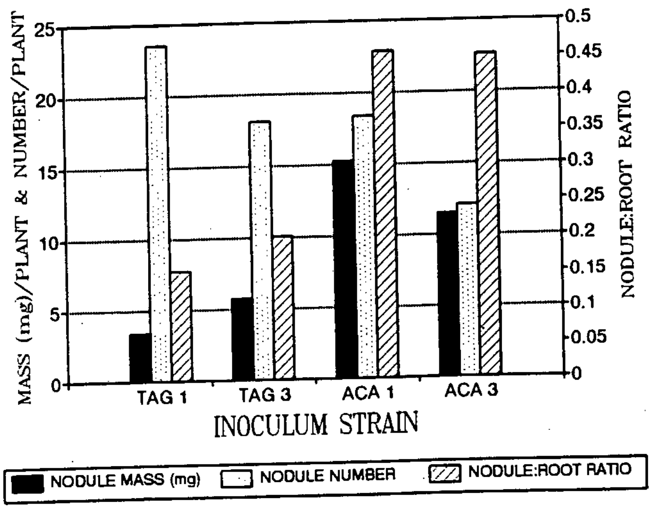


Figure 3. Nodule mass, number and nodule:root ratio for *C. palmensis* (TAG) and *A. saligna* (ACA) (1 = inoculum XIA1; 3 = inoculum XHZ1).

Figure 3 indicates that although the number of nodules per plant was similar or more for *C. palmensis*, the mass of these nodules was lower than that of *A. saligna*. These very small nodules consequently contributed a smaller percentage to the below-cotyledon dry mass, as is shown by the lower nodule:rest-of-root ratio for *C. palmensis* than *A. saligna* (Figure 3).

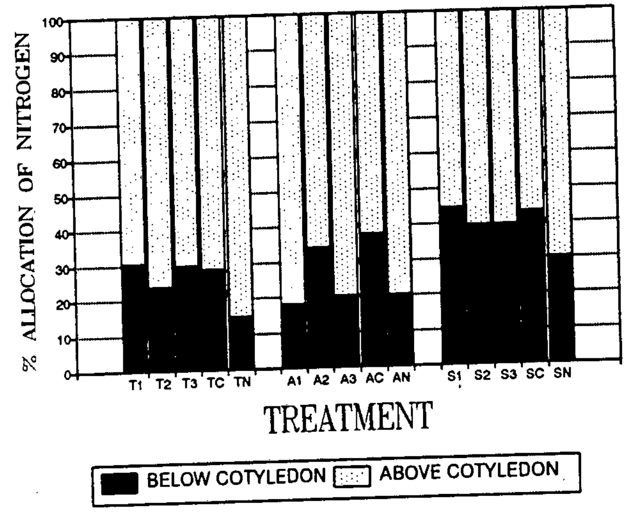


Figure 4. Nitrogen allocation pattern for *C. palmensis, A. saligna* and *S. sesbania*. Calculated as the proportion of the total plant nitrogen content (mg N plant $^{-1}$) allocated above and below the cotyledon, expressed as a percentage.

Figure 4 indicates that, for all species, the amount of nitrogen allocated to the roots was lower in the nitrate-fed plants when compared to the control plants. In the non-nitrate *C. palmensis* treatments, an average of about 30% of the total nitrogen was found to be allocated to the roots and 70% to the stems (Figure 4).

The pattern is, however, different in *A. saligna*. Here, in plants where nodulation had been initiated, the allocation pattern was found to reflect that of the nitrate-fed plants, 20% to roots and 80% to stems. This is in contrast to the non-nodulating plants of this species, which showed a higher percentage allocation (about 35%) of N to the roots (Figure 4).

The trends for *S. sesban* indicate a general trend of a slightly larger percentage (about 40%) of nitrogen allocation to the roots than in the other two species (Figure 4).

The results of the one-way analysis of variance for seed N content indicated a significant difference ($p < 0.0001$) between the three species.

The results of the two-way analysis of variance between species and treatment for total plant N-content indicated significant differences ($p < 0.0001$) between species and between treatments. Significantly different results ($p < 0.0001$) were also obtained for two-factor interactions, indicating that the effect of the different treatments were not the same across the species.

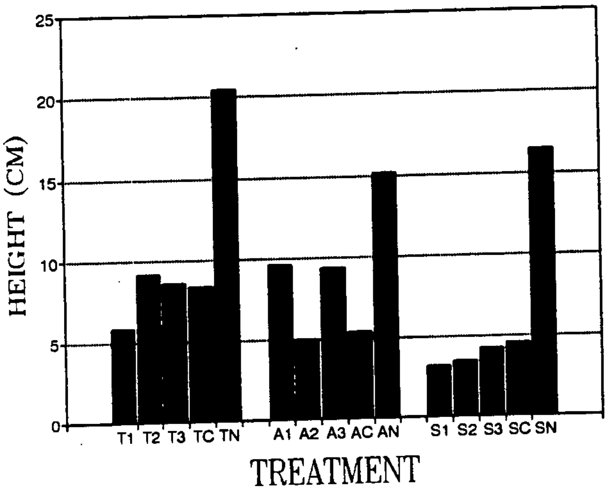


Figure 5. Plant height (cm) for *C. palmensis, A. saligna* and *S. sesban* for the five treatments, after 12 weeks growth.

Growth data

Figure 5 graphically illustrates the height differences between treatments for each species. It is clear that the nitrate-fed plants grew substantially taller than plants in the other treatments. The height of *A. saligna* plants in the two treatments that were nodulating (1 and 3) was almost twice that of the non-nodulating plants (Figure 5).

From these results it can be seen that fixed nitrogen was being put into shoot growth in terms of elongation as measured by plant height. The relatively lower values for plant height in treatment 1 for *C. palmensis* (Figure 5) is explained by the fact that the degree of branching was higher, and the leaves were smaller and more clustered in plants under this treatment, when compared to other treatments. No other above-ground differences (eg. increased insect damage) which might explain this phenomenon were observed. Although the sand was sterilized, the possibility of soil pathogen-induced effects are not ruled out.

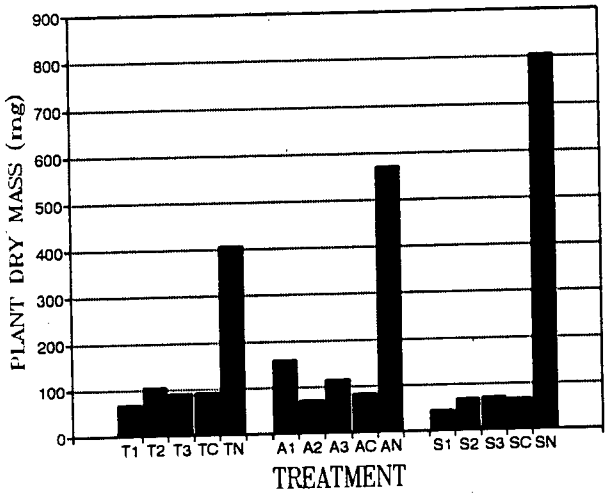


Figure 6. Total plant dry mass (mg) for *C. palmensis, A. saligna* and *S. sesban* after 12 weeks growth in acid washed sand, under different treatments.

In Figure 6, the most obvious trends are the much larger plant dry mass values for nitrate-fed plants, as would be expected. Increases in biomass in nodulated plants is also higher in *A. saligna* than in non-nodulated plants (Figure 6). This trend is, however, not reflected by *C. palmensis*, the other nodulating species.

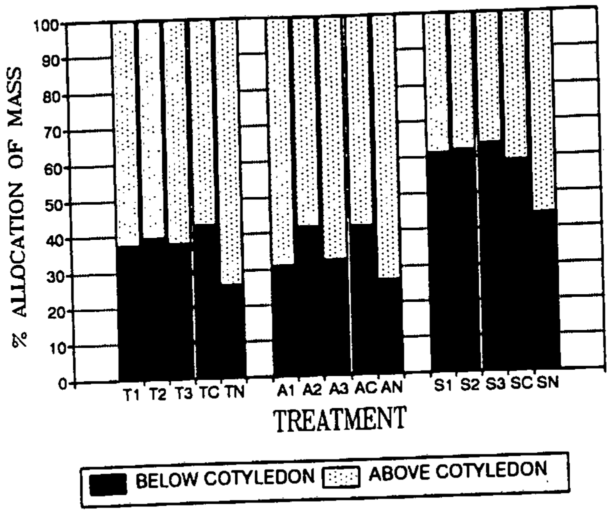


Figure 7. Biomass allocation patterns for roots and shoots of *C. palmensis, A. saligna* and *S. sesban* after 12 weeks growth. The values represent the proportion of plant dry mass allocated below and above cotyledons for the five treatments.

From Figure 7 it can be seen that for all three species there was a lower proportion of biomass allocation to roots in the nitrate-fed plants. This trend may also be seen in Figure 8 where the root:shoot ratio was lowest for the nitrate treatment.

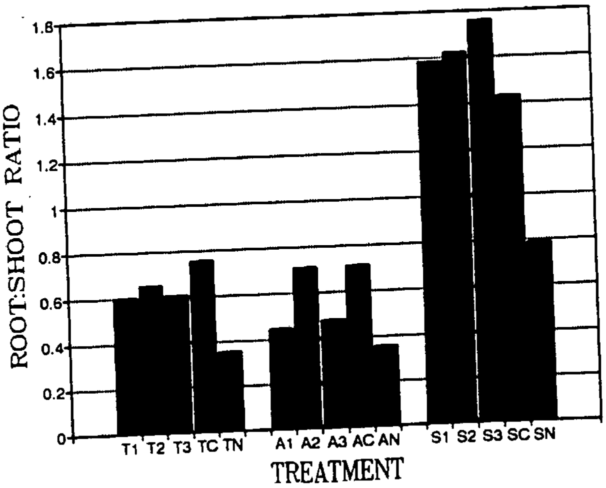


Figure 8. Root:shoot ratio comparison between *C. palmensis, A. saligna* and *S. sesban* under five treatments after 12 weeks growth. Values represent the ratio of below cotyledon (root) to above cotyledon (shoot) dry mass.

The trends of lowered biomass allocation to the roots (Figure 7) and lower root:shoot ratios for the nitrate-fed plants (Figure 8) are reflected by *A. saligna* plants treated with inocula 1 and 3 (Figures 7 and 8). Patterns of biomass allocation (Figure 7) for all plant species reflect N allocation patterns (Figure 4), indicating a correlation between plant nitrogen content and growth.

The graph of the biomass allocation patterns (Figure 7) shows different trends for the three species, indicating that *S. sesban* has an different intrinsic growth strategy during these early stages, when compared to *C. palmensis* and *A. saligna*. *S. sesban*, when nitrogen-stressed, was found to allocate a substantially higher proportion of its biomass to roots than to stems (60% as opposed to 40% in *C. palmensis* and *A. saligna*). This trend is also reflected in the root:shoot ratio graph (Figure 8).

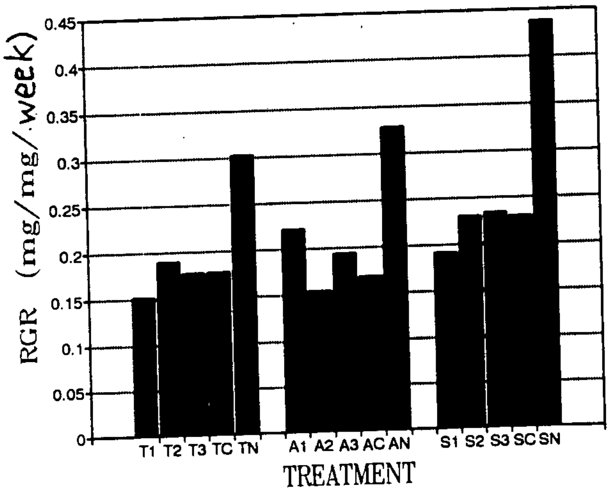


Figure 9. Relative growth rate values (mg mg week $^{-1}$) for *C. palmensis*, *A. saligna* and *S. sesban* under different treatments after 12 weeks growth.

Trends in relative growth rate show that nitrate-fed plants had the highest relative growth rates compared to other treatments, in all species (Figure 9). *S. sesban* showed the highest relative growth rate, followed by *A. saligna* and *C. palmensis* respectively. The R.G.R.'s for the other *S. sesban* plants produced an interesting result in that in general they also had higher R.G.R.'s than *C. palmensis* and *A. saligna* although they were showing signs of being N-stressed (yellowing).

The results of the two way analysis of variance for the height data indicated highly significant differences between species and between treatments ($p < 0.0001$) as well as highly significant differences ($p < 0.001$) for the two-factor interactions. The analysis of variance for the biomass data produced a similar trend of results with the exception of a slightly lower significance level ($P < 0.05$) for the differences in biomass between the species.

DISCUSSION

Legume-rhizobia symbiosis

The most significant result of this experiment is the fact that only two of the inocula (1 and 3), induced nodulation and that this occurred in only two of the species (*C. palmensis* and *A. saligna*). The fact that inoculum 2 failed to induce nodulation in either of these species suggests that either the rhizobium failed to locate/recognise the roots of these species or that the bacterial spores are no longer active.

The nodulation which *did* occur with the other inoculants, however, demonstrates that nodulation of one species by more than one rhizobial strain as well as nodulation across legume sub-families by a single rhizobial strain, is possible.

Genera of rhizobia are divided into species and biovars, but there are conflicting ideas about the classification at this level (Sprent and Sprent 1990). Until recently, there appeared to be a broad distinction between legumes and rhizobia of tropical and temperate regions with fast-growing rhizobia supposedly more common in temperate regions

(*Rhizobium*) and slower-growing ones in the tropics (*Bradyrhizobium*) (Sprent and Sprent 1990). However, following recent studies on mimusoid legumes (Barnet 1988, in Sprent and Sprent 1990), this distinction is no longer valid. Young and Johnston (1989) point out that as new legume-rhizobia symbioses are continually being described, it seems that many more genera will eventually be required to accommodate all root-nodule bacteria.

The results of this experiment suggest that *A. saligna* is capable of accepting a broad range of rhizobia which can induce nodulation. Barnet *et al.* (1985) discovered nodulation of *A. saligna* by both fast and slow-growing strains of rhizobia. Also, this species (from Australia) is easily nodulated by indigenous strains of South African rhizobia (pers. obs.).

Chamaecytisus palmensis, which is native to the Canary Islands, was nodulated by bacteria isolated from the Scottish broom, *Cytisus scoparius*, which occurs over a wide area of Europe and the Canary Islands (Wiersama *et al.* 1990). This suggests a similarity in the host-recognition systems of the associated rhizobia of these species which would both be present in the soil in which *C. palmensis* naturally occurs.

Sprent and Sprent (1990) point out some of the difficulties involved in the colonization of a legume root by a rhizobial bacterium. They also note that even if there are sufficient numbers of rhizobia near the host root surface, success of colonization of the root surface might be impeded due to competition between rhizobia, or between rhizobia and other micro-organisms.

Why was there no nodulation induced by the rhizobia isolated from *Chamaecytisus supinus*? This plant occurs naturally in central and eastern Europe (Wiersama *et al.* 1990), restricted to temperate, rather than Mediterranean regions. As there was little possibility for rhizobial competition in this experiment, and as *A. saligna* appears to accept a wide variety of nodulating rhizobia from Mediterranean regions, it is suggested that the strain XIB2 (isolated from a plant restricted to a temperate region) could be incompatible due to a number of reasons.

C. palmensis and the plants from which the inocula for this study were isolated (*Chamaecytisus supinus, Cytisus scoparius* and *Cytisus decurrens* (*decumbens*)), although taxonomically similar, originate from different geographic regions, in which specific host-recognition patterns and compatibility by their associated rhizobia, has evolved.

The genera *Chamaecytisus* and *Cytisus* are closely related and there is some overlap in the nomenclature between these species, *Chamaecytisus supinus* also being referred to as *Cytisus supinus* (Wiersama *et al.* 1990). The natural range of *Cytisus decurrens* could not be located from the literature, in fact not one reference to this species could be found. It is, however, suggested that the distribution of this species would include the Mediterranean region, as is suggested by the ability of its associated rhizobia to nodulate *C. palmensis* and *A. saligna.* It is likely that due to a typographical error *C.decurrens* should read *Cytisus decumbens* (Durande) . This latter species *is* native to the Mediterranean region of Europe (Synge 1956) and would therefore fit the above suggestion of the evolution of the legume-rhizobium symbiosis in *C. palmensis.*

The complete lack of nodulation in *S. sesban* suggests that a different rhizobial strain is required to inoculate this species. This species, with many sub-specific varieties, occurs over a wide range of mostly tropical or subtropical climates in Africa and Asia (India) (Wiersama *et al.* 1990). The particular variant used in this experiment comes from Malawi (Armstrong pers. comm.) and is therefore probably *Sesbania sesban* subspecies *sesban* var. nubica (Wiersama *et al.* 1990). It seems that the rhizobial symbiosis required for this tropical species is more specific than for *C.*

palmensis and *A. saligna*. In describing the tribes of the legume sub-family Papilionoideae, the tribes Robinieae (includes *S. sesban*) and Genisteae (includes *Chamaecytisus* and *Cytisus*) are fairly isolated from each other (Young and Johnston 1989). It has been noted that members of the Robinieae have only shown rhizobial symbioses with fast-growing bacterial strains (Young and Johnston 1989) and that these associations are specific (Nuti and Casella 1989).

The specificity of the legume-rhizobium symbiosis is not well understood and is extremely complex and diverse (Young and Johnston 1989). In looking at the genetic bases of host range specificity Young and Johnston (1989) have noted the following;

i) No single explanation accounts for the ability of one bacterial strain to nodulate a particular host.

ii) Factors that specify host-range differences between one pair of rhizobial strains may be quite different from those that distinguish host range between others.

Nodule development and N-fixation

As has been shown (Figure 3), the mass of nodules formed was relatively smaller in the case of *C. palmensis* than *A. saligna,* although the nodule mass of the latter species was found to be small in comparison with nodules of other *Acacia* spp. grown for two months under experimental conditions (Dreyfus and Dommergues 1981).

The relatively low mean temperatures inside the glasshouse and the fact that the plants were grown during winter (June - August) meant that optimal conditions for growth and nodulation were not provided in this experiment.

In examining the results for total nitrogen content (Figure 1) and nitrogen content increase (Figure 2) it can be inferred that N-fixation was occurring in *A. saligna* but not in *C. palmensis*, although both species had been nodulated by inocula 1 and 3 (Table 1).

In understanding the possible reasons for this result the stages of nitrogen-fixation need to be examined. Sprent (1989) has summarised the stages of nodule formation as follows;

colonization - attachment - hair curling - infection - nodule initiation - bacterial release - bacteroid differentiation - nitrogen-fixation - nodule operation

From this it can be seen that although nodulation may have already occurred, nitrogen-fixation only begins following release of rhizobia from infection threads and their division and differentiation into bacteroids. In this experiment the *C. palmensis* nodules may have still been at the development stage when plants were harvested.

Another possible explanation for the observed phenomena might be species-specific differences in the ability of plants to overcome "nitrogen hunger" during the early stages of development. In this study, the inoculated plants were forced to obtain their nitrogen purely by fixation as no additional nitrogen was given. Although nitrogen fertilizers may inhibit nodulation, small amounts of combined nitrogen may stimulate nodulation by tiding the plant over this "nitrogen hunger" period when nodules may put a heavy drain on plant resources (Sprent and Sprent 1990). *A. saligna* appears to be less susceptible to "nitrogen hunger", this species placing its cotyledonary nitrogen reserves into nodule growth at an early stage (cotyledons abscised early) whereas *C. palmensis* seemed to draw on cotyledonary reserves over an extended period (a few cotyledons were still present at harvest).

A third possible explanation for the differences in nodule formation and nitrogen-fixation is given by Sprent (1989). Two possible strategies are described, following the onset of nitrogen-fixation. Plants may produce nodules which export the products of nitrogen-fixation immediately, but nodule growth and infection of new cells continues for varying lengths of time. Alternatively, plants may produce nodules where the first nitrogen fixed is used for nodule growth and only after this nodule expansion is well advanced does export begin. *A. saligna* seems to be adopting the former strategy and if *C. palmensis* had reached the stage of beginning to fix nitrogen this species would probably adopt the latter strategy.

Cases of ineffective nodulation (formation of nodules but failure to fix nitrogen) may also be explained by parasitic associations of soil micro-organisms (Staphorst pers. comm.). Although the sand was autoclaved in this experiment, total sterility of the glasshouse was not achieved and the possibility of nodule parasites in *C. palmensis* can not be ruled out.

The above discussion points out the fact that the formation of nodules on the roots of a potential N-fixing plant does not necessarily mean that N-fixation is taking place. Nodulation of roots by a rhizobium without effecting N-fixation suggests only a partial compatibility in the legume -rhizobium symbiosis.

N-fixation, N allocation and growth

The lower percentage of root-allocated nitrogen in the nodulated *A. saligna* plants (Figure 4) suggests a "self-sufficiency" in terms of nitrogen requirement, enabling a higher percentage of nitrogen to be allocated to the shoots (80%) than in non-fixing plants (about 65%) (Figure 4).

In the nitrogen-fixing *A. saligna* plants, increases in biomass were found to be slightly higher than in the control and inoculum 2 treatments (Figure 6), and these trends are more obvious in terms of height differences (Figure 5).

This suggests that nitrogen-fixation is taking place in treatments 1 and 3 and that as a consequence, the plants have begun to allocate more of their total nitrogen to stem growth.

Although the nitrogen content in the nitrogen-fixing *A. saligna* plants was relatively higher than the non-nodulating plants (Figure 1), the growth of these plants, measured as biomass, was not substantially different for these plants (Figure 6). In addition, the nitrogen-fixing *A. saligna* plants showed relatively lower growth rates (Figure 9) than the nitrate-fed plants. but higher than non-fixing plants

Thus, although they were fixing nitrogen, N-fixing *A. saligna* plants were not supplying the optimal amounts for maximum plant growth. The reason for this is probably due to the fact that assimilated carbon in the nitrate-fed plants could be used solely for growth, whereas in the nitrogen-fixing plants, a percentage of assimilated carbon would be required for the maintenance of nodules. Ryle *et al.* (1981) have noted that the energy requirement for nitrogen fixation in nodules may reduce the carbon resources retained for shoot growth in comparison with similar plants utilizing abundant combined nitrogen.

Hirose (1988) has noted a strong correlation between plant nitrogen concentration and relative growth rate. The same general trends can be observed in this study if we compare the nitrogen content values (Figure 1) with the relative growth rate values (Figure 9). In comparing the nitrogen-fixing *A. saligna* plants, the relatively higher R.G.R. value for the inoculum 1-treated plants might suggest that this is a slightly more superior rhizobium than inoculum 3. However, more experimental evidence would be required to confirm this.

Root:shoot ratios

In examining the root:shoot ratios for the three species and the five treatments, a great variability in values is to be seen (Figure 8). In all species, the nitrate-fed plants were found to exhibit lower root:shoot ratios than plants in the other treatments. The reason for this seems that as plants were receiving a full compliment of nutrients required for growth, relatively more effort could be put into shoot growth instead of root growth for exploration of the soil.

Ryle *et al.* (1981) note that a lack of nitrogen leads very rapidly to an increase in root:shoot ratio, while plants receiving high levels of applied nitrogen generally exhibit minimal root:shoot ratios. In the case of *S. sesban* the large root:shoot ratios were recorded for plants which were noticeably nitrogen-stressed. This result is not consistent with those of Fownes and Anderson (1991) who found a greater allocation of biomass to stem growth (67%) after 15 weeks growth for *Sesbania sesban* var *nubica*. Differences in the results may be explained by the fact that in the experiment of Fownes and Anderson (1991), effective nodulation was achieved, and in addition, the growth medium contained nitrogen. By comparison, plants in this study were visibly nitrogen-stressed, having neither a soil, nor an atmospheric nitrogen source.

The trend of reduced root:shoot ratio with increased nitrogen content (as in the nitrate-fed plants) is also notable in the N-fixing *A. saligna plants* (Figure 8). However, this root:shoot ratio is higher for the nitrogen-fixing plants than for the nitrate-fed plants (Figure 8). Similar differences in root:shoot ratios in relation to nitrogen source have been found in white clover by Ryle *et al.* (1981). In that study, root:shoot ratios were found to be consistently higher in nodulated plants than in nitrate-plants from quite an early stage of development (Ryle *et al.* 1981).

Seed size, seed nitrogen and R.G.R.

The R.G.R. value trends for the nitrate-fed plants (Figure 9) are negatively correlated with seed nitrogen content (Table 1). However, if the seed size is taken in account, it can be seen that seed nitrogen concentration values are similar (T=7.1%, A=6.2%, S=6.1%, Table 1) for the three species. When the concentration of embryo (seed - testa) nitrogen is examined (T=14.7%, A=9.7%, S=13.7%, Table 1) no clear correlation between these values and R.G.R.'s are observed (Figure 9). These results indicate that intrinsic differences in growth rates between species cannot be predicted by seed nitrogen content alone.

Implications for agroforestry

The results of this study indicate that specific legume-rhizobia symbioses are required by some legume tree species but not by others. Although one combination of inocula was found to nodulate more than one species (*C. palmensis* and *A. saligna*), another species (*S. sesban*) would seem to require different inocula for N-fixation. The implication of such a system for agroforestry is that it would be advantageous to grow combinations of species with common rhizobial symbionts. These could be combinations of perennial legumes intercropped with annual legumes.

An example would be to intercrop a perennial tree, *Acacia albida*, which is nodulated by a poorly-specific, slow-growing cowpea rhizobia (Dreyfus and Dommergues 1981; Nuti and Casella 1989), with the cowpea (*Vigna unguiculata*) or peanut (*Arachis hypogaea*), which both require the same cowpea rhizobium (Young and Johnston 1989).

In perennial systems such as with a *C. palmensis-A. saligna* combination (Snyman 1992) the advantage in this case, is that fewer inocula need to be supplied to the soil to induce nodulation in both species. From a management perspective, it is easier to deal with fewer inocula when inoculating either seed or soil. In terms of nitrogen-fixing potential it may also be beneficial to introduce fewer rhizobial strains to the soil which would reduce the negative competitive effects of many rhizobia competing for host plants. Such competition may occur between different introduced rhizobia or between introduced and indigenous strains (Sprent and Sprent 1990). It has been noted that highly effective rhizobia might not achieve the desired productivity due to competition by less effective indigenous strains (Danso *et al.* 1992; Staphorst pers. comm.).

Although it is advantageous to minimise the rhizobial requirements in agroforestry systems, it is nevertheless a complex issue in which the rhizobial strain selection should be a critical part of the evaluation of a tree legume species. Highly specific nodulation requirements of a particular species or group of legume trees might be an advantage, as the introduced inoculum would be effective in out-competing the indigenous strains. Danso *et al.* (1992) note that many soils lack rhizobia for specific tree legumes eg. *Leucaena leucocephala* and that this would allow the full response to inoculation with effective rhizobia.

Short note

Although *A. saligna* is seen as a useful species by certain individuals (Armstrong 1992), others (Cowling 1992; Donnelly and Morris 1992) see it as an undesirable weed. The fact that the rhizobial strains for *C. palmensis* (as supplied by the PPRI) also induces nodulation in *A. saligna* might be of concern in terms of the management of these potentially invasive aliens (Staphorst pers. comm.).

CONCLUSION

Nodulation of *C. palmensis* was achieved by rhizobial strains XIA1 and XHZ1. No nodulation occurred with rhizobial strain XIB2 and it seems that no advantage would be gained in its continued distribution by the Nitrogen Fixation Unit. The same strains, XIA1 and XHZ1, were found to induce nodulation in *A. saligna*. The nodules in the case of *C. palmensis* were very small at the time of harvest and no significant nitrogen accumulation in nodulating plants was detected. However, nitrogen-fixation *had* been initiated at the time of harvest in the case of *A. saligna*, with similar amounts of nitrogen, 7.5 and 7.2 mg N plant $^{-1}$, being fixed with strain XIA1 and XHZ1 respectively. No nodulation was observed in *S. sesban*, indicating that a different (specific) rhizobium is required to induce nodulation in this species.

Effective nodulation in *C. palmensis has* been observed in the field using the three recommended rhizobial strains (Armstrong pers. comm.; Staphorst pers. comm.). As this result was not borne out in this study, further tests including field trials would be required in order to determine the effectiveness of these strains for inducing N-fixation of *C. palmensis*.

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