# CHAPTER IV

## ASSOCIATIONS OF MICROORGANISMS WITH TREES - AN AID TO SOIL AMELIORATION

In arable systems, nitrogen fixation through Rhizobium/legume symbioses currently provides a higher nitrogen input to soils (as plant residues) on a global basis than nitrogen fertilizer applications. Management of legumes and of legume residues is therefore a key factor in sustained crop production.

Many tropical soils are inherently deficient in nitrogen, a primary soil nutrient for plant growth. Where legume crops are not part of the agricultural system, legume trees could be valuable in adding nitrogen to the agroecosystem. Not all legumes fix nitrogen. The potential nitrogen - fixing abilities of legume trees in the three legume sub-families, Caesalpinioideae, Mimosoideae and Papilionoideae are discussed by J. I. Sprent in Section I of this chapter. In Section 2, Sesbania sesban, a legume tree, is evaluated as a soil conditioner in relation to its broad tolerance of extreme physiological conditions as well as its nitrogen fixing abilities.

Legumes are not the only group of woody species capable of fixing nitrogen. Casuarina, a non-leguminous tree is nodulated by the nitrogen-fixing filamentous soil bacterium, Frankia. Casuarina also grows well under extreme soil conditions such as high salinity, and therefore has considerable potential in soil amelioration. The biological and environmental factors affecting the symbiosis between Casuarina and Frankia are discussed in Section 3 of this Chapter. This symbiosis is further investigated in section 4 by P. A. Rosbrook. In this section, the sources of Frankia capable of nodulating and effectively fixing nitrogen in symbiotic associations with various Casuarina species are screened.

Another way in which microorganisms in association with trees can improve soil productivity is through the mutualistic association of fungi with roots, known as mycorrhizae. Mycorrhizae increase the trees' absorptive capacity and uptake of nutrients and water from the soil and promote tolerance to drought, salinity and heavy metals. Mycorrhizae also bind soil particles, forming soil aggregates, and can therefore be of primary importance in forming and maintaining soil structure.

Because of their ability to take up nutrients which are otherwise unavailable to the plant, and to directly take up nitrogen and phorphorous from litter, mycorrhizae play an important part in conversion of nutrients and in nutrient cycling. The role of mycorrhizae in soil amelioration is considered in section 5 and mycorrhizae research in India is reviewed.

# 1. NITROGEN FIXING LEGUME TREES: PROBLEMS AND MISCONCEPTIONS

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## SUMMARY

A number of important legume trees, particularly in the sub-family Caesalpinioideae, cannot nodulate. Others, which have the potential to nodulate, may not do so in extreme environments. The occurrence of nodulation is discussed especially in relation to the type of nodule produced and the problems which need to be solved before their nitrogen fixing potential can be optimised.

## INTRODUCTION

The family Leguminoseae probably evolved in the tropics, early in the evolution of flowering plants (Pohill et al, 1981). From there it spread into temperate and artic regions, becoming progressively more herbaceous in habit. These temperate, herbaceous species, together with some sub-tropical crop relatives, almost all of which are form the sub-family Papilionoideae, have dominated research into legume physiology. It is becoming increasingly clear that tree legumes do not obey many of the rules of their herbaceous descendants. This is particularly true of their potential to fix nitrogen. The purpose of this paper is to put the problems and the potentials for nitrogen fixing trees into perspective.

## TAXONOMIC FRAMEWORK, CAESALPINIOID SPECIES

It has been known for many years (see data summarised by Allen et al, 1981) that Caesalpinioid legumes nodulate less frequently than their Mimosoid and Papilionoid counterparts. This still holds, although a number of new reports of nodulated species have been made recently (Faria et al, 1984 and in preparation). In many cases the literature is ambiguous. Bauhinia for example is generally considered to be a non-nodulating genus (Allen et al, 1981, list 27 species reported to lack nodules and Faria et al, 1984 have added a futher 4) and yet Quinones (cited by Domingo, 1983) claims to have isolated rhizobia from nodules of B.purpurea (one of the species for which Allen et al, 1981 cite three negative reports of nodulation). Parkinsonia aculeata is listed as nodulated by Halliday et al (1981), but the references given to support this all report absence of nodules, so one suspects that a '+' was included in the table instead of a '-'. Extensive searches for nodules in Brazil (Faria pers. comm.) and Central America (Roskowski, pers. comm.) have yielded negative results. However, there are unconfirmed reports that this species nodulates in the Sudan. Since it is being recommended for introduction into arid areas (Hughes et al, 1984) it is desirable that this conflict is resolved. We have grown P.aculeata with a rhizobial inoculant from NifTAL (the centre for rhizobial inoculants in Hawaii) and with many strains of rhizobia from Brazil and none has yielded functional nodules. However, outgrowths from the junctions between main and lateral roots, superficially like nodules of Arachis (peanut, groundnut) are often seen.

One possibility is, therefore, that these pseudonodules, which result from division of a phellogen, have been mistaken for nodules. Although it has serious limitations as a quantitative assay, an acetylene reduction test (as used by Faria et al, 1984) should always be performed on any structure suspected of being a nodule. An alternative is to fix nodules and examine them for the presence of infected cells, consulting a suitable authority in the case of unusual nodule structures.

A further possibility in the case of Parkinsonia is that it nodulates in some countries but not in others. Geographical restrictions on nodulation have been known amongst actinorhizal nodulated plants for a number of years (Bond, 1976) and appear to hold also for some legume genera. Pterocarpus spp. (Papilionoideae) for example, apparently nodulate in Africa, Asia and Venezuela (Allen et al, 1981) but not in Brazil (Faria et al, 1984). Another possible source of confusion is misidentification of the legume - some are very difficult to identify, especially when not flowering. The taxonomy of many of these groups is under revision. The genus Cassia for example has recently been divided into three, Cassia, Senna and Chamaechrista (Irwin et al, 1982). All the nodulated cassias have, for other reasons, been placed in Chamaechrista.

Many trees of established and potential commerical significance are apparently non-nodulated members of the Caesalpinioideae. These include Baikaea plurijuga which yields valuable timber (Allen et al, 1981). Of the five legume trees listed by Hans (1985) as important fruit species in Zambia, three, (Dialium engleranum, Guibortia colespermum and Tamarindus indica) are Caesalpinioid and one (Cordyla africana), classifed as Caesalpinioid by Hans (1985) has now been transferred, with other members of the tribe Swartzieae to the Papilionoideae (Cowan, 1981). In none of these could Corby (1974) find nodules in the neighbouring state of Zimbabwe. There are no confirmed reports of nodules on Cassia siamea, a tree widely grown in Africa and elsewhere. The fact that many important Caesalpinioid legumes do not nodulate does not, of course, detract from their value as crops in comparison with non-legume species. Bauhinia for example yields high quality forage (Robinson, this volume). Further, their success in some nutrient poor soils may relate to a general efficiency in nutrient acquisitition and in this way they should be evaluated against non-legumes (see also Okigbo, 1984). Such trees may improve nutrient cycling and nitrogen levels in upper soil layers, but will not increase total soil N.

Amongst nodulated Caesalpinioid trees, work has concentrated so far on describing nodule morphology and on isolating rhizobia (Corby, 1974; Allen et al, 1981). Infection processes, nodule structure and nitrogen fixing activity have been largely ignored, mainly because of lack of facilities in areas where these plants grow. Faria and Sprent (in preparation) have found considerable similarity in internal structure within Caesalpinioid nodules, but major differences from nearly all other known nodule structures. Some nodules are themselves black and woody, due to extensive sclereid production and thick walled xylem elements. Work on estimating the nitrogen fixing potential of these nodules is commencing, but field studies are urgently needed in all those countries where the plants grow naturally. Only then can improvement programmes be seriously undertaken.

## MIMOSOID TREES

Most of these have the potential to nodulate, although there may be geographical variation in the genus Parkia. Nodules have been found in Asia and

Hawaii (see references in Allen et al, 1981), but Norris (1969), confirmed by Faria et al, 1984 could find none in Brazil.

Mimosoid trees are very important in arid and semi-arid regions throughout the world and some genera, such as Prosopis have great drought and salinity tolerance. However, such evidence as is available (discussed in Sprent, 1985, 1986) suggests that even though they can nodulate, they may not do so under extreme environmental conditions. Even when nodulated, active nitrogen fixation only appears to occur when water is available. However, they have many important attributes, including being multipurpose plants and having deep roots. Investment of plant resources in root systems in generally nutrient poor soils is of more potential value than investment in nodules which only alleviate N deficiency (Sprent, 1985). The key to the success of Mimosoid trees in dry and/or saline environments is more likely to result from deep roots than nitrogen fixation.

## PAPILIONOID TREES

Most of these are nodulated although there are some exceptions (particularly in some of the more primitive tribes which contain trees, Faria et al, 1984). The type of nodule varies considerably. Common amongst the tribe Dalbergieae, which includes the important timber, incense and fodder genus Dalbergia, are small nodules of limited growth, located in the axils of lateral roots (Faria et al, 1984). They appear to form very freely, not only on young roots, but also on old roots among earlier formed, senescent nodules. This property might give them a versatility not possessed by species which nodulate only on young roots. Erythrina, normally included in the tribe Phaseoleae, has nodules similar to those of Dalbergia. An intersting special case of this type of nodule is found on the stems of the genera Sesbania (Tsien et al, 1983) and Aeschynomene (Vaughn et al 1985). These too are associated with root initials. They form most readily when soils are flooded, reducing the extent of root nodulation. In the case of Sesbania rostrata different rhizobia and modes of infection may be used for stem and root nodules on the same plant (Olsson et al, 1985).

Nodules on most of the other Papilionoid trees (e.g. Sophora, Robinia, Wisteria) that have been examined, show indeterminate growth, as do most Mimosoid nodules: many are branched and perennial. Often, like Mimosoid nodules, the older parts become corky on the surface as compared with looser aerating tissue on the surface of the young, active nitrogen fixing regions. Indeterminate growth, as in advanced herbaceous species such as the clovers, enable plants to recover from some forms of environmental stress, such as drought, by growing new nitrogen fixing tissue (Sprent, 1976, 1985).

## TREE LEGUME RHIZOBIA

Like their nodules, tree legume rhizobia do not conform to the familiar patterns found in temperate crop species. Growth rate (fast, intermediate, slow or very slow, compared with fast or slow) varies both within and between host genera (Allen et al, 1981: Trinick, 1982; Barnet et al, 1985). Many are rather promiscuous, nodulating a variety of species. If this promiscuity is similar to that on herbaceous genera, then there is likely to be a big range in nitrogen fixing efficiency. This has been shown for example in introducing soybean (Glycine max) to Nigeria, where many poorly effective or ineffective nodules form with indigenous rhizobia which normally nodulate cowpea (Vigna *unguiculata*) (Devine, 1984). These problems need to be studied and taken into account in any programme to enhance nitrogen fixation by nodulated legume trees.

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# 2. NITROGEN FIXATION AND BIOMASS PRODUCTION BY SESBANIA SESBAN IN ALKALI SOILS

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## SUMMARY

(1) Sesbania sesban was evaluated in pot and laboratory studies for nodulation, N fixation in an alkali soil and tolerance to salinity and alkali at germination. Field experiments on green-manure potential and biomass production were carried out at an alkali site.

(2) On an average, S. sesban had 36 nodules plant$^{-1}$, nodule biomass (dry) of 92 mg plant$^{-1}$, 2.3 g dry wt. plant$^{1}$ and N content of 3.5% at the 30 day growth stage. Native rhizobia produced excellent nodulation and actively competed with inoculated strains.

(3) S. sesban could tolerate EC 11.0 (0.7% salts) and pH 10.0 at germination. It produced a biomass (oven-dry) of 5.2 and 12.7 t ha$^{-1}$ at 100 and 200 days growth respectively. In long term experiments it produced air-dry biomass of 87 t ha$^{-1}$ in 3 years. At 100 days growth the green-manuring potential was (kg/ha) 64 N, 5 P and 52 K. At 200 days it was (kg/ha) 59 N, 4 P and 38 K.

## INTRODUCTION

Sesbania sesban (Linn.) Merrill (Syn. S. aegyptiaca) is a soft-wooded, quick growing, short-lived tree cultivated throughout the plains of India. It branches copiously, growing to a height of 4.5 - 6m. It forms root nodules by symbiotic association with Rhizobium sp. capable of fixing atmospheric nitrogen. it is often grown as a perennial green manure crop. The plant stands lopping well and produces regrowths quickly. It tolerates a wide range of soil conditions, including acidity, periodic flooding and water-logging (Anonymous, 1983). It can endure 0.4-10% salt concentration at the seedling stage and 0.9-1.4% near maturity (Jen et al., 1965). The plant has been reported to grow up to 5m in 12 months and the yield recorded in India was up to 75 t ha$^{-1}$ (10% moisture) in 1 year (Anonymous, 1983).

Work on the growth and nitrogen fixation of S. sesban in alkali soils is lacking. An initial report (Kumar, 1983) showed that S. aegyptiaca (syn. S.sesban) could tolerate pH 9.9 at germination and pH 9.5 at later growth stages in pot experiments. In the present studies, S. sesban was screened for its tolerance to salinity and alkali at germination in laboratory studies and for its use for foliage and fuel wood production in field experiments on an alkali site. A comparative evaluation of nodulation and nitrogen fixation by eight strains of S. sesban is also reported.

## MATERIAL AND METHODS

### Germplasm

Eight varieties of S. sesban wee collected from different sources in India and from the United States Department of Agriculture. Before germination, the seeds needed to be soaked in water overnight or scarified in concentrated sulfuric acid for different periods (Table 1).

### Nodulation and nitrogen fixation by S. sesbans

Surface soil of the CSSRI Farm (Sandy loam, $pH_2$ 9.2, ESP 32, EC 0.3 mmhos $cm^{-1}$, organic C 0.4%, total N 0.04%, available N 90.0 kg $ha^{-1}$ was used for a pot experiment to assess nodulation and N fixation by S. sesban. Before sowing, an effective strain of the Rhizobium sp. for S. sesban was inoculated on the seeds using standard techniques (Vincent, 1970). Uninoculated seeds served as a control. Plants were uprooted at 30 days to score for nodulation, biomass, and nitrogen acumulation in the shoots. Rhizobium sp. isolated from the nodules was used for antibiotic marker pattern studies and the native and the inoculated Rhizobium sp. were compared.

### Salinity and alkalinity tolerance at germination

For studying the effect of salinity, the seeds were allowed to germinate on filter papers kept continuously soaked in solutions of four salinity levels (EC 5, 11, 15 and 18 mmhos $cm^{-1}$). The salinized solutions were prepared with NaCl, $Na_2SO_4$ and $CaCl_2$ keeping a Na:Ca ration of 4:1 and Cl : $SO_4$ ratio of 4:1. Distilled water (EC 0.02 mmhos $cm^{-1}$) was included as a control. For checking the effect of alkalinity, $Na_2CO_3$ solution of pH 8.0, 9.0, 10.0 and 11.0 were used.

### Green manuring potential and biomass accumulation (Short-term study)

To evaluate the potential of S. sesban CSSRI to supply nutrients through green manure and to study biomass production in short periods of growth in the summer and in the rainy season, a field experiment was set up in the summer of 1985. The soil was sandy loam, pH 8.5, ESP 15, EC 0.2 mmhos $cm^{-1}$, organic C 0.5%, total N 0.06% and available N 55kg $ha^{-1}$. S. sesban was sown at four seed rates ( 5, 10, 15 and 20 kg/ha) by broadcasting the seeds in thin standing water in 6 x 6m plots. The treatments were replicated four times in a randomized block design. In one replication plants were harvested at 100 days and N, P, K and biomass accumulation through leaves and stem estimated. At 200 days the plants from another replication were harvested and nutrient and biomass accumulation in leaves and stem were evaluated. In calculations of green-manuring potential only the leaves and the tender 'top' portions of the stems were considered.

The experiment started on 5 May 1985 and terminated on 20 November, 1985. The mean maximum and minimum temperatures during the first 100 days growth ranged from 32.7 to 40.7°C and 23.2 to 27.0°C respectively. The mean maximum ranged from 27.0 to 32.2°C and the mean minimum from 10.7 to 23.0°C during the last 100 days of growth. The crop was irrigated as and when there were symptoms of stress.

### Biomass accumulation (Long term study)

Seeds of S. sesban CSSRI were sown in August 1982 by dibbling at a distance of 1m, maintaining 3m distance between the rows over an area of one hectare. Biomass accumulation in 3 years was recorded.

## RESULTS AND DISCUSSION

### Nodulation and nitrogen fixation by S. sesbans

There was a range of variation among the strains in the nodule no., nodule biomass, plant dry weight and shoot N content at 30 days (Table 1).

TABLE 1: Nodulation and N fixation by S. sesbans at 30 days growth stage in an alkali soil (pH 9.2, ESP 32).

| Acq. No. | Source and origin | Germination treatment | Nodulation No.plant | Nodulation Dry wt. (mg) of nodules | Biomass g $plant^{-1}$ | Shoot %N |
|---|---|---|---|---|---|---|
| 05 | MPKV, India | W | 17 | 53 | 2.7 | 3.7 |
| 06 | GAU, India | W | 18 | 110 | 1.6 | 3.2 |
| 07 | CSSRI, India | $A_{20}$ | 17 | 68 | 1.1 | 2.7 |
| 09 | IGFRI, India | $A_{15}$ | 45 | 125 | 0.7 | 3.4 |
| 31 | USDA 215608, India. | $A_{45}$ | 71 | 116 | 3.1 | 4.0 |
| 32 | USDA 228236, India. | W | 63 | 124 | 3.0 | 2.4 |
| 33 | USDA 233551 Okinawa. | $A_{15}$ | 37 | 75 | 2.6 | 4.4 |
| 34 | USDA 279601, China | $A_5$ | 21 | 74 | 3.9 | 4.0 |
| Mean | | | 36 | 92 | 2.3 | 3.5 |
| S.D. | | | 20 | 28 | 1.0 | 0.6 |

a

W = germinated by soaking in water; $A_5$, $A_{15}$, $A_{20}$, $A_{45}$ = germinated by soaking in concentrated sulphuric acid for 5, 15, 20, 45 min. respectively.

(Modified from Ghai, Rao and Batra, 1985 a).

On average, dry matter accumulation was 2.3g per plant and N content 3.5%. There were, on the average, 36 nodules/plant weighing 92 mg dry weight. In all the strains, nodulation was profuse even in the uninoculated controls and the nodules were mostly confined to the tap root. The intense pink colour of the nodules suggested their active nitrogen fixing ability. The rhizobia corresponding to S. sesbans were thus already present in abundance in tha alkali soil. In the competition studies based on antibiotic marker patterns, it was found that the native strains competed actively and prevented nodulation by effective strains of the inoculated Rhizobium sp.

## Salinity and alkali tolerance at germination

Response to different levels of salinity at germination was studied up to 10 days. There was no effect on germination up to EC 11 mmhos $cm^{-1}$ (0.7 % salt concentration) beyond which there was a drastic reduction (Table 2) at EC 15 (1% salt) and EC 18 (1.2% salt).

TABLE 2: Effect of salinity and alkalinity on germination of S. sesban

| Salinity (mmhos $cm^{-1}$) | Days 1 | 5 | 10 | Alkalinity (pH) | Days 1 | 4 | 7 |
|---|---|---|---|---|---|---|---|
| | % germination | | | | % germination | | |
| Control | 78.0 | 100 | 100 | Control | 47.5 | 84.2 | 84.2 |
| 5 | 40.2 | 94.7 | 100 | 8.0 | 52.6 | 88.2 | 91.1 |
| 11 | 3.9 | 68.6 | 97.5 | 9.0 | 60.5 | 99.0 | 99.0 |
| 15 | 1.2 | 27.3 | 67.4 | 10.0 | 48.6 | 92.2 | 92.2 |
| 18 | 0 | 7.8 | 24.6 | 11.0 | 7.9 | 39.6 | 60.5 |

(modified from Ghai, Rao and Batra, 1985 b).

This agrees well with Jen et al. (1965) who reported that S. aegyptiaca (Syn. S. sesban) tolerated a salt concentration of 0.4-1.0% at seedling stage. S. sesban was subjected to alkalinity stress up to 7 days. Increase in alkalinity up to pH 9.0 had a beneficial effect. Maximum germination took place at pH 9.0, however it could tolerate pH 10.0. Beyond this, there was a decreased as well as delayed germination. Kumar (1983) showed that germination of S. aegyptiaca is unaffected up to a soil pH of 9.4 beyond which there was reduction. The plants could tolerate even pH 9.9, showing 72% germination as compared to germination in a normal soil of pH 7.6.

Successful establishment of a seed-propagated tree species in salt-affected soils depends on its capacity to initiate and complete the germination process since this phase is often relatively more sensitive, and often more decisive than subsequent growth stages. The results of the present study and that of the earlier ones show that S. sesban germinates quickly, has a high survival rate and is quite tolerant to salinity and alkalinity.

## Green-manure potential and biomass accumulation (Short-term study)

At 100 days growth stage, 10 kg seed rate $ha^{-1}$ was optimal as it resulted in maximum height (2.3 m) and girth (14 mm) of the plants and produced maximum biomass (oven-dry), 5.2 t $ha^{-1}$ (Table 3). The total N, P and K and S accumulation was 84, 7.2, 7.5 and 17.2 kg/ha of which the green manure potential was 68 kg N, 4.9 kg P and 53 kg K and 11.4 kg S, respectively per hectare (Table 4).

TABLE 3: Biomass accumulation in S. sesban (100 days growth)

| Seed rate (kg/ha) | Height (m) | Girth (mm) | BIOMASS $t.ha^{-1}$ Stem Upper | Stem Lower | Leaf | Total |
|---|---|---|---|---|---|---|
| 5 | 1.8 | 12 | 0.6 | 1.2 | 0.6 | 2.4 |
| 10 | 2.3 | 14 | 1.4 | 2.5 | 1.3 | 5.2 |
| 15 | 2.1 | 11 | 1.2 | 2.1 | 1.0 | 4.3 |
| 20 | 1.8 | 9 | 1.3 | 2.4 | 1.1 | 4.8 |

TABLE 4: Nutrient accumulation (kg/ha) in S. sesban at 100 day growth.

| Seed rate (kg/ha) | Leaf | | | | Stem (Upper) | | | | Stem (Lower) | | | | Total | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | P | K | S | N | P | K | S | N | P | K | S | N | P | K | S | |
| 5 | 25 | 1.6 | 15 | 3.5 | 6 | 0.7 | 9 | 1.7 | 8 | 1.1 | 10 | 2.8 | 39 | 3.4 | 34 | 8.0 | 84. |
| 10 | 55 | 3.4 | 33 | 7.5 | 13 | 1.5 | 20 | 3.9 | 17 | 2.3 | 22 | 5.8 | 85 | 7.2 | 75 | 17.2 | 184. |
| 15 | 42 | 2.6 | 25 | 5.8 | 11 | 1.3 | 18 | 3.4 | 14 | 1.9 | 18 | 4.8 | 67 | 5.8 | 61 | 14.0 | 147. |
| 20 | 46 | 2.9 | 28 | 6.4 | 12 | 1.4 | 19 | 3.6 | 16 | 2.2 | 21 | 5.5 | 74 | 6.5 | 68 | 15.5 | 164. |

At 200 days the plants grew to an average height of 3.2m, girth 17mm and produced a biomass (oven-dry) of 12.7 t $ha^{-1}$ of which the stem contributed 12.1 (Top 2.6, middle 3.7, bottom 5.8) and leaves 0.6 t $ha^{-1}$. The differences among the various seed rates were non-significant. The total N, P, K and S accumulation at 200 days was 120, 9.2, 117 and 17.7 kg $ha^{-1}$ of which the green-manure potential was 59 kg N, 4.1 kg P, 38 kg K and 7.9 Kg S $ha^{-1}$ (Table 5).

TABLE 5: Nutrient accumulation (kg $ha^{-1}$) in S. sesban at 200 days growth (average of seed rates)

| Plant Part | N | P | K | S | Total |
|---|---|---|---|---|---|
| Leaf | 32 | 1.8 | 10 | 4.0 | 47.8 |
| Stem | | | | | |
| Top | 27 | 2.3 | 28 | 3.9 | 61.2 |
| Middle | 27 | 2.1 | 33 | 5.2 | 67.3 |
| Bottom | 34 | 3.0 | 46 | 4.6 | 87.6 |
| Total | 120 | 9.2 | 117 | 17.7 | 263.9 |

**Biomass accumulation (Long-term study)**

Sesbania sesban grew to an average height of 7 m (range, 5.2 m to 8.2 m) and girth of 58 mm (range, 32-80 mm) in the alkali soil. Biomass production (air-dry) was 87.0 t $ha^{-1}$. Height and girth increments measured during the experiment showed that there was no appreciable growth after two years.

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## 3. NITROGEN FIXATION IN CASUARINACEAE

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### SUMMARY

Some 29 species of Casuarinaceae are now known to nodulate. Although species of Casuarina were usually well nodulated in the field, nodulation of Allocasuarina was variable and often absent. Frankia sources from Casuarina usually nodulated other Casuarina species; similarly cross-inoculation was frequent with Allocasuarina but there was litle cross-inoculation between genera. Large differences in effectiveness of Frankia-Casuarina combinations occurred - some Frankia highly effective on C. equisetifolia were ineffective on C. cunnninghamiana and vice versa. Inoculation of each species of Casuarina with selected Frankia strains is necessary for effective nitrogen fixation.

Soil temperature affected growth of C cunninghamiana differently when dependent on the symbiosis than when given fertilizer nitrogen. Nitrogen fixation did not occur below 20°C. Soil phosphate status was important in determining distribution of Allocasuarina and Casuarina and in nodulation and nitrogen fixation. Interactions with mycorrhizal fungi in uptake of phosphate (and other nutrients necessary for plant growth or nitorgen fixation processes) are discussed.

Frankia sources differed in their tolerance of high soil salinity. Selection of Frankia for highly saline soils is likely to be important to Casuarina growth in such soils.

Future directions for nitrogen fixation research and management are discussed.

### INTRODUCTION

The symbiotic fixation of atmospheric nitrogen by Frankia (an actinomycete) in root nodules formed on Casuarinaceae is a key factor in the successful growth of these trees and shrubs in a wide variety of nitrogen deficient soils in warm temperate and tropical climates. With fast growing species such as Casuarina equisetifolia*, Dommergues (1963) calculated at least 58 kg N $ha^{-1}$ $yr^{-1}$ were fixed and it is apparent both from this and from laboratory studies that fixation rates can approach those of effectively nodulated legumes. If members of the Casuarinaceae are to achieve their full potential in production of fuelwood, in soil conservation, in soil rehabilitation, and in bestowing productivity on saline soils, it is important to understand the biological and environmental factors affecting the symbiosis. This paper addresses some of these.

## BIOLOGICIAL FACTORS

### Occurrence of Nodulation

Some 29 of the 68 species of Casuarinaceae have now been recorded as nodulating (Bond, 1976; Lawrie, 1982; Reddell, Bowen and Robson, 1986). It is highly probable that some do not nodulate but as the absence of nodulation in the field may reflect only the absence of an appropriate Frankia, or soil conditions unsuitable for nodulation of the particular species, dogmatic conclusions on non-nodulation cannot be drawn. Certainly, there are large differences in the ease and the extent of nodulation between genera and species in the Casuarinaceae (Lawrie, 1982; Reddell et al, 1986). In a study of 187 Australian sites, embracing 22 species of Casuarinaceae, Reddell et al, (1986) found most plants of the four Casuarina species examined were nodulated at the 60 out of 88 sites. However, with only 5 of the 17 species of Allocasuarina examined (120 sites) did nodulation occur in more than 50 % of the sites - nodulation was not observed with 8 species (2 to 9 sites per species). Furthermore, in no species of Allocasuarina was more than 40% of the individual plants nodulated (see also Lawrie, 1982.)

Allocasuarina was particularly frequent in soils with low available phosphorus; 70% of all sites carrying Allocasuarina has less than 5 ppm available phosphorus, while only 42% of Casuarina sites were in this category. Subsequent glasshouse studies indicated that two of the major factors determining field nodulation were the presence/absence of appropriate Frankia and soil phosphate status, although other factors such as low soil pH undoubtedly are important also (Bond, 1957; Coyne, 1973).

Why is the variation in frequency of nodulation Allocasuarina, (both between sites and within sites) so marked? Perhaps Allocasuarina species consist of mixed populations of individuals that are susceptible and resistant to Frankia infection (a hypothesis which could be tested via vegatative propogation from individual plants) or perhaps nodulation of this genus may be particularly sensitive to small variations in site conditions. Because of this frequently poor nodulation it should not be assumed that the contribution of casuarinas to the nitrogen economy of a natural ecosystem is always large.

### Specificity in Nodulation

Very few pure cultures of infective Frankia have been isolated from Casuarina nodules so far, and inocula used in studies on specificity have necessarily been a suspension from a crushed nodule (usually raised from a single nodule on plants in sterile soil). As nodules may sometimes contain more than one strain of Frankia (Reddell and Bowen, 1985, a), results from such studies on specificity will need to be confirmed by later studies using a range of pure cultures of Frankia. There is no evidence that Frankia which form nodules on other actinorhizal plants (eg. Alnus, Myrica) also nodulate Casuarina. Some specificity in nodulation within the Casuarinaceae was indicated by Coyne (1973). Reddell and Bowen (1985, b) using nodules from 25 species of Casuarina and 11 species of Allocasuarina to inoculate 8 and 6 species of Casuarina and Allocasuarina respectively, found very high cross-inoculation within Casuarina and frequent (but less) cross inoculation within Allocasuarina but little cross inoculation between these genera. Some Frankia had wider host ranges than others, nodulating outside the genus or series of origin; in particular, 3 sources from A. torulosa provided 7 of the 10 instances in which Allocasuarina sources formed nodules on Casuarina, and thus may be a bridging species. With Casuarina there appeared to be no barriers between series, and while this was probably the case also with Allocasuarina, the data were too few to be certain.

## Effectiveness

To examine whether different Frankia-Casuarina combinations differed in effectiveness, Reddell and Bowen (1985, c) inoculated C.equisetifolia ssp. incana and C.cunninghamiana, (both of which are important species in a number of developing countries) with five different sources of Frankia. This glasshouse study showed striking differences between Frankia-Casuarina combinations in their effectiveness (Table 1).

Although strain cb nodulated C.equisetifolia profusely it was completely ineffective but was easily the most effective of this species on C.cunninghamiana; strains kb, and ca, although highly effective on C.equisetifolia either did not nodulate C.cunninghamiana (kb) or were poorly effective (ca).

TABLE 1. The effects of 5 Frankia sources on growth, nodule formation and nodule activity with C. equisetifolia and C.cunninghamiana; 240 days after sowing (Reddell and Bowen),

| Inoculum Source* | Plant Species+ | Shoot Dry Wt (g) | No. Nodule per Plant | Nodule d.wt per plant (mg) | N fixed $g^{-1}$ nodule |
|---|---|---|---|---|---|
| kb | EQU | 1.55 | 49 | 117 | 0.38 |
| | CUN | 0.00 | 0 | 0 | - |
| ca | EQU | 1.39 | 94 | 146 | 0.23 |
| | CUN | 0.70 | 41 | 104 | 0.14 |
| hw | EQU | 0.19 | 73 | 63 | 0.06 |
| | CUN | 0.07 | 0 | 0 | - |
| ch | EQU | 0.38 | 108 | 98 | 0.9 |
| | CUN | 0.08 | 0 | 0 | - |
| cb | EQU | 0.11 | 66 | 22 | 0.03 |
| | CUN | 2.27 | 19 | 195 | 10.03 |
| Uninoculated | EQU | 0.10 | 0 | 0 | |
| | CUN | 0.11 | 0 | 0 | |

* Sources kb, ca, hw, were from C.equisetifolia, ch was from C.timorensis cb, was from C.cunninghamiana

\+ EQU = C.equisetifolia, CUN = C.cunninghamiana

These results are of great applied significance:

(i) they indicate that Frankia strains must be selected to match the particular species one wishes to introduce, in order to maximise tree growth. Perhaps some failures with casuarinas are due to the lack of inoculation with a suitable Frankia - we have observed instances of this. At present, a Frankia selection programme with 10 species of casuarinas is in progress in our laboratory. It may be that effectiveness groupings will emerge ie. clusters of species for which the one Frankia may be effective on all.

(ii) they raise the possibility that in some places where casuarinas have been introduced (successfully), the existing Frankia strains may be of only moderate effectiveness. In such cases problems may occur with the introduction of more highly effective strains. One of our programmes (sponsored by the Australian Cente for International Agricultural Research in collaboration with the Royal Thai Forest Department), is to examine the relative effectiveness of Frankia nodulating C.junghuhniana in the many locations in which this species has been planted in Thailand.

(iii) In many natural Australian woodland ecosystems, productivity is determined largely by soil nitrogen (and soil phosphorus) status. In many cases the major accession of nitrogen is from casuarinas and it is likely that the productivity of the ecosystem (and the rate of successional development) will be determined largely by the effectiveness of the indigenous Frankia strains.

In the study above, all sources of Frankia formed nodules at similar times and differences in effectiveness were not due to this. Table 1 shows that "effectiveness" was a function both of total nodule weight and of nitrogen fixed $g^{-1}$ nodule. With the ineffective cb - C.equisetifolia combination, high numbers of small nodules and low fixation efficiency occurred; with the cb - C.cunninghamiana combination, low numbers of large nodules with high fixation rates occurred; other pairings showed a mixture of these properties. The nature of high/low fixation $g^{-1}$ nodule remains to be studied - is low fixation efficiency due to a low fixing activity per Frankia cell in the nodule or is it merely that there are relatively few Frankia cells in the nodule?

## ENVIRONMENTAL FACTORS

Many edaphic and climatic factors can affect nitrogen fixation, either directly by their effects on establishment of the infection, the various stages leading to nitrogen fixation, and the physiology of symbiosis or indirectly by effects on plant growth and assimilates available to the nodule. Below, we give three examples of environmental effects on nitrogen fixation.

### Soil Temperature

Table 2 (from Reddell, Bown and Robson, 1985) shows the effect of differing soil temperatures on C.cunninghamiana growth when dependent on nitrogen fixation and when supplied with nitrogenous fertilizer ("applied N"), and on nodulation and nitrogen fixed $g^{-1}$ nodule.

(i) Reasonable growth occurred at 15°C soil temperature when plants had N applied, but when dependent on N-fixation, no response occurred until

20°C. Furthermore, the optimum soil temperature for growth with applied N was 20°C, but with the symbiotic system it was 25°C. These data indicate that the physiology of the interacting soil/micro-organism/plant system is markedly different in many respects from the "simple" soil/plant system. In many soils, casuarinas are dependent almost entirely on nitrogen fixation, and factors such as soil temperatures at times when soil moisture is adequate for plant growth must be considered in site/casuarina species/Frankia selection. Reddell et al. (1985) suggested that the sensitivity of the symbiotic system to soil temperatures below 20°C may define the limits to the natural distribution of C.cunninghamiana and other Casuarina species. It will be necessary to perform screening programmes to select highly effective Casuarina species-Frankia combinations at soil temperatures relevant to the areas to be planted.

TABLE 2: Effect of soil temperature on shoot and nodule dry weights/of Casuarina cunninghamniana, and N fixed $g^{-1}$ nodule Means ±S.E. mean (Reddell, Bowen and Robson, 1985) 148 days.

| N Treatment | Soil temp (°C) | Shoot D.Wt g/plant | Nodule D.Wt mg/plant | g N $g^{-1}$ nodule |
|---|---|---|---|---|
| Nil | 15-30 | 0.05±0.01 | 0 | |
| $NH_4NO_3$ | 15 | 0.97±0.10 | 0 | |
| | 20 | 2.13±0.67 | 0 | |
| | 25 | 1.27±0.70 | 0 | |
| | 30 | 1.15±0.24 | 0 | |
| Frankia cb | 15 | 0.04±0.01 | 3±1 | - |
| | 20 | 1.57±0.27 | 181±34 | 0.35±0.10 |
| | 25 | 2.57±0.18 | 307±75 | 0.30±0.06 |
| | 30 | 0.80±0.13 | 65±8 | 0.37±0.02 |
| Frankia tn | 15 | 0.03±0.01 | 1±0.4 | - |
| | 20 | 0.34±0.13 | 34±9 | 0.11±0.01 |
| | 25 | 1.33±0.23 | 133±27 | 0.35±0.11 |
| | 30 | 0.81±0.15 | 75±26 | 0.33±0.05 |

(ii) Short-term studies on nitrogen fixation by excised Casuarina nodules have demonstrated an optimum temperature of 35°C (Waughman, 1977) for nitrogen fixation. The studies above (with a different species) indicating a 25°C optimum with the whole plant system, suggests a danger in extrapolating from studies on excised nodules to whole plants and to field situations. The main determinant of the optium soil temperatues and other "climatic factors" for fixation may be via effects on the plant assimilation of $CO_2$ and its translocation to the root.

(iii) The effects of soil temperature on fixation (Table 2) were largely on nodule development (which differed with Frankia source) and not on the fixation process itself, although with Frankia source tn the nitrogen fixation $g^{-1}$ nodule was also depressed at 20°C. Although some nodules formed with both strains at 15°C, these were small and fixed little, if any, nitrogen.

**Soil Nutrients**

As for legume nitrogen fixation, some elements eg. molybdenum may be needed uniquely or in relatively greater supply for nitrogen fixation than for plant growth and assimilation. The resolution of plant v. nitrogen fixation requirements has been discussed by Robson (1983) in relation to legumes. Table 3 from Diem and Gauthier (1982) indicates the importance of mycorrhizal infection. Bond and Hewitt (1962, 1967) have shown the necessity of Co and Cu for the fixation process in Casuarina. Mo is probably also necessary.

TABLE 3: Inoculation of seedlings of C.equisetifolia with Frankia nodules and/or an endomycorrhizal fungus (Glomus mosseae) (Diem and Gauthier, 1982) (6 months)

| Treatments | Nodule Dry wt mg/plant | PLANT Dry wt g/plant | PLANT Total N mg/plant | PLANT Total P mg/plant |
|---|---|---|---|---|
| Control | $0^a$ | $2.69^a$ | $22.0^a$ | 2.6 |
| + Frankia | $57^b$ | $4.23^b$ | $50.7^b$ | $2.1^b$ |
| + Frankia + mycorrhiza | $132^c$ | $7.69^c$ | $96.1^c$ | $3.9^c$ |
| + Frankia + Phosphate | $107^c$ | $7.49^c$ | $120.5^c$ | $3.0^b$ |

Values in columns having the same letter superscripts differ with probability = 0.01 (Duncan test)

Soil phosphate status has a central role in distribution of casuarinas - Reddell et al (1986) found Casuarina occured much more frequently than Allocasuarina on sites with more than 10 ppm bicarbonate available phosphate (see above). Frequently, species of Casuarina are much faster in growth than Allocasuarina and it is apparent that small additions of phosphate to a soil may allow the successful introduction of much faster growing species.

**Mycorrhizas and cluster roots** Mycorrhizal infections of roots (which occur with most of the higher plants) and microbial stimulation of root branching on a few plant families ('cluster' or 'proteioid' roots, Lamont, 1982) (Malajczuk and Bowen, 1974) are particularly important in increasing the absorption of poorly mobile ions from soil.

Although mycorrhizas may increase the uptake of many such ions eg. Zn, K, Cu, Mo (see Bowen, 1980), phosphate uptake has received particular attention, and indeed occupies a special place, not only because phosphorus and nitrogen are the most frequently deficient nutrients in soils but also because high phosphate levels depress mycorrhiza formation. Certain root characteristics such as an abundance of fine roots with well developed root hairs (eg. with gramineous plants) assist in effective uptake of poorly mobile ions from soil, but root microbial associations have almost always been involved also in the evolution of root systems to cope with nutrient deficient soils. Mycorrhizas are also important in competition between species for nutrients and have probably been important in the evolution of mixed ecosystems (Bowen, 1980).

In 83 Australian soils examined by Reddell et al (1986), casuarinas at only 14 sites had neither mycorrhizas nor cluster roots. Cluster roots, ectomycorrhizas and vesicular-arbuscular mycorrhizas (V.A.M.) (the two major mycorrhiza types, encompassing the vast majority of higher plants) occurred both on Casuarina and Allocasuarina, frequently on the same plant. Cluster roots were relatively uncommon. Ectomycorrhizas were most frequent with Allocasuarina and V.A.M. dominated somewhat in Casuarina. Table 3 indicates the importance of mycorrhizas to phosphate uptake and thence to nitrogen fixation - it is little wonder that casuarinas are pioneer plants par excellence. Little is known, so far, about the specific mycorrhizal requirements for casuarinas.

The technology of inoculation with ectomycorrhizal fungi has now been worked out (see Theodorou, 1971; Marx et al, 1982). V.A.M. cannot yet be grown in laboratory media and the logistics of large scale inoculation with direct sowing of agricultural crops, are daunting. However, inoculation of forest nurseries with selected V.A. fungi should be practicable. There are exciting possibilities ahead.

Many other soil "chemical" factors will probably affect nodulation and nitrogen fixation eg soil pH, (Coyne, 1973) and levels of soil nitrogen. Such factors have yet to be studied in detail. It is probable that there will often be mycorrhizal interactions in those factors - Bowen (1980, 1985) pointed out that growth of mycorrhizal fungi into soil should be regarded as a general alternative root strategy, and that such growth by selected mycorrhizal fungi will often compensate for poor root growth in deleterious soil situations, eg high soil acidity, high aluminium and highly saline soils and where root disease organisms occur. That is, the plant scientist has two genomes to manipulate, not just one.

## Soil Salinity

Some casuarinas grow in extreme soil situations, one of which is high soil salinity. For example, Casuarina obesa occurs naturally (and is nodulated) in soils with up to 28 mg $Cl^{-}$ $g^{-1}$ soil (28,000 ppm). Several other species also grow in saline soils or in sites where they would be subject to considerable cyclic salt eg C. glauca, C.equisetifolia.

Reddell, Foster and Bowen (1986) examined the effect of salinity on nodule growth and nitrogen fixation with two Frankia sources. Table 4 shows a quite different effect of increasing salinity on fixation by these two Frankia: the less effective strain (cb) had greatly impaired nitrogen fixation beyond 0.15 mg NaCl $g^{-1}$ soil but strain ys was unaffected at 10 times this concentration. Decreased nitrogen fixation occurred via an effect on nodule growth (both strains) and via fixation $g^{-1}$ nodule. (cb only). Electron microscopy showed that with strain ys, nodule cells containing Frankia has very little chloride accumulation, and it is possible that with some Frankia-Casuarina combinations, physiological/anatomical changes occur in the cell wall/plasmalemma which exclude chloride. In a parallel (unreported) study uninoculated C.obesa supplied only with fertilizer N grew only in much lower salt concentrations that those infected with Frankia ys; this needs further study.

TABLE 4: The effect of increasing soil NaCl concentration on N content in shoots, nodule dry weight and $N_2$ fixation in Casuarina obesa seedlings inoculated with two sources of Frankia (± SE) 24 weeks after inoculation, 16 weeks after the commencement of salinity treatments. (Reddell, Foster and bowen, 1986).

| Frankia source | NaCl added mg NaCl g $soil^{-1}$ | Nitrogen content of shoots | Dry weight of nodules mg $pot^{-1}$ | mg shoot per mg nodule mg $pot^{-1}$ |
|---|---|---|---|---|
| ys | 0 | 126±5 | 550±23 | 0.23±0.01 |
| | 0.15 | 190±4 | 562±48 | 0.35±0.02 |
| | 1.5 | 109±32 | 292±55 | 0.37±0.04 |
| | 15 | 17±3 | 57±14 | 0.30±0.03 |
| cb | 0 | 30±5 | 203±48 | 0.15±0.03 |
| | 0.15 | 32±6 | 176±62 | 0.18±0.05 |
| | 1.5 | 8±3 | 123±37 | 0.07±0.02 |
| | 15 | 1 | 25±2 | - |

It is clear that selection of salt tolerant Frankia strains will have an important bearing on the use of casuarinas to bestow productivity on highly saline soils. Such soils could well be important fuelwood producing sites for many developing countries.

## FUTURE DIRECTIONS

We have indicated above some of the plant/Frankia factors and environmental factors which affect nitrogen fixation and play a critical role in the successful growth of casuarinas. Below, we comment briefly on some future research needs in the management of nitrogen fixation by casuarinas.

We have demonstrated specificity both in nodule formation and in effectiveness and therefore the need for Frankia selection and inoculation programmes. Both of these activities will be assisted enormously by the development of methods for the easy isolation and growth of Frankia nodulating the Casuarinaceae. At present this is difficult, not impossible (eg Diem, Gauthier and Dommergues, 1982; Zhang, Lopez and Torrey, 1984). Inoculation technology, to date, has used crushed nodules as inoculum; this will change drastically in the next few years. With isolation and cultivation of Frankia from casuarinas, not only will highly efficient inoculum production and application technologies be developed, but also sound research on Frankia strain identification and Frankia ecology will be possible.

Environmental effects on fixation have obvious important consequences. It is appropriate to further examine soil factors such as those above, in order to appreciate the critical steps being affected. In order to manage N fixation more effectively, either by Frankia selection or site management, good methods must be developed for studying the effect of environment on growth of Frankia in the rhizosphere, the infection process, nodule genesis, nodule development and nitrogen fixation processes. Nodulation/nitrogen fixation can fail at any of these.

The measurement of nitrogen fixation in the field, and questions such as the rate of nitrogen availability to associated plants are further areas requiring study. "Traditional" methods used in glasshouse studies: plant growth responses in closed pots, the use of $^{15}N$ gas or $^{15}N$ isotope dilution methods, and acetylene reduction assay of nitrogenase activity have serious shortcomings in field conditions, partly because of losses from open systems, partly because of logistic and sampling problems associated with perennials ($^{15}N$ dilution methods) and partly because such tests such as acetylene reduction give only one point in time (even if one could overcome problems of acetylene introduction into soil). Even with its faults (ie losses from the system), the best method so far has been the increase of nitrogen in the soil and the plant biomass at a field site (see Gauthier et al, 1978). "Newer" approaches to measurement of nitrogen fixation eg the use of $^{15}N$ natural abundance (Rennie, Paul and Johns, 1976) have a particular attraction with perennials but this awaits critical examination.

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# 4. A FRANKIA SOURCE SELECTION PROGRAMME TO IMPROVE NITROGEN FIXATION BY CASUARINACEAE

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## SUMMARY

1. A Frankia source selection program for select species of Casuarinaceae is outlined at both a glasshouse and field level.

2. Preliminary field results show a 30% increase in plant height due to inoculation at 16 months for one site and a negative response to inoculation at a second site.

3. The direction of future research is outlined.

## INTRODUCTION

Various species within the family Casuarinaceae have potential for colonizing and stabilizing adverse soils. For example Casuarina equisetfolia commonly occurs on coastal sand dunes where it tolerates salt spray, infertile soils and drought. C.obesa has been observed to colonize highly saline soils whilst C.cristata grows well in extremely arid areas with as little as 200 mm annual rainfall.

Some 29 species within the family Casuarinaceae are known to be capable of entering into a symbiotic relationship with the actinomycete, Frankia. The symbiosis results in nitrogen being fixed in a similar manner to Legume-Rhizobium symbioses and in many instances the symbiosis is thought to be an important factor contributing to the successful establishment and growth of Casuarinas on adverse sites. However there is an urgent need to learn more about the symbiosis so that it can be successfully utilized and managed and so that the improvements to be gained by soils such as improved soil nitrogen and decreased soil erosion can be maximised.

Currently CSIRO, Division of Soils has a Frankia strain selection programme for 10 species of Casuarinaceae. The need for the programme arose from work by Reddell and Bowen (see 3) which showed that different crushed nodule sources varied in their ability to nodulate and fix nitrogen on two species of Casuarina. Further, a nodule source effective at fixing nitrogen on one species, could be quite ineffective on another species. Thus to optimize nitrogen fixation by Casuarina, it is necessary to select sources of Frankia effective at fixing nitrogen for the species in question.

## FRANKIA STRAIN SELECTION

(1) Glasshouse and Laboratory Programme

The species included in the programme are listed in Table 1 along with data on their climatic range. The Frankia sources being screened include nodules from different species, climatic regions, and soils, and seven isolates.

TABLE 1: The 10 species being studied plus altitude and climatic data

| Species | Alt (m) | | Climatic zones* | Frosts /yr | Rainfall 50 percentiles | Season[A] |
|---|---|---|---|---|---|---|
| C.cristata subsp. cristata+ | 175-325 | | Wsa<br>Wsh | 2-50 | 550-650 | U-S |
| C.cunninghamiana+ | 0-1000 | | Wsh<br>Wsa | 0-50 | 500-1500 | S |
| C.equisetifolia var.+ equisetifolia | 0-100 | | Hh<br>Hsh<br>Wsh | 0 | 1000-2150 | S |
| C.equisetifolia var. incana+ | 0-100 | | Wsh | 0-3 | 100-1500 | S |
| C.glauca+ | 0-30 | | Wh<br>Wsh | 0-5 | 900-1150 | S |
| C.junghuhniana° | 0-3000 | m | Hh | | | S |
| C.obesa+ | 0-300 | | Wsh<br>Wsa | 0-12 | 250-500 | W |
| C.oligodon° | -2,500 | m | Hh | | | |
| Allocasuarina decaisneana+ | 250-700 | | A | 1-12 | 200-250 | U-S |
| A.torulosa+ | 0-1100 | | Wsh<br>Wh | 0-20 | 950-1250 | S |

* A, arid; Wsa, Warm semi-arid; Wh, Warm humid; Wsh, warm sub-humid, Hh, Hot humid; Hsh, Hot sub-humid

A S, summer; W, winter; U, uniform

\+ Climatic data from Doran, J.C. and Hall, N. (1983)

° Climatic data from National Research Council (1984).

The screening process commences in the glasshouse in a closed pot system in which plants are supplied with all essential elements except nitrogen. The questions addressed by the glasshouse trials are:

1. Which of the species of Casuarinaceae being studied are the Frankia sources capable of nodulating?

2. What is the relative effectiveness of the Frankia sources at fixing nitrogen when associated symbiotically with each species?

For most species, nodules are visible three to eight weeks after inoculation. Visible differences in plant growth as a result of different Frankia sources are evident by five months after inoculation.

In terms of growing up inoculum for large scale field work, crushed nodule inocula have many limitations, including extremely long time periods to grow up the quantity of nodules needed, and the lack of a method for assessing the viability of nodules. As a result, emphasis is being placed on isolation of Frankia from effective nodule sources. The first recorded isolation of a Frankia strain which was capable of reinfecting a species of Casuarina was that of Diem et al. (1983). Since then, further reinfective isolates have been reported from species of Casuarina (Zhang et a., 1984) and Allocasuarina (Zhang and Torrey, 1985). However, the total number of reinfective isolates is small. Work is currently underway to develop a routine procedure for isolation and thereby increase the number of Frankia isolates available.

(2) Field Program

The field program aims to study the effect of inoculation and different Frankia sources on growth and nitrogen fixation by selected species of Casuarinaceae over a longer time period. Plants are raised in the nursery in tubes filled with a peat/vermiculite mix and fertilized with all essential elements except nitrogen. Inoculation takes place either when the seedlings are transplanted into tubes, or, immediately, prior to field planting, and involves watering on either a crushed nodule suspension or an isolate grown in liquid medium. Seedlings are planted into the field approximately six months after emergence.

Preliminary results are available for two field trials. The first trial involves C.cunninghamiana growing at a temperate site which is waterlogged during winter. At 16 months after planting into the field a 30% increase in tree height resulted from inoculation. Wood volume was calculated by using the formula for a cone and entering basal diameter and height measures. The 30% increase in height at 16 months was equivalent to a 180% increase in wood volume.

The second site is situated in the subtropics and consists of C.cunninghamiana supplied with three nitrogen sources (uninoculated, no applied nitrogen; uninoculated, applied nitrogen), each in the presence and absence of applied phosphorus. Plant height measures show that at 12 months after planting into the field, no response to nitrogen was obtained unless phosphorus was added. More importantly there was a negative rather than a positive response to inoculation. Further work is underway to ascertain what is preventing the nodulated plants from fixing nitrogen.

## FUTURE WORK

Future work will examine the effect of various soil and environmental factors on nodulation and nitrogen fixation. Production of inoculum for large scale use and methods of application in the nursery will also be studied.

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# 5. CURRENT STATUS OF MYCORRHIZA RESEARCH IN INDIA

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## SUMMARY

(1) Types of mycorrhizas and their importance in plant nutrition are briefly mentioned.

(2) Mycorrhiza research in different laboratories in India is briefly reviewed.

(3) Some areas where mycorrhizal associations may play a role in amelioration of soils are highlighted.

(4) Possible areas for future research in mycorrhizal associations with trees, are suggested.

## INTRODUCTION

Mycorrhiza literally means 'fungus root'. Mycorrhiza results from a mutualistic symbiosis between roots and certain fungi. There are two main types of mycorrhizas; 1. Ecto and 2. Vesicular-arbuscular mycorrhizas. Ectomycorrhizas (EM) are mostly found in forest trees in the families Pinaceae, Salicaceae, Betulaceae, Fagaceae and Tiliaceae. Basidiomycetous fungi are the common fungal symbionts. Mycorrhizal associations stimulate branching of the roots. This along with fungal hyphae increases the absorption surface of the root. Some ectomycorrhizas are known to decompose organic matter. The ectomycorrhizal sheath also offers protection against root pathogens. EM fungi are culturable and some of them are produced industrially and available in the market in some countries of the world (Marx, 1980).

Vesicular arbuscular mycorrhizas (VAM) are commonly associated with most plants important in agriculture, horticulture and forestry. They are observed by clearing and staining roots, and looking for vesicles and arbuscules. VAM are produced by Zygomycetous fungi. They are obligate symbionts and hence maintained as "pot cultures" on suitable hosts. They improve plant growth through better uptake of P, Zn, Cu, water and making the root zone inimicable to root pathogens. They also improve the activity of N fixing organisms in the root zone. Plants respond well to inoculation with efficient strains of VAM even in unsterile soil with native endophytes (Manjunath et al., 1984). Recent studies show that VAM help in aggregation of soil (Bethlenfalvay, Personal communication). The bottleneck in VAM reaching practical agriculture is the inability to culture the fungus on laboratory media.

## REVIEW OF MYCORRHIZA RESEARCH IN INDIA

### University of Agricultural Sciences, Bangalore

In the Department of Agricultural Microbiology, work on VAM was initiated in the year 1977 by Dr. D. J. Bagyaraj. The work done so far brought out the following:

1. The occurence of VAM in most of the economically important crops and trees, including reporting of their association for the first time in pepper, cardamom and betlevine.

2. Improved growth of crop plants and tree seedlings with VAM inoculation.

3. Isolation, screening and selection of efficient mycorrhizal fungi for citrus and leucaena.

4. Possibilities of increasing yield and/or saving phosphatic fertilizer application through VAM inoculation.

5. Suppression of the root pathogenic fungus Sclerotium rolfsii and rootknot nematode Meloidogyne spp. through VAM.

6. Synergistic interaction between VAM and rhizobia, azotobacters and phosphate solubilizing bacteria with consequential benefit to plant growth (Bagyaraj, 1984).

7. In a recent screening trial, Glomus mosseae was found to be the best mycorrhizal fungus improving the growth of 4 cultivars of Leucaena in a P deficient marginal soil.

8. Recently, 100 different species of tree seedlings growing in a marginal soil, were examined for mycorrhizal associations. About 60% of them were found to be colonized by VAM and the rest by EM. Aspects currently being investigated are:

    1. Mycorrhizal dependency of plants of economic importance,
    2. Microorganisms commonly associated with the pot cultures of VAM,
    3. Methods for producing 'clean' inoculum of VAM, and
    4. Response of tree species to EM and VAM inoculation.

### Forest Research Institute, Dehra Dun

Mycorrhiza research was initiated in 1950's under the leadership of Dr. B. K. Bakshi. Surveys to find out the occurrence of EM were carried out. The mycorrhizal fungi associated with different tree species were identified. In the nursery, fungicides were found to retard the growth of mycorrhiza only initially. Inoculation was found to reduce the nursery period of silver fir (Abies pindrow) from 6 growing seasons to 3 or 4. VAM association was observed in 8 conifers and 28 broad-leaved species and their spore population was more in summer. Two new species Glomus multicaulis and Sclerocystis sinnosa were reported (Bakshi, 1974).

**Tropical pine research centre, Dehra Dun**

Dr. Thaper found that exotic conifers and hardwoods established mycorrhiza with native endophytes. Trimming of silver fir roots at the time of field planting considerably removed mycorrhiza. A survey for mycorrhiza in pine nurseries in different parts of the country suggested insufficient native ectomycorrhizal propagules in Indian soils. Pinus caribaea responded better to inoculation at acidic pH than at alkaline range. Glomus macrocarpum was the most common VA mycorrhizal fungus in forest soils (Thaper & Khan, 1985). Rate of destruction of mycorrhiza was more in poor, than quality sites. Fertilizers reduced VA mycorrhizal colonization in Kauri pine (Agathis robusta).

**North Eastern Hill University, Shillong**

Prof. R.R. Mishra and his group are studying the ecology of mycorrhizal associations in timber trees, weeds and orchids, and the role of mycorrhiza in plant nutrient uptake.

**Delhi University, New Delhi**

Prof. K.G. Mukerji has established an excellent school on fine structure and taxonomy of VAM. The laboratory is also involved in the studies of VAM spores (Mukerji and Ardey, 1985).

**Jawaharlal Nehru University, New Delhi**

Dr. A.K. Varma investigating the VAM of Aravally hills and xerophytes reported Melia azadirachta harboured Gigaspora sp. in the root system, while Ficus bengalensis and Murraya paniculata harboured Glomus sp. thus showing host preference by these fungi. A new species Gigaspora coralloidea occurring in xerophytes was reported from this laboratory. Surface sterilized Glomus macrocarpum spores were found to harbour enteric bacteria, pseudomonads and streptococci (Varma et al., 1981).

**Haryana Agricultural University, Hissar**

Dr. B.L. Jalali, surveying soils of Haryana for VAM, observed more root colonization and spores in nutrient deficient soils. Inoculation of plants with efficient strains of VAM improved growth and P uptake. Addition of rock phosphate with VAM further improved plant growth. Fungitoxicants Brassicol, Emisan, Bavistin and Thirma depressed VAM. VAM reduced the severity of disease caused by the root pathogens Fusarium oxysporum and Rhizoctonia solain. Dual inoculation with Rhizobium and VAM improved nodulation, N fixation and plant growth in legumes (Jalai and Thareja, 1985).

**Govind Ballabh Pant University of Agriculture and Technology, Pantnagar**

Dr K. Seetharamaiah of this Institute has carried out extensive studies on interaction between VAM and root knot nematodes. A time course study confirmed that mycorrhizal colonization begins 1-3 days after inoculation and reaches a maximum within 3-4 weeks. Optimum inoculum level needed for growth improvement was 200 spores/plant and adding inoculum at the planting hole was found to be better than mixing the inoculum with entire soil.

**Indian Agricultural Research Institute, New Delhi**

Dr. N.S. Subba Rao and his associates have recently started studying the interaction between VAM and N fixing bacteria, especially Azospirillum. Synergistic interaction between the two organisms was observed.

**International Crops Research Institute for Semi Arid Tropics, Hyderabad**

Dr. K. Krishna observed plant genotype dependent differences in VAM colonization. Different plant genotypes within a single species varied in their ability to harbour VAM. This suggests that during breeding programmes, inheritance patterns of VAM colonization may be worth looking at (Krishna et al., 1984).

**Tamil Nadu Agricultural University, Coimbatore**

Dr. Kandaswamy and Mr. Santhanakrishnan are studying growth responses of species to VAM inoculation. Other areas of study include: 1. Interactions of VAM with other beneficial soil microbes, 2. Biological control of soil-borne diseases using VAM, 3. Possibilities of reducing P-fertilizer application through VAM inoculation.

**Sri Pushpam College, Poondi**

Prof. K. Kannan and his associates have initiated studies on EM and VAM. Recently they observed VAM in some rhizomatous medicinal plants of Zingiberaceae and Araceae.

**Sheep Breeding Research Station, Sandynallah**

Mr. R. Narayanan isolated EM fungi associated with Pinus patula plantations in Nilgiris and Palani hills. Amanita muscarea, Laccaria laccata, Hebeloma crustuliniforme, Rhizopogon sp. and Suillus sp. were found to be the common mycorrhizal associates. Results from the mycorrhiza synthesis and nursery inoculation studies showed Pisolithus tinctorious to be the best mycorrhizal fungus of EM with pesticides and methods for mass production of P.tinctorious are in progress (Narayanan & Bhattacharyya, 1984).

**Central Plantation Crops Research Institute, Kasargod**

Dr. C. R. Ramesh and Dr. Rohini Iyer who are investigating the mycorrhizal associations in plantation crops have found Endogone australis and Gigaspora gilmori to be the two dominant fungal associates. Coconut, cacao, cinnamon and black pepper were the crops examined. Spores were abundant in poor soils and younger roots showed higher percentage root colonization (Ramesh, 1984). From the Kayangulam centre, Dr. V. G. Lily reported the occurrence of mycorrhiza in coconut for the first time.

**Central Agricultural Research Institute, Port Blair**

Dr. Ramesh has recently initiated VAM research in Port Blair. He is screening for efficient strains of VAM to be used as inoculants for oil palm. VAM as a bio-control agent is also being investigated.

## SOME AREAS WHERE MYCORRHIZA MAY PLAY A ROLE IN THE AMELIORATION OF SOILS

### Revegetation of strip mines and other industrial waste lands

Legumes are a special case from the point of view of plant ecology as they can be supplied with N and P by the naturally existing symbionts, Rhizobium and VAM. Therefore plants colonizing mine spoils and other industrial wastelands are usually legumes, indicating the importance of VAM for revegetation purposes in these soils.

### Establishment and improvements in marginal soils

Tropical soils are highly P deficient. Mycorrhizae have the ability to scavenge a larger volume of soil beyond the P depletion zone and absorb P from concentrations much lower than that which can be absorbed by the root hairs. Thus they help in the introduction of legumes to nutrient deficient soils and thereby bring about improvement of these soils.

### Reclamation of eroded soils

Loss of top soil and vegetation is a consequence of erosion. Eroded soils tend to be depleted of VAM. Reintroduction of these fungi help in the establishment of plants and also improve soil structure, thereby reducing soil erosion (Powell, 1980).

### Tolerance to other stress situations

Mycorrhizal plants have a high tolerance to salinity, low pH, and soil temperature (Lambert & Cole, 1980). Mycorrhizal plants are also known to tolerate water stresss and transplant shock better than non mycorrhizal plants (Menge et al., 1978).

### Decomposition of litter and direct cycling of nutrients

Ectomycorrhizal fungi have been reported to play a role in the decomposition of organic matter and in the direct nutrient cycling (Smith, 1980).

### Transport of nutrients between trees

A network of mycorrhizal mycelia may link one tree with the other thus transporting P between plants (Whittingham & Read, 1982). In leguminous trees, these hyphae may have a role in transporting N and P.

## AREAS FOR FUTURE RESEARCH

1. Selection of efficient strains of EM and VAM for the tree species important in soil amelioration.

2. Compatibility of EM and VAM with pesticides commonly used in forest nurseries.

3. Methods of inoculum production and inoculation.

4. In case of inoculation failures, follow up research to investigate reasons for failure.

5. Establishment of germplasm collection centres of mycorrhizal fungi.

6. Mycorrhizal dependency of forest tree species.

7. Dual inoculation of VAM and N fixers such as Rhizobium and Frankia in tree species.

8. Role of mycorrhiza in biological control of soil borne pathogens in the forest tree nursery.

9. Possibilities of introducing VAM through trees in alley cropping systems advocated in agro-forestry which will act as sources of mycorrhiza for crop plants sown season after season.

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