# RHIZOBIAL NODULATION OF *ACACIA* TREE SPECIES IN SUDAN: SOIL INOCULUM POTENTIAL AND EFFECTS OF PEAT

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**DEANS, J. D., ALI, O.M., LINDLEY, D.K. & NOUR, H.O.A. 1993. Rhizobial nodulation of *Acacia* tree species in Sudan: soil inoculum potential and effects of peat.** Soil cores were removed in 20 *cm* fractions to 1*m* depth from beneath five apparently unnodulated mature *Acacia mellifera* trees growing in the clay plains of east Sudan. Seedlings of *Acacia mellifera* grown in this soil in pots at Khartoum and in a tropicalised glasshouse near Edinburgh, Scotland, produced root nodules regardless of the depth or tree from which the soil had been taken. Supplementary nutrition and inoculation with compatible *Rhizobia* had no significant effect on nodulation, although nutrition increased seedling growth. It was concluded that the inoculum potential of the soil in Sudan was high, but that nodulation in the field was inhibited by lack of water. Seedlings of *A. mellifera, A. senegal* and *A. seyal* grown in a tree nursery in Sudan produced substantial numbers of nodules when peat was added to the Nile silt/sand medium . Improved aeration seemed the most likely reason for the stimulation of nodule production.

Key words: *Rhizobium* - *Acacia senegal* - *Acacia mellifera* - *Acacia seyal* - nitrogen fixation - nodulation

**DEANS,J.D., ALI,O.M., LINDLEY,D.K. & NOUR,H.O.A. 1993. Nodulasi Rhizobil spesies pokok *Acacia* di Sudan: potensi inokulum tanah dan kesan tanah gambut.** Kor tanah atau lapisan kerak tanah pada dalaman 20 *cm* hingga 1 *m*, di bawah lima pokok matang *Acacia mellifera* yang tidak mempunyai nodul yang tumbuh di tanah liat bahagian timur Sudan telah diambil bagi maksud ujian. Anak benih *Acacia mellifera* yang tumbuh dari tanah ini dalam tabung di Khartoum dan di rumah kaca dekat dengan Edinburg, Scotland, didapati mengeluarkan nodul akar tanpa mengambilkira kedalaman atau pokok dari mana tanah tersebut di ambil. Nutrien sampingan dan penginokulatan dengan *Rhizobia* yang serasi tidak menunjukkan kesan yang bererti atas nodulasi, walaupun penggunaan nutrien didapati berupaya meningkatkan pertumbuhan anak benih. Walaupun potensi inokulum tanah di Sudan adalah tinggi, nodulasi di kawasan ini sering terganggu oleh masalah kekurangan air. Anak benih *A.mellifera, A. senegal* dan *A.senyal* yang tumbuh di tapak semaian di Sudan didapati menghasilkan nodul yang banyak apabila tanah gambut dicampurkan kepada endapan Nile/medium pasir. Pengudaraan yang baik mungkin merangsang pengeluaran nodul.

## Introduction

Nitrogen fixing leguminous trees are frequently used to rehabilitate degraded land in the tropics. A recurring feature of many arid and semi arid zone studies is the inability to find root nodules on plants with the capacity to fix nitrogen symbiotically with appropriate species of *Rhizobium.*

Chatel and Parker (1973) reported that the survival of rhizobia could be severely restricted by moisture deficits and Hamdi (1970) found that mobility of the bacteria was restricted at soil water potentials lower than - 0.8 *Mpa.* Nodules are rarely found in the dry surface horizons (Felker & Clark 1982) but it has been suggested that they occur at greater depth where moister soil conditions prevail. In a laboratory study, Felker and Clark (1982) demonstrated that nodulation was possible in moist conditions at 3.2 *m* depth even although nodules were absent from the drier upper regions of their artificial soil profile.

Other factors which limit or restrict nodulation are:- (1) unsuitable pH (Habish 1970), (2) high soil temperature, (Bowen & Kennedy 1959), (3) large concentrations of soil nitrogen (Bernhard-Reversat & Poupon 1980), (4) deficiencies of essential trace elements, for example, cobalt (Dilworth *et al.* 1979), and molybdenum (Evans & Russell 1971), (5) oxygen availability (Dart & Day 1971), and (6) the absence of appropriate strains/species of *Rhizobium.* Even where nodules exist in moist conditions at greater depth in the soil profile, the benefits of nitrogen fixation may be limited, because most of the potential sites for nodulation will occur higher in the profile where the majority of the roots are found. Bonnier (1957) working in Zaire found that only about half of the leguminous species present in the Yangambi forest bore root nodules. Such observations led Nye and Greenland (1960) to conclude that it was unlikely that symbiotic nitrogen fixation would make a significant contribution to the nitrogen economy of the drier forest areas.

While excavating the surface root systems of five *Acacia senegal* and *Acacia mellifera* growing in tree fallows in the clay plains of east Sudan, the authors examined about 3 $m^3$ of soil from the top metre of the profile and found no evidence of rhizobial nodulation. Also, despite the presence of many roots, nodules were absent from 20 soil cores which were located about 1 *m* from tree stems and had been extracted to 1 *m* depth. These excavations took place in November, about two weeks after the annual rains. Although the top 20 *cm* of the soil profile was very dry, physiologically available moisture (soil water potential between 0 and -1.5 *Mpa*) was present below this depth.

The studies described below attempted to elucidate why these root systems were not nodulated. Two experiments were conducted to test the capacity of soil from the clay plains in Sudan to nodulate *Acacia* root systems, *i.e.* the inoculum potential of the soil, and a third experiment was designed to examine nodule production on *Acacia* species produced in a Sudanese tree nursery with different soil media.

## Materials and methods

### *Inoculation potential of soil in eastern Sudan*

In November 1989, soil cores (7 *cm* diameter) were removed from beneath tree crowns to a depth of 1 *m* at a distance of 1 *m* from the stem of each of five *Acacia mellifera* trees which were at least 30 years old. The trees were growing near Jebel Dali, in the clay plains of the Blue Nile province in east Sudan, latitude *c.* 13°N longitude *c.* 33°30' E.

The soil, with pH ranging between 8.5 and 9.5 is a dark 'cracking clay' Vertisol with vertical cracks at least 2 *cm* wide at the surface and extending below 1 *m* in depth.

The soil cores were split into 20 *cm* lengths to provide samples from five differing depths down each profile. Soil samples were individually air dried and then ground to pass a 2 *mm* screen. The samples were then mixed before being split into two portions. One portion was used for experiments in Khartoum, the remainder for experiments in Scotland.

Annual precipitation at the site averages *c.* 550 *mm* and air temperatures range between 16°*C* in winter and 45°*C* in summer. At the time of sampling, air temperature at midday was 42°*C* and the temperature of the top 1 *cm* of the soil was 60°*C*, declining to about 30°*C* at 30 *cm* depth. A light shower of rain had fallen at the site two weeks prior to sampling.

### *Experiments in Khartoum*

In Khartoum, during the first week of December 1989, freshly collected seeds of *A. mellifera* were soaked for 15 *min* in 10 *N* sulphuric acid before being rinsed in several washes of tap water. The seeds were then soaked in water for two days in the laboratory. Subsequently, seeds which had swollen were sown into clean plant pots containing the previously ground soil samples. Because amounts of soil were limited, washed sand which had been sieved to pass a screen and then sterilised in an oven at 200°*C* was added to the base of each pot. The plant pots were protected from extreme heat by a double loose fitting paper surround and then arranged in a fully randomised five by five Latin square design (five cores by five depths) which was mounted on a clean wooden board to prevent contamination from underlying soil. The board was located outside within the campus of the University of Khartoum. The pots were watered twice daily until the end of the experiment 13 weeks later. In total, three plants failed to develop. At the end of the experiment the number of root nodules produced by each seedling was recorded. The data were subjected to analysis of variance using a General Linear Models procedure to test for differences in nodule production according to soil depth and core origin.

*Experiments in the United Kingdom*

In the United Kingdom, the sieved air dried soil samples which had been transported from Khartoum were increased in volume by the addition of 'Perlite' which had been washed several times in deionised water before being sterilised in an autoclave at 121°*C*. The soils from equivalent depths in the differing cores were combined and mixed to provide single large samples from each of the five differing depths in the profile. Pots (50 *mm* diameter) which had been sterilised by immersion in 95% ethyl alcohol were filled with the soil/Perlite mixture and two seeds of *A. mellifera* from the same source as that used in Khartoum were scarified with sandpaper and then soaked in deionised water for two days before being sown into each pot on 9 April 1990. The pots were maintained in a glasshouse at Bush Estate near Edinburgh, Scotland, latitude 55°52'N, longitude 3°13'W. Temperatures in the glasshouse were maintained at 35°*C* day, and 25°*C* night, relative humidity was maintained at about 40% but reached 60% for short periods following watering. Photoperiods were natural and averaged about 16 hours. Supplementary lighting which produced an additional 150 $\mu E\ m^{-2}\ s^{-1}$ at plant level was provided. Plant numbers were reduced to one per pot after germination.

Four experimental treatments were applied:- (1) control, *i.e.* watered with distilled water at pH 8; (2) fertilised, *i.e.* watered as above but on two occasions each week, watered with a balanced nutrient solution containing nitrogen at 80 $mg\ l^{-1}$ with other elements in proportion to *N* (Ingestad 1979). All other nutrients necessary for growth and nodulation were included; (3) inoculated, *i.e.* each plant received 2 *ml* of water containing $1.4^9$ live rhizobia per *ml*. The rhizobia were a mixture of five strains with proven ability to nodulate *A. mellifera.* The bacteria were placed at five locations around the tap root of each plant on the 16 April; (4) inoculated and fertilised as above.

Three replicates of each treatment at each depth were set out in a fully randomised design. Sufficient space was allowed between plants to avoid cross contamination by splashing during watering and the pots were mounted on a 2 *cm* metal grid so that excess water fell immediately to the floor 1 *m* below. The presence of the grid avoided possible contamination of adjacent pots by excess water/ nutrient solution which drained through.

Plants were harvested on 18 June, nine weeks after sowing. At harvest, plants were separated into roots and shoots. The root systems were then washed out over a 200 $\mu m$ screen and the number of nodules present on each root system was recorded. Root and shoot dry weights were recorded after oven drying to constant weight at 95°*C*. Data were analysed by analysis of variance to test for differences between treatments and depths. Additionally, covariance analysis using plant dry weight as covariate was employed to remove the effects of differing plant size when considering nodule production.

### *Nodulation of seedlings in a Sudanese nursery*

Plants of *Acacia seyal, A. senegal* and *A. mellifera* were raised from seed in a polythene house situated in a tree nursery at Soba near Khartoum, latitude 15° 30' N, longitude 32° 40' E. Seeds were sown, either, one to each 1 *l* polythene pot which contained a mixture (2:1) of Nile silt and sand, (the traditional medium for producing tree seedlings), or one to each plastic "Ensopot" container which contained Nile silt to which peat was added at approximately 25% by volume. The trial was set up in the third week of December 1989, and the pots were watered twice daily until harvested 75 days later (the first week in March), 1990. Shoot length, numbers of leaves and number of root nodules were recorded at harvest.

## Results

### *Inoculum potential of soil in eastern Sudan*

At Khartoum, the number of nodules produced on seedlings grown in soil from the clay plains was variable. Overall, nodule numbers ranged between 0 and 48, the mean value was 14. There was no discernible pattern of nodulation with depth in the profile, neither were there any significant differences associated with the tree under which the soil had been removed. However, nodules were produced on about 80% of the seedlings.

In the more controlled glasshouse conditions in the United Kingdom, there were no significant differences in nodule numbers between treatments (Table 1) and, as at Khartoum, there were no significant differences in nodule numbers at differing depths in the soil profile. The greatest numbers of nodules were produced by the control plants. Thus, the native indigenous population of *Rhizobium* was at least as effective at nodulation as the strains which were applied in the inoculum. Although the differences between treatments were not significant (p=0.05), enhanced nutrition tended to depress nodule production.

**Table 1**. Mean root, shoot and total dry weights (*g*) for 9-week-old *Acacia mellifera* seedlings grown under four regimes in a glasshouse in Scotland, and number of root nodules on each plant

| Treatment | Root weight | Shoot weight | Total weight | Nodule numbers |
|---|---|---|---|---|
| Control | 0.27 | 0.56 | 0.83 | 33.1 |
| Fertilised | 0.33 | 0.78 | 1.10 | 23.8 |
| Inoculated | 0.31 | 0.65 | 0.95 | 26.9 |
| Inoculated and fertilised | 0.38 | 0.86 | 1.24 | 28.5 |
| LSD p=0.05. | 0.05 | 0.10 | 0.14 | 9.5 |

Between treatments, there were significant differences in the sizes of the plants after nine weeks of growth (Table 1). Root, shoot and total dry weights were smallest for control plants. All other treatments positively stimulated plant growth, and inoculation combined with supplementary nutrition produced significantly larger plants than those in the control and the inoculated treatments. Although there was a slight positive stimulation to growth in response to inoculation, there were no significant differences of dry weight between control and inoculated plants. The effects of combined inoculation and fertilisation appeared additive rather than synergistic, *i.e.* adding the separate responses for the two treatments to the size of control plants produces an estimated 1.22 *g* dry weight, which is very similar to the actual size found for plants given the combined treatment, *i.e.* 1.24 *g*.

Because control plants had significantly smaller root systems than plants in two other treatments, they had fewer potential sites for nodule production and a lower probability of contact beween root tissues and rhizobia. Accordingly, covariance analysis of nodule production, using plant dry weight as covariate was undertaken to remove effects of plant size from the analysis. This analysis produced adjusted mean values for numbers of nodules on each plant in each treatment as follows: 37.4, 22.3, 28.6 and 24.1 for control, fertilised, inoculated and inoculated and fertilised respectively. There were significant differences between these means ($p = 0.001$, LSD = 9.2), clearly demonstrating an adverse effect of fertilisation on nodule production.

Even although the plants were only nine weeks old at the end of the study in the United Kingdom, many nodules were in an advanced state of decay. This unfortunately meant that other commonly quoted descriptors of $N_2$ fixation potential, *e.g.* nodule dry weight, would produce data of little value.

When intact nodules were sliced open, there was evidence of pink pigmentation indicating the presence of leghaemoglobin and suggesting that the nodules were effective in fixing $N_2$.

### *Nodulation of seedlings in a Sudanese nursery*

Analysis of variance revealed that the substitution of peat for sand in the Nile silt potting medium, which is traditionally used for the production of containerised *Acacia* seedlings in Sudan, significantly increased nodule production ($p=0.001$, Table 2). Although *A. seyal* seemed capable of consistent nodule production in the absence of peat, substituting 25% peat by volume for sand in the potting medium increased the mean number of nodules on each plant from 15 to nearly 25, an increase of about 67%. The other species seemed incapable of consistent nodule production in the absence of peat.

Growth of shoots and the numbers of leaves produced were significantly ($p=0.001$) increased by the addition of peat to the growing medium, (Table 2).

## Discussion

The experiments at both Khartoum and Edinburgh confirmed that rhizobia capable of forming nitrogen fixing nodules occur in the soils of the clay plains of east Sudan. The number of nodules produced by each plant in this study was very variable as also reported by Miettenen *et al.*, (1992) who worked in central Sudan and reported that up to half of the seedlings grown in mixtures of sand and clay failed to produce nodules. It appears that the failure to find nodules in the field is not caused by absence of suitable bacteria. It seems equally implausible that soil pH or the absence of essential trace elements was responsible because nodule formation occurred in the unamended soil both in Khartoum and in Scotland. Similarly, soil temperature is not implicated because conditions in both experiments were similar to those in the field.

**Table 2.** Mean numbers of leaves and nodules and mean shoot length (*cm*) for three species of *Acacia* grown under two growing regimes in a Sudanese tree nursery. Figures in parenthesis are standard errors

| Species | Growing regime | Shoot length | Number of leaves | Number of nodules |
|---|---|---|---|---|
| *Acacia seyal* | Silt+Sand | 10.0(0.7) | 7.4 (0.4) | 15.0 (4.5) |
| *A. seyal* | Silt+Peat | 17.1(2.3) | 9.4 (0.4) | 24.6 (4.3) |
| *A. senegal* | Silt+Sand | 9.1(0.7) | 7.6 (0.7) | 0.0(0) |
| *A. senegal* | Silt+Peat | 17.3(1.9) | 15.0 (1.4) | 37.0 (8.4) |
| *A. mellifera* | Silt+Sand | 8.3(0.3) | 8.2 (0.4) | 0.8 (0.5) |
| *A. mellifera* | Silt+Peat | 16.8 (1.9) | 14.4 (1.3) | 52.2(12.1) |

Although nutrient addition tended to decrease nodule production, the total nitrogen concentration of the soil in the clay plains was very small. It was less than 0.06% in the surface 20 *cm* and declined to about 0.03% at about 50 *cm* depth (Lindley *et al.* in preparation). These concentrations are an order of magnitude lower than those reported to restrict nodulation beneath the crowns of old *Acacia* trees in Senegal (Bernhard-Reversat & Poupon 1980), and can probably be eliminated as the causal agent for nodulation failure.

The greatest difference between conditions in the field and in the pot studies reported here was the moisture content of the soil. Whereas in the pot studies, soil moisture was maintained close to field capacity, in the field, soil water potential near the surface was less than - 4.0 *MPa* increasing to -1.2 *MPa* at about 60 *cm* depth and - 0.85 *MPa* at 1 *m*. It is possible that the favourable hydrological conditions in the pots were responsible for the nodulation observed. Moore *et al.* (1967) observed that, whereas in dry years nodulation of *A. harpophylla* was poor, in wetter years substantial nodulation occurred.

That the nodules produced in the study in the United Kingdom seemed to be short lived suggests that the failure to locate nodules in the field may be associated with their apparent ephemeral nature. If, as seems likely, the root systems of *Acacias* behave in the same manner as those of trees in rainfed tropical orchards, most of the fine root system located near the soil surface will die as the seasons change from wet to dry (Howard 1924/5). In consequence, the likelihood of finding nodules near the soil surface will vary seasonally, and will probably be greatest just after the onset of the annual rains when resumption of fine root growth in the surface horizons occurs (Howard 1924/5).

It was disappointing to find that of the three species of planting stock raised in the nursery, only *A. seyal* seemed capable of nodulation under the traditional nursery regime. This result suggests that many routinely produced seedlings will be transplanted in an unnodulated condition. Satisfactory nodulation of *A. senegal* and *A. mellifera* appeared to require addition of peat to the growing medium. It seems unlikely that the peat which was used in this experiment could have increased the population of compatible rhizobia. The peat originated in Scandinavia and had a pH less than 4.0 which is known to limit the viability of *Rhizobium* species (Holding & Lowe 1971) and inhibit nodulation. Habish (1970) observed that *A. mellifera* nodulated best at pHs near neutrality and failed to nodulate whenever the pH was less than 5.5. Although the inclusion of peat reduced the pH of the mixed growing medium from 8.0 to 7.8, this is unlikely to have had a major influence on nodule abundance. It seems more likely that the influence of peat was indirect, either through increasing the soil porosity, or reducing its bulk density. Such effects, either singly or in combination, could account for the improved growth in the peat amended medium and would improve oxygen flux to the potential nodulation sites.

While this reasoning is plausible, because of the striking effects that peat had on nodulation, further studies to identify the causal relationship are warranted. If the effects on nodulation were brought about indirectly as suggested, it may be advantageous to substitute locally available alternatives for imported peat which may be prohibitively expensive. For example, tree bark is a commonly used substitute in many parts of the world and the bark of tree ferns has been used as an organic component of growing media in the tropics (Landis 1990). The compost used by Miettinen *et al.* (1992) who successfully produced nodulated plants on a large scale was based on sawdust, peanut shells and cow manure.

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## References

BERNHARD-REVERSAT, F. & POUPON, H. 1980. Nitrogen cycling in a soil-tree system in a Sahelian savannah. Example of *Acacia senegal.* Pp.363 - 369 in Rosswall, T. (Ed.) *Nitrogen Cycling in West African Ecosystems.* SCOPE/UNEP International Nitrogen Unit, Royal Swedish Academy of Sciences, Stockholm. 450 pp.

BONNIER, C. 1957. *Symbiose Rhizobium-légumineuses en région équatoriale.* I.N.E.A.C. Séries Science No. 72. 68 pp.

BOWEN, G.D. & KENNEDY, M. M. 1959. Effect of high soil temperatures on *Rhizobium* spp. *Queensland Journal of Agricultural Science* 16: 177-197.

CHATEL, D.L. & PARKER, C.A. 1973. Survival of field grown rhizobia over the dry summer period in Western Australia. *Soil Biology and Biochemistry* 5: 415 - 423.

DART, P.J. & DAY, J.M. 1971. Effects of incubation temperature and oxygen tension on nitrogenase activity of legume root nodules. *Plant and Soil* special volume: 167-184.

DILWORTH, M.J., ROBSON, A.D. & CHATEL, D.L. 1979. Cobalt and nitrogen fixation in *Lupinus angustifolius* L. II. Nodule formation and function. *New Phytologist* 83: 63 - 79.

EVANS, H.J. & RUSSELL, S.A. 1971. Physiological chemistry of symbiotic nitrogen fixation by legumes. Pp. 191 - 245 in Postgate, J.R. (Ed.) *The Chemistry and Biochemistry of Nitrogen Fixation.* Plenum Press, London. 326 pp.

FELKER, P. & CLARK, P.R. 1982. Position of mesquite (*Prosopis* spp.) nodulation and nitrogen fixation (acetylene reduction) in 3-*m* long phraetophytically simulated soil columns. *Plant and Soil* 64: 297-305.

HABISH, H.A. 1970. Effect of certain soil conditions on nodulation of *Acacia* spp. *Plant and Soil* 33: 1- 6.

HAMDI, Y.A. 1970. Soil water tension and the movement of rhizobia. *Soil Biology and Biochemistry* 3: 121-126.

HOLDING, A.J. & LOWE, J.F. 1971. Some effects of acidity and heavy metals on the *Rhizobium*-leguminous plant association. *Plant and Soil* special volume: 153 - 166.

HOWARD, A. 1924-25. The effect of grass on trees. *Proceedings of the Royal Society of London,* Series B 97: 284-321.

INGESTAD, T. 1979. Mineral nutrient requirements of *Pinus sylvestris* and *Picea abies* seedlings. *Physiologia Plantarum* 45: 373 - 380.

LANDIS, T.D. 1990. Growing media. Pp. 41 - 85 in Landis, T.D., Tinus, R.W., MCDonald, S.E. & Barnett, J.P. (Eds.) *Containers and Growing Media.* Volume 2, *The Container Tree Nursery Manual.* Agricultural Handbook 674. U.S. Department of Agriculture Forest Service, Washington D.C. 88 pp.

MIETTINEN, P., KARSISTO, M. & MUSA, M.G. 1992. Nodulation of nine nitogen-fixing tree species grown in central Sudan. *Forest Ecology and Management* 48: 107 - 119.

MOORE, A.W., RUSSELL, J.S. & COALDRAKE, J.E. 1967. Dry matter and nutrient content of a subtropical semiarid forest of *Acacia harpophylla* F. Muell. (brigalow). *Australian Journal of Botany* 15: 11-24.

NYE, P. H. & GREENLAND, D.J. 1960. *The soil under shifting cultivation.* Technical Communication No. 51. Commonwealth Agricultural Bureau, Farnham Royal, Buckinghamshire, England. 156 pp.