THE ECOLOGY OF NITROGEN-FIXING SYMBIOSES UNDER ARID CONDITIONS OF KENYA

by

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Dedication

'Kuom Mama, Baba, Miriam, Leonida, Shona'

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Declaration

This thesis is my own composition, the results presented are of investigations conducted by myself, work other than my own is clearly indicated by reference to the relevant workers or their publications and it has not been presented in any previous application for a higher degree.

David Warambo Odee

Statement

I certify that David Warambo Odee has spent 9 terms of full time research work under my supervision and has fulfilled the conditions of Ordinance No. 14 (University of Dundee) and is thus qualified to submit this thesis for the degree of Doctor of Philosophy.

Professor Janet I Sprent

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SUMMARY

The rhizobial populations and nodulation status of both indigenous (mainly *Acacia* species) and some introduced woody legume species were assessed under glasshouse conditions in soils collected from 12 sites located in different ecological zones of Kenya. The soils were collected on 6 dates spanning a period of 3 years (1989-1992) and at least once every year covering wet and dry seasons.

The rhizobial populations among the sites, as estimated by the most-probable-number (MPN) technique, varied from <3.6 to $>2.3 \times 10^5$ cells g^{-1} soil. There were some intra-site variations in population estimates depending on the trap host species and the method used in sampling the soils. Nodulation also varied across the sites with test species showing frequently higher nodulation ability in native soils. *Sesbania sesban* (L.) Merr. was the most prolific nodulating species while *Acacia tortilis* (Forsk.) Hayne was very erratic in nodulation. Most species showed interplant and intraspecific variability within a single soil source. Nodule shapes were consistent within each species irrespective of soil source but nodulation pattern on the root systems differed when grown in axenic conditions. A comparison of nodule internal structure between *Acacia polyacantha* Willd. and *Faidherbia albida* (Del.) A. Chev. showed differences in the distribution and sizes of infected cells vis-a-vis uninfected cells.

Over 480 isolates were recovered from nodules of test plant species in the various soils. The bulk of the isolates (91%) were watery-, milky-translucent and curdled milk types with moderate to copious extracellular polysaccharide slime (EPS) production. The rest were creamy or white opaque with none or moderate EPS production. They showed a wide range of growth rates on yeast mannitol agar

(YMA) plates: very fast-growing (34.3%), fast-growing (47%), intermediate between fast- and slow-growing (8.4%) and slow-growing (10%). Characterization by intrinsic antibiotic resistance (IAR) and salt (NaCl) tolerance showed that *Bradyrhizobium* was generally more sensitive to antibiotics (streptomycin, kanamycin and ampicillin) and NaCl than *Rhizobium*. Numerical analyses of the IAR and NaCl sensitivity data showed in most cases, clustering of the isolates according to their cultural and morphological characteristics. There was no apparent relationship of isolates within phenons due to site and date of collection, but isolates from *S. sesban* tended to cluster within the same phenon.

Nitrogen fixation potential as determined by acetylene reduction assay (ARA) ranged from 0.2 $\pm$ 0.01 (*F. albida*) to 19.1 $\pm$ 1.2 (*Sesbania sesban*) μmoles plant^{-1} h^{-1}. The woody legumes were vesicular-arbuscular mycorrhizal (VAM). An intraspecific comparison between two provenances of *A. tortilis*: Marigat and Mwatate in a native soil (Marigat) showed that the native provenance was inferior in nitrogen fixation attributes (i.e. nodulation and acetylene reduction rates) but superior in phosphorus (P) uptake.

The results in this work portray the major influences of site (habitat), host and microsymbiont in the nitrogen fixation symbioses under the Kenyan arid and semi-arid lands (ASALs) conditions. The ecological and practical implications are discussed in each chapter.

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background

Many parts of Kenya are faced with severe soil deterioration, erosion and desertification. High human and livestock populations have brought pressure to bear on the meagre medium to high potential lands culminating in shortening of crop rotations and bringing marginal lands under cultivation. Repeated cultivations without fallows and removal of forest trees, either for forest-based products or as expansion for agricultural activities, have resulted in deterioration of soil physical and chemical status due to increased leaching and run-off.

Forest and woodland areas have greatly diminished while the demand for forest-based products has increased. This is reflected in the supply and demand picture in which a deficit of, eg. woodfuel, is forecast to rise from 14 million m^3 y^{-1} in 1990 to 45 million m^3 y^{-1} in 2000 [Kenya Forestry Research Institute (KEFRI) strategic plan, 1990]. In addressing the above problems, KEFRI's core research programmes include expansion of forest management into the arid and semi-arid lands (ASALs) which constitute about 80% of the total land area. The research programmes have prescribed studies on woody species embracing seedling production and establishment, selection of appropriate species and provenances to be incorporated into the agroforestry and farming systems. These strategies are expected to provide

fodder, woodfuel, timber and protection of the environment. Fig. 1.1 shows one of the ASALs sites.

Because nutrient deficiency, especially nitrogen, is a major factor limiting plant establishment and growth in arid lands, the use of nitrogen fixing trees (NFTs) would be the norm and not the exception. However, the microsymbiont status of NFTs in Kenyan ASALs either in natural stands or plantations have been little studied. NFTs have an ecological advantage in colonization of impoverished soils when they form effective symbioses with rhizobia (and *Frankia* for actinorhizal plants) because of their ability to fix atmospheric nitrogen and the potential of enriching the soil with combined nitrogen. Estimated values of nitrogen fixation by trees range from less than 1 kg N ha^{-1} a^{-1} up to 600 kg N ha^{-1} a^{-1} (see collated data in Duhoux and Dommergues, 1985; Giller and Wilson; 1991; Sutherland and Sprent, 1993).

1.2 Distribution of woody legumes in this study

The major woody legume species used were of the genus *Acacia* (Mimosoideae). This genus is widely distributed, consisting of 1200 species globally and occurring in the Americas, the Caribbean and Pacific Islands, Africa, Madagascar and the Mascarenes, Asia, Indo-Malesian region and Australia (Ross, 1981; Coe and Beentje, 1991). In Kenya, the genus *Acacia* is prominent in the woody flora either in pure stands or in mixture with other shrubs and trees. There are 43 *Acacia* species in Kenya (Bogdan and Pratt, 1974; Coe and Beentje, 1991) represented in most habitats from the lowland riparian to highland montane conditions. Other species used to a

Figure 1.1 Lodwar riverine site located in a dryland area (*Acacia tortilis* trees in the background).

lesser extent were of the genera *Sesbania* (Papilioniodeae) and *Prosopis* (Mimosoideae). *Sesbania* species occur in tropical and subtropical regions which have alternating wet and dry periods (Evans, 1990). *Prosopis* species are xerophytic, occurring in arid areas and are native to Asia, North Africa, Central and South America (Leakey and Last, 1980).

1.3 Nitrogen fixation status

The nitrogen fixing ability of woody legumes in their natural habitats in Kenyan soils from various ecological zones had not been elaborately investigated before this study. Earlier work had been done under axenic conditions in assessing symbiotic effectiveness between exotic or introduced species of agroforestry interest and rhizobial strains isolated from Kenyan conditions (Odee, 1989).

In this work, the ecology of nitrogen fixing symbioses was studied under glasshouse conditions using whole soil collected from sites with native woody legumes. In a preliminary survey of nodulation of woody legumes in their natural habitats in the early part of the study, recovery of nodules was very low. Even with the partial excavation of root systems to a depth $\leq$ 1 m, nodules were only recovered from *Acacia polyacantha* and *Sesbania sesban* in the slightly wetter sites in the Lake Victoria region (Odee and Sprent, 1992). This is because in arid lands, mature trees and shrubs are often deep rooted to exploit the deep water resources (Nilsen *et al*, 1983). In my study sites, mature woody legumes had limited fine lateral roots in the top 1 m layer of soil, and most were deep taprooted (Fig. 1.2). Other

Figure 1.2 Structural root systems of representative leguminous tree species growing at ten Kenyan sampling sites.

(a) *Acacia polyacantha* at Nyamonye
(b) *Sesbania sesban* at yala swamp
(c) *Acacia senegal* at Loruk
(d) *Acacia tortilis* at Marigat
(e) *Acacia tortilis* at Lodwar revierine
(f) *Acacia nubica* at Lodwar shrubland
(g) *Acacia tortilis* at Kibwezi savanna
(h) *Acacia xanthophloea* at Kibwezi riverine
(i) *Acacia elatior* at Bura
(j) *Acacia zanzibarica* at Bura savanna


Bar represents 0.1 m

Soil surface

Pit floor


Taproot(s) penetrating down to groundwater


Lateral roots penetrating horizontally to the river channel


Dotted section indicate root penetrating into vertical or horizontal plane of pit

a)
b)
c)
d)
e)
f)
g)
h)
i)
j)

workers (Virginia *et al*, 1986; Jenkins *et al*, 1987; Dupuy and Dreyfus, 1992) have shown that microsymbionts namely rhizobia and mycorrhizas are associated with deep roots of legume trees in the phreatic zones.

The use of surface soil (to 30 cm) to grow plants under glasshouse conditions presented the best alternative to study the interaction of the indigenous nitrogen fixing microsymbionts and the native legumes in their natural habitats. These glasshouse based experiments would be expected to closely simulate the seedling growth and establishment phase in field conditions. Furthermore, the results would have direct implications for the improvement of nitrogen fixing symbioses in the same habitats.

1.4 General aims

The general aims of the work presented in this dissertation were firstly, to determine the indigenous rhizobial populations and nodulation of important woody legumes in soils from their natural habitats representing different ecological zones of Kenya. Secondly, to delineate root nodule morphology and structure, and characterize their rhizobial occupants using cultural and physiological phenotypes. Finally, to evaluate the nitrogen fixation potential of various species and provenances. Mycorrhizal status of some species and provenances that have been assessed are also presented.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Site description and sampling

Twelve sites were selected for the nodulation survey under field conditions and collection of soil and plant samples for various experimental purposes. The major sites are shown in Fig. 2.1. Muguga, which is centrally located, is the headquarters of the Kenya Forestry Research Institute (KEFRI) and also the site for some of the laboratory and glasshouse experiments. Ramogi has two sites (not shown in the map) which are near lake Victoria: Nyamonye on the shores of the lake and Yala swamp, a site reclaimed from a swamp in continuum with lake Victoria. These sites lie within the zone mainly designated as arid and semi-arid lands (ASALs) of Kenya (KEFRI, 1990). They are characterized by low rainfall and exhibit diverse climatic and edaphic conditions (Table 2.1). The other three sites are in the proximity of some of the major sites (ranging from 2 - 7 kilometres) namely Bura riverine, Kibwezi riverine and Lodwar riverine, which as the names suggest are riparian. Lodwar riverine is however, a seasonal river and has of late been drying up. These riparian sites are quite in contrast to the proximal sites in terms of micro-climate and vegetation due to localised moisture availability.

The sites support natural woody legume vegetation of mainly *Acacia* species with the exception of the coastal sites of Gede and Jilore which at sampling had stands of *Casuarina equisetifolia* grown for fuelwood and building poles. These two sites are categorized as the most disturbed.

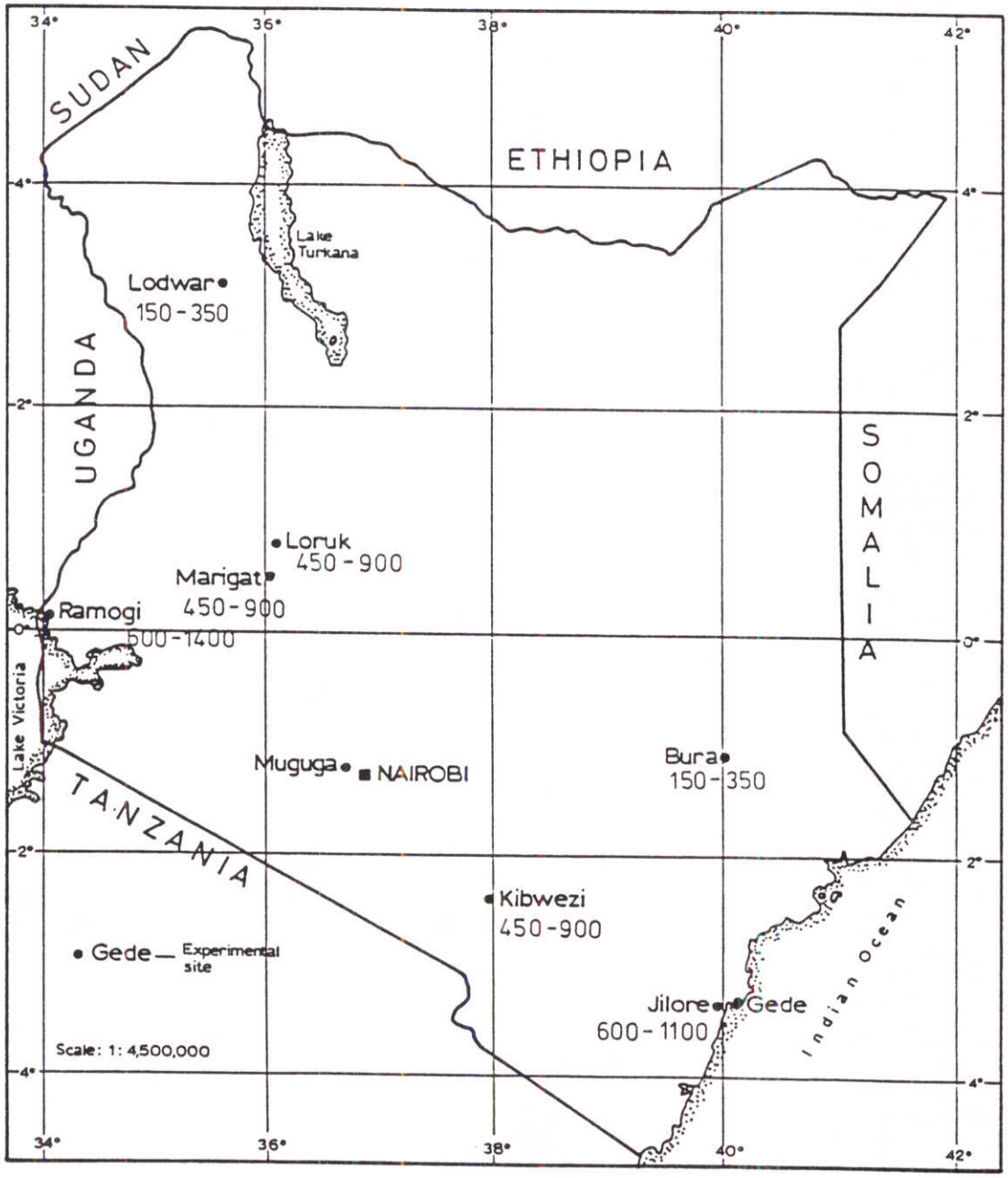


Figure 2.1 Location of experimental sites. Numbers indicate the annual range of rainfall (mm) for the area.

TABLE 2.1. Characteristics of the Kenyan sites

Site	Mean Annual Rainfall[a] (mm)	Mean Annual Temperature[a] (°C)	Elevation in Metres above sea level	Climate[a]	Disturbance History[b]	Vegetation
Nyamonye[c]	600-1100	21-23	1,175	Semi-humid to semi-arid	1,2	A thicket of *Acacia polyacantha* and isolated *A. seyal* trees. Low shrubs of *Lantana camara* and *Vernonia amygdalinia*
Yala swamp[c]	800-1400	21-23	1,225	Semi-humid	1,2,3	Isolated *Sesbania sesban* trees with a *Cassia didymotyra - V. amygdalinia* undergrowth
Kibwezi savanna	450-900	22-24	941	Semi-arid	3	*Acacia tortilis - Commiphora sp.* woodland savanna with isolated *Terminalia brownii* trees and a woody undergrowth of *Acalypha fruticosa*
Kibwezi riverine	450-900	22-24	911	Semi-arid (locally moist)	1,2	Very tall *A. xanthophloea* trees with a lush undergrowth composed of *V. colorata*, *Solanum dasyphyllum* and *Hypoestes verticillaris*
Bura savanna	150-350	23-24	120	Very arid	1	*A. zanzibarica* woodland savanna with isolated *Dobera glabra* trees
Bura riverine	150-350	23-24	120	Very arid (locally moist)	1,2	Tall *A. elatior* trees with an undergrowth composed of *Salvadora persica*, *Cordia sinensis*. *Maerua triphylla* and *Securinega virosa*
Marigat	450-900	23-25	1,116	Semi-arid	2	Tall *A tortilis* trees and an undergrowth comprising of planted *Leucaena leucocephela* and *Cassia siamea* interpersed with natural woody shrubs (*Cadaba farinosa*, *A. fruticosa* and *L. camara*)

Table 2.1.
Continued

Loruk	450-900	23-25	1,186	Semi-arid	1	Mixed acacia shrub (*A. reficiens*, *A. mellifera*, *A. nubica* and *A. brevispica*). Also directly under the acacias are perenial herbs. (*Abutilon mauritianum* and *Bareleria acanthoides*)
Lodwar shrubland	150-350	25-31	518	Very arid	1,2	Isolated *A. nubica* and *C. rotundifolia* shrubs.
Lodwar riverine	150-350	25-31	478	Very arid (occasionally moist)	1,2	*A. tortilis* woodland with isolated trees and shrubs (*S. persica*, *Cordia sinesis*, *Hyphaenae sp.* and *Cadaba rotundifolia*)
Gede White sand	600-1,100	23-24	30	Semi-humid to semi-arid	1,2	*A Casuarina equisetifolia* stand planted on a six-year rotation. Short mixed grassland cover.
Jilore red soil	600-1,100	23-29	120	Semi-humid to semi-arid	1,2,3	*C. equisetifolia* stand with isolated shrubs (*Mundulea sericea* and *Hunteria zeylanica*)

[a]Source: Exploratory soil map and agro-climatic zone map of Kenya, Kenya Soil Survey (Sombroek *et al*, 1982)
[b]Site disturbance history categorized as follows:
1. grazing and browsing by livestock and/or game
2. Management - eg felling, coppicing, clearing of undergrowth, agricultural activities etc.
3. Fire history - man made or sporadic
[c]Both sites are near Ramogi (see Figure 2.1)

During the preliminary survey of the sites, plant specimens of the major flora were collected and identified at the East African Herbarium, Nairobi.

2.2 Soil sample collection

Soil samples were obtained from the surface 15-30 cm at each site (~900 m^2 depending on the size and composition of the natural flora) covering both under trees (mostly legumes) and away from canopies. A composite soil comprising at least 20 samples per site was transported back to KEFRI within the shortest time possible for further work. Each soil was collected with aseptic precautions to avoid cross-contamination between soils from different sites. Sampling was repeated at each site on several occasions and at different times and seasons (wet and dry) between 1989 - 92 inclusive. Representative subsamples were used for standard analyses according to the methods described in Anderson and Ingram (1989).

2.3 Growth of plants

2.3.1 Seed sources

Seeds of various woody legume species and provenances comprising mainly the indigenous *Acacia* species, were obtained from KEFRI's seed centre. These seeds were collected either from natural stands or plantations. Some of the seeds were collected on site from single trees or families. Seeds

of herbaceous legumes were also accessed from other sources for comparative work (see appendix 1).

2.3.2 Seed pretreatment and germination

Healthy looking seeds of uniform weight were selected for germination. Most of the seeds species used were hard-coated and therefore required some form of pretreatment to promote germination. The following choice of methods described by Doran *et al* (1983) were used depending on the requirement of individual species:

(a) seeds were immersed in boiling water and allowed to cool gradually overnight;

(b) seeds were nicked or scarified with hot-wire and;

(c) seeds were scarified in concentrated sulphuric acid.

For experiments requiring axenic conditions, seed scarified other than with the acid method were surface sterilized for 2 min in 2% sodium hypochlorite and then germinated on 0.75 % (w/v) agar plates.

2.3.3 Growth of plants in Leonard jars and enclosed tubes

Plants were grown in modified Leonard jars (see appendix 2) and 3 cm x 20 cm pyrex tubes in experiments involving rhizobial strain/isolate testing and Most Probable Number (MPN) estimation in site soils. The Leonard jar top was filled with a washed mixture of vermiculite:sand (2:1) as a rooting medium and the bottom half (jar) contained *ca* 250 cm^3 of N-

free solution (Somasegaran and Hoben, 1985) with the following composition: $CaCl_2.H_2O$ (1 mol m^{-3}), KH_2PO_4 (0.5 mol m^{-3}), $C_6H_5O_2Fe.H_2O$ (0.01 mol m^{-3}), $MgSO_4.7H_2O$ (0.25 mol m^{-3}), KH_2SO_4 (0.25 mol m^{-3}), $MnSO_4.H_2O$ (1 mmol m^{-3}), H_3BO_3 (2 mmol m^{-3}), $ZnSO_4.7H_2O$ (0.5 mmol m^{-3}), $CuSO_4.5H_2O$ (0.2 mmol m^{-3}), $CoSO_4.7H_2O$ (0.1 mmol m^{-3}) and $Na_2MoO_2.2H_2O$ (0.1 mmol m^{-3}). The pH of the solution was adjusted to 6.8 - 7.0 with NaOH (1 kmol m^{-3}).

The tubes were also filled to one-third with the same rooting medium and added 5 cm^3 each of quarter-strength of N-free nutrient solution and the top plugged with cotton wool. The growth units were then autoclaved at 121°C and 103 kPa for 1 h and used to plant axenically germinated seedlings.

2.3.4 Growth of plants in polyvinylchloride (PVC) pots

PVC pots of 13 cm dia and 11 cm height and 700 cm^3 capacity were used to grow plants where strict axenic conditions were not required. The rooting medium was the same but supplied with nutrient solution according to McInroy *et al* (1988). The base composition of this solution was $CaSO_4.2H_2O$ (2.8 mmol m^{-3}), KH_2PO_4 (4 mol m^{-3}), K_2HPO_4 (0.4 mol m^{-3}), $MgSO_4.7H_2O$ (3.2 mol m^{-3}), $C_6H_5O_7Fe.5H_2O$ (5 mmol m^{-3}), $CoSO_4.4H_2O$ (0.02 mmol m^{-3}), H_3BO_3 (5 mmol m^{-3}), NaC1 (10 mmol m^{-3}), $NaMoO_4.2H_2O$ (0.5 mmol m^{-3}), $CuSO_4.5H_2O$ (0.1 mmol m^{-3}), $MnSO_4.4H_2O$ (0.02 mmol

m^{-3}), $ZnSO_4.7H_2O$ (0.1 mmol m^{-3}). The pH was adjusted to 6.8 - 7.0.

2.3.5 Growth of plants in polythene seedling bags

Germinated seedlings were grown in seedling bags containing 1.5 kg of field site soil and watered to field capacity. The bags were perforated at the bottom to allow free drainage of excess water.

2.3.6 Growth conditions

Plants were grown under glasshouse conditions at KEFRI in Muguga or in Dundee. At KEFRI, the temperature maxima were 30/18 °C (day/night) and natural light of *ca* 12 h photoperiod. In Dundee plants were grown at 28/18 °C (day/night) and 200-400 μmol m^{-2} s^{-1} and 14 h photoperiod.

2.4 Determination of nitrogenase activity

Acetylene reduction assay (ARA) was used to detect and measure the nitrogenase activity in nodules of nodulated plants grown in seedling bags or Leonard jars. A modification of the method by Grove and Malajczuk (1987) was used in which the whole plant in its growth unit was enclosed in a sealable airtight bag (40.6 cm x 61.0 cm). A rubber septum was securely glued onto the upper half side of the bag for removal and introduction of gas. Air equivalent to 10% of the total air volume contained therein was removed and an equivalent volume of acetylene introduced into the bag to

Figure 2.2 Apparatus for acetylene reduction assay

give a 9:1 air-acetylene mixture. The assay was conducted under glasshouse conditions as described for plant growth. Gas samples of 10 cm^3 were removed after 30 min of incubation and stored in vacutainers (Becton Dickinson VACUTAINER Systems, Rutherford, New Jersey). The apparatus used is shown in Fig. 2.2. Acetylene and ethylene were determined in the samples by a gas chromatography (Varian aerograph 1200 series, with a Porapak T 80/100 mesh column) using flame ionization detection.

2.5 Harvesting of plants and assessment

2.5.1 Harvesting

Tube - and Leonard jar-grown plants were harvested at the end of week 6 - 8, whereas those grown in PVC pots and seedling bags were harvested at the end of week 8 - 12.

2.5.2 Nodulation assessment

The seedling bag was removed from the soil-root matrix and a gentle stream of water from a hose used to wash off the soil to expose the nodules. If nodulation occurred, nodules were carefully detached with a piece of root/rootlet bearing the point of attachment so as not to cause any superficial wounds or drainage. The detached nodules were then counted and their shapes described.

2.5.3 Height and fresh and dry weight determinations

The plants were cut at the level of the growth medium to separate them into shoot and root. The shoot was further separated into leaves and stem after measuring the height. The fresh weights of the plant parts were then determined separately. The dry weight of the same plant parts were determined after drying in an air-circulation drying oven at 60 °C for at least 48 h to constant weight. The total dry weight of the root system was estimated from the ratios of the fresh and dry weight of the remainder of root system if nodules and fine roots were sub-sampled for isolation of rhizobia and mycorrhizal infection assessment respectively.

2.5.4 Determination of carbon and nitrogen content

The dried plant parts were ground to a fine and uniform powder consistency such as to pass through 0.5mm sieve with Retsch mixer mill MM2 (supplied by Glen Creston Ltd., Stanmore, Middlesex). Each sample was thoroughly mixed before weighing out 0.5mg - 0.8mg on a Cahn Ventron microbalance and placed into a tin boat. The percent of carbon and nitrogen was then determined using a Carlo Erba Strumentazione Elemental Analyzer (Model 1106).

2.5.5 Determination of phosphorus

Phosphorus was determined using the method described by Anderson and Ingram (1989). About 0.2 g of either ground plant or soil sample was subjected to a wet Kjeldahl oxidation at 360 °C for 2 h. The digest was

made up to 100 cm^3 in a volumetric flask with water and thoroughly mixed. One cm^3 of sample was mixed with 4 cm^3 of ascorbic acid and 3 cm^3 of molybdate reagent and the mixture left to stand for 1 h for colour to develop. Standards were prepared from oven dried (105 °C for 2 h) potassium dihydrogen orthophosphate (KH_2PO_4). The working standards contained 0, 1, 3, 4 and 5 mg cm^{-3} phosphorus (P). Phosphorus concentration in the standard and samples were determined colorimetrically at 880 nm.

2.6 Microscopy

2.6.1 Light microscopy

Fresh nodules with brown-pink infected tissue were cut into slices (1-2 mm thick) and fixed for at least 2 h in freshly prepared 250 mol m^{-3} glutaraldehyde in 50 mol m^{-3} KH_2PO_4 buffer (pH 6.8). Nodules were then washed off 3 times in a buffer alone followed by dehydration in ethanol series. After which the slices were placed in white resin (London Resin Company) on a specimen rotator for 14 d at room temperature with 2 resin changes. Polymerization of resin was done at 60 °C for 48 h.

Sections were cut with a glass knife on a Reichert OM U3 microtome, placed on glass slides and dried on a hot plate. The sections were then stained with toluidine blue (33 mol m^{-3}) for 5 - 10 s, drained, washed and the slide dried in a weak Bunsen burner flame. The stained sections were then viewed and photographed with a camera mounted on an Olympus BH2 microscope.

2.6.2 Transmission electron microscopy (TEM)

Nodule slices were subjected to the same treatment as for light microscopy except for post-fixation in aqueous osmium tetroxide (79 mol m^{-3}) for 1 h prior to dehydration. Ultra-thin sections were cut and collected on formvar coated copper grids. The sections were left to air dry briefly before being stained with uranyl acetate for 30 min, followed by lead citrate for 10 min at room temperature. They were then viewed and photographed using a JEOL 1200EX TEM.

2.6.3 Scanning electron microscopy (SEM)

Nodules were cut in half, fixed and dehydrated as for light microscopy, then passed through an ethanol/freon 113 series until transferred into pure freon. They were then dried using a critical point drier (Polaron Equipment, Watford, Herts, UK). After which they were mounted on aluminium stubs and coated with gold palladium in a polaron E 5100 sputter coater before being viewed and photographed using a JSM35 SEM.

2.7 Rhizobial isolation, maintenance and characterization

2.7.1 Isolation and maintenance

Isolation was done aseptically in a laminar flow cabinet. Those nodules collected in the field and desiccated in silica gel were rehydrated in sterile distilled water for at least 12 h prior to isolation. Fresh and rehydrated nodules were transferred into 95% ethanol for 5-10 s and then

into 1% NaOC1 for 6 min. Each nodule was then successively rinsed in sterile distilled water (at least 6 changes) to remove traces of the sterilant, each time sterilizing the forceps by dipping it into 95% alcohol followed by flaming with Bunsen burner. The surface sterilized nodule was then placed in a drop of sterile water in a sterile petri-dish and then crushed with a blunt tipped forceps. A loopful of the crushed nodule was the picked and streaked across the surface of yeast extract mannitol agar (YMA) plates. The composition of YMA was according to Vincent (1970) and shown in appendix 3. The streaked plates were incubated at 28 °C until colonies appeared. Different types of isolates were reisolated on diagnostic YMA media before being transferred to YMA slants in universal or Mcartney bottles. The isolates were then stored at 4 °C for further work.

2.7.2 Intrinsic antibiotic resistance (IAR) of rhizobia

Stock solutions of the following antibiotics were prepared: streptomycin (Sigma), kanamycin (Sigma) and ampicillin (Merck). The solutions were filter-sterilized using 0.22 μm millipore filters and stored in one-use aliquots at -20°C. At each agar plates preparation, an aliquot of each antibiotic was thawed at room temperature and added aseptically to freshly prepared sterile molten YMA (yeast, extract and agar from Difco Laboratories, Detroit, Michigan, USA) at 50 °C to give the desired final concentration.

Rhizobial isolates were grown in yeast extract mannitol broth (YMB = YMA minus agar, see also appendix 3) to late exponential phase and

diluted accordingly before being used to inoculate the agar plates with a Denley multipoint inoculator (Denley Instruments Ltd, Billingshurst, Sussex). The plates were then incubated at 28 °C for 7 d. Up to 20 isolates were tested on a single plate. There were two replicates for each antibiotic x concentration combination. Growth on antibiotic plates was compared with control (antibiotic free) plates and scored as follows: ++, growth greater than control; +, growth same as control: +/-, growth less or weaker than control, and -, no growth.

2.7.3 Salt tolerance level determination

The level of tolerance to sodium chloride (NaCl) was determined on YMA plates containing the following levels of NaCl concentrations in mol m^{-3} 17, 85.6, 171, 342 and 513. Rhizobial isolates were grown and inoculated as for IAR. Growth was compared with YMA plates with the normal concentration (1.7 mol m^{-3}) and scored as above.

2.8 Mycorrhizal infection assessment

Soil grown plants were assessed for root length infection by vesicular-abuscular mycorrhizal (VAM) fungi. A sample of 100 1 cm root fragments from each plant was randomly selected and cleared and stained using the technique of Koske and Gemma (1989). Water was drained off the roots and covered in 446 mol m^{-3} potassium hydroxide (KOH), and autoclaved at 121 °C and pressure of 103 kPa for 3 min. The KOH was drained off and roots rinsed well in tap water until the brown colouring

disappeared in the rinse water. The roots were covered in another reagent; alkaline hydrogen peroxide at room temperature for 10 - 20 min until they bleached. The reagent was composed of 10 cm^3 of 30% hydrogen peroxide (H_2O_2), 3 cm^3 of 30% ammonium hydroxide (NH_4OH) solution and 587 cm^3 H_2O. This reagent was rinsed out in tap water and the roots covered in 1% hydrochloric acid (HCl) for about 1 h. The acid was decanted and the roots covered with 0.5% trypan blue in acidic glycerol (500 cm^3 glycerol, 50 cm^3 of 1% HCl and 450 cm^3 H_2O) and autoclaved again for 3 min. The roots were then left in the stain for at least 12 h and stored in acid glycerol awaiting VAM infection assessment.

Root length VAM infection was estimated using the gridline intersect method (Giovannetti and Mosse, 1980). The method involved spreading out a root sample onto a petri-dish marked with a 1.2 cm x 1.2 cm grid. A thin film of water was added to aid the dispersion and uniform spread of the fragments so that no root obscured another. The total root length was estimated by scanning the horizontal and vertical gridlines under a stereo microscope (Carl Zeiss, JENA) and counting the fragments that crossed the gridlines. The process was repeated with the same sample but counting only the VAM infected fragments which were identified by blue staining VAM features namely: vesicles, arbuscules and intercellular hyphae within the root cortices. The percentage root infection was calculated from the ratio of the length of the infected fragments to the total length of the root. Formation of a fungal sheath and the presence of a Hartig net were diagnostic features for ectomycorrhizal (ECM) fungi.

2.9 Data analysis

Several computer packages were used in the analysis and presentation of data. MINITAB was used for descriptive statistics. STATGRAPHICS 5.0 was used for analysis of variance. The GENSTAT-SHARE was used to determine the phenotypic relatedness of rhizobial isolates and SIGMA PLOT 5.0 was used for graphical presentations.

CHAPTER 3

3.0 NATURAL RHIZOBIAL POPULATIONS AND NODULATION OF WOODY LEGUMES IN KENYAN SOILS

3.1 INTRODUCTION

Leguminous crop plants are grown widely, especially in protein-poor countries, as they are excellent sources of protein (Lowendorf, 1980). Because herbaceous legumes provide protein with minimal N-fertilizer requirement, they have received much more research attention than woody legumes, such that scientific information regarding herbaceous crop legumes - rhizobia symbioses has been regarded as the norm, notwithstanding that tree-rhizobial symbioses may be different (Sprent, 1993).

Nitrogen fixing trees (NFTs), just as their herbaceous counterparts, can enhance soil fertility through the return of N-rich litter if nodulated with effective rhizobia. In addition they can also provide protein (seed and leaves) for man and livestock, fuelwood and building poles.

In Kenya, there is an increase in the use of NFTs, but like the global trend, there is a paucity of information relating to their symbiotic status under local conditions. Most of the work reported on natural rhizobial populations and nodulation has been on agriculturally important pasture and grain legumes (McDonald, 1935; Bumpus, 1957; Morrison, 1966; Souza, 1969) and nodulation status of the legumes have been described as erratic (Keya and van Eijnatten, 1975; Keya, 1977). More recently *Prosopis*

juliflora, an introduced woody legume species grown in eastern Kenya for fuelwood, was reported to nodulate with the indigenous rhizobia (Miettinen *et al*, 1988).

This study involved a survey of natural stands of indigenous leguminous trees, mainly *Acacia* spp. growing in sites located in different ecological zones of Kenya. Because of the failure to recover nodules from the majority of the putative nodulating species in their natural habitats, soils were collected under and around the trees for use in determination of natural rhizobial populations and nodulation status of both indigenous and some introduced species in these soils.

3.2 MATERIALS AND METHODS

3.2.1 Soil collection and analysis

Soils in this study were collected from the experimental sites at 6 collection dates spanning a period of 3 years (1989 - 1992) and at least once every year covering wet and dry seasons. The soils were analyzed and used for nodulation experiments immediately after each collection.

The soils were analyzed using the methods described in Anderson and Ingram (1989). The pH was measured in calcium chloride ($CaCl_2$) as follows: 10 g of soil was suspended in 20 cm^{-3} of 10 mol m^{-3} $CaCl_2$ solution and measured using a Corning 240 pH meter. Conductivity was measured with a portable conductivity meter, organic carbon by the Walkley-Black wet

oxidation method, total nitrogen by Kjeldahl digestion technique and phosphorus by sodium bicarbonate ($NaHCO_3$) extraction method. The predominant exchangeable cations namely potassium (K^+), sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were determined by flame photometry.

3.2.2 Enumeration of indigenous rhizobial populations.

Indigenous rhizobial soil populations were determined using the plant infection technique (Brockwell, 1980). Thirteen *Acacia* spp. and provenances, 1 *Faidherbia albida* provenance and 1 *Sesbania sesban* provenance were tested with varying numbers of soils collected from 10 experimental sites where some of the test species grew naturally.

Each soil sample was mixed thoroughly and quartered. Ten grams of the soil sample was suspended in 90 cm^3 of sterile distilled water (diluent) in a 250 cm^3 conical flask and shaken in an orbital shaker at 100 rev min^{-1} for 10 min. Then 1 cm^3 of the suspension was aseptically pipetted into a 9 cm^3 diluent contained in a Mcartney bottle and shaken for a further 2 min. This sample was serially diluted 5 times resulting in 6 tenfold serial dilutions of 10^{-1} - 10^{-6}. Each dilution level of the soil was mixed thoroughly by sucking up and down with a pipette to distribute the solution before using 1 cm^3 to inoculate 3 - 5 d old seedlings that were previously pretreated, germinated and transferred into Leonard jars (see chapter 2). Each dilution level was used to inoculate 2 test plants per jar replicated 3 times. Three jars with 2 test plants each were included as uninoculated controls, to check for

exogenous rhizobial contamination.

All plants were grown in a KEFRI glasshouse (see chapter 2 for growth conditions) with replenishment of sterile N-free nutrient solution and distilled water as required. They were harvested after 8 wk of growth to assess nodulation and growth appearance. The number of nodulated plants at each dilution level of inoculating solution was recorded and used as an ordered code (from low dilution to high dilution) to estimate the rhizobial population. The computer program, the Most Probable Number Enumeration System (MPNES) by Woomer *et al* (1990) was used to generate solutions and confidence limits (P = 0.05) of the population estimates of the Most Probable Number (MPN) data. The codes were also subjected to a Range of Transition (ROT) test which assesses the reliability of nodulation results (Scott and Porter, 1986; Woomer *et al*, 1988b).

3.2.3 Nodulation assessment of woody legume species.

Eighteen woody legume species with variable numbers of provenances, mainly indigenous or naturalized in Kenya, were assessed for nodulation ability in various Kenyan soils under glasshouse conditions. Seeds were aseptically pregerminated and planted in polythene seedling bags containing composite site soil (see chapter 2). Two plants per bag containing site soil replicated 4 times for the first 4 sampling occasions and 8 times for the fifth and subsequent sampling occasions were maintained for each species/provenance. The plants were grown under the same growth conditions as MPN experiments and watered regularly to field capacity.

They were harvested at the end of week 12 after germination and assessed for nodulation as described in chapter 2.

3.3 RESULTS

3.3.1 Soil analysis

The soil pH of the sites varied from 4.96 at Yala swamp to 7.80 at Lodwar shrubland and electrical conductivity ranged from 0.10 mS cm^{-1} at Jilore to 1.0 mS cm^{-1} at Lodwar riverine (Table 3.1). Carbon and N were highest at the highly vegetated Kibwezi riverine site (13.92% and 1.09% respectively) and lowest at the least vegetated Lodwar shrubland site (0.11% and 0.01% respectively). Similarly, P was highest at Kibwezi riverine but lowest at Jilore. C:N ratio ranged from 10 at Bura savanna to 13 at Nyamonye. C and N were highly correlated ($r=0.99$, $P=0.001$), and they were also correlated with Ca^{2+} ($r=0.60$, $P=0.05$; $r=0.61$, $P=0.05$, respectively). P was correlated with Ca^{2+} ($r=0.80$, $P=0.05$), K^{+} ($r=0.72$, $P=0.05$), Mg^{2+} ($r=0.64$, $P=0.05$), pH ($r=0.64$, $P=0.05$) and electrical conductivity ($r=0.59$, $P=0.05$). Electrical conductivity was also correlated with Mg^{2+} at $r=0.67$, $P=0.05$.

BLE 3.1. Properties of soils at 12 sampling sites. Values are means (± standard error of the mean) of 5 collection dates except Nyamonye site (n = 4 collection dates). Conductivity values are means of 3 later collection dates

Site	pH in $CaCl_2$ 1:2	Conductivity mS cm^{-1}	Organic matter %	C %	N %	P ppm	K me $100g^{-1}$	Na me $100g^{-1}$	Ca me $100g^{-1}$	Mg me $100g^{-1}$
Nyamonye	5.60 ± 0.15	0.41 ± 0.03	5.63 ± 0.53	3.15 ± 0.16	0.24 ± 0.02	1.06 ± 0.18	6.00 ± 0.64	12.76 ± 0.60	17.75 ± 1.49	7.28 ± 0.94
Wala swamp	4.96 ± 0.13	0.41 ± 0.11	5.20 ± 0.96	2.86 ± 0.44	0.25 ± 0.03	4.62 ± 0.42	5.00 ± 0.51	13.48 ± 0.93	12.70 ± 0.65	5.23 ± 0.42
Narigat	7.02 ± 0.27	0.28 ± 0.08	1.24 ± 0.21	0.67 ± 0.09	0.06 ± 0.01	7.58 ± 0.23	15.28 ± 1.20	14.32 ± 1.03	22.62 ± 2.41	7.32 ± 0.93
Goruk	7.34 ± 0.16	0.42 ± 0.07	2.56 ± 0.28	1.42 ± 0.18	0.14 ± 0.02	5.96 ± 0.71	16.04 ± 1.25	12.14 ± 0.63	14.76 ± 2.74	4.80 ± 1.36
Godwar grubland	7.80 ± 0.17	0.86 ± 0.02	0.20 ± 0.03	0.11 ± 0.02	0.01 ± 0.001	7.46 ± 0.37	7.28 ± 0.68	13.38 ± 1.63	10.80 ± 1.16	3.12 ± 0.49
Godwar riverine	7.48 ± 0.09	1.00 ± 0.01	2.54 ± 0.18	1.44 ± 0.11	0.13 ± 0.02	7.94 ± 0.18	25.52 ± 1.50	23.88 ± 1.07	29.56 ± 4.24	13.50 ± 4.37
Gibwezi savanna	6.88 ± 0.21	0.17 ± 0.03	4.52 ± 0.54	2.62 ± 0.20	0.24 ± 0.02	9.24 ± 0.74	21.00 ± 1.91	12.00 ± 0.87	25.13 ± 2.94	6.16 ± 0.52
Gibwezi riverine	7.16 ± 0.16	0.94 ± 0.05	28.84 ±3.15	13.92 ±1.41	1.09 ± 0.07	11.64 ±1.05	26.32 ± 1.70	15.32 ± 0.79	39.32 ±10.38	11.32 ± 1.02
Gura savanna	7.64 ± 0.20	0.45 ± 0.03	1.10 ± 0.20	0.60 ± 0.09	0.06 ± 0.01	6.68 ± 0.26	16.38 ± 0.58	22.98 ± 3.10	28.50 ± 2.37	8.14 ± 0.61
Gura riverine	7.72 ± 0.28	0.52 ± 0.04	4.06 ± 0.20	2.30 ± 0.14	0.22 ± 0.03	7.14 ± 0.27	24.30 ± 1.42	19.72 ± 0.80	30.22 ± 0.78	7.46 ± 0.52
Kede white sand	7.44 ± 0.16	0.18 ± 0.02	1.36 ± 0.13	0.76 ± 0.06	0.07 ± 0.01	1.50 ± 0.20	14.90 ± 2.54	11.32 ± 1.47	6.38 ± 1.73	1.42 ± 0.25
Kilore red soil	5.84 ± 0.18	0.10 ± 0.01	1.21 ± 0.28	0.67 ± 0.15	0.06 ± 0.01	0.43 ± 0.09	5.44 ± 1.33	12.12 ± 0.41	3.22 ± 0.49	1.40 ± 0.17

TABLE 3.2. Indigenous rhizobial populations at 10 Kenyan sites

Site	Soil Collection date	Method[a] of soil collection	Trap host Species	Provenance	Plant infection count (log_{10} g^{-1} of soil)
Yala swamp	13.6.90	1	*Sesbania sesban*	Yala swamp	5.38
	13.6.90	4	*Sesbania sesban*	Yala swamp	5.38
	13.6.90	1	*Acacia xanthophloea*	Athi river	0.87
	13.6.90	4	*Acacia xanthophloea*	Athi river	0.55[c]
	3.5.91	2	*Sesbania sesban*	Yala swamp	>5.38
	3.5.91	2	*Acacia polyacantha*	Nyeri	0.55[c]
Kibwezi savanna	6.6.90	1	*Acacia tortilis*	Marigat	3.97
	6.6.90	1	*Acacia reficiens*	Katilu	2.05
	6.6.90	1	*Acacia polyacantha*	Nyeri	4.44
	10.5.91	2	*Faidherbia albida*	Kainuk	1.54[b]
	10.5.91	3	*Faidherbia albida*	Kainuk	3.43
Kibwezi riverine	6.6.90	1	*Acacia xanthophloea*	Mai mahiu	2.32
	6.6.90	1	*Acacia polyacantha*	Nyeri	4.16
Bura savanna	22.6.90	1	*Acacia zanzibarica*	Garissa	3.19
	22.6.90	1	*Acacia polyacantha*	Nyeri	5.38
	23.5.91	2	*Acacia zanzibarica*	Garissa	>5.38
	23.5.91	2	*Acacia polyacantha*	Nyeri	>5.38
Bura riverine	22.6.90	1	*Acacia tortilis*	Kibwezi	5.38
	22.6.90	1	*Acacia polyacantha*	Nyeri	5.38
Marigat	28.4.90	1	*Acacia tortilis*	Marigat	0.87
	28.4.90	1	*Acacia reficiens*	Katilu	4.60
	28.4.90	1	*Acacia polyacantha*	Kitui	3.15
	5.5.91	2	*Acacia tortilis*	Marigat	0.87
	5.5.91	2	*Acacia polyacantha*	Nyeri	3.54[b]
	5.5.91	3	*Acacia tortilis*	Marigat	2.04
	5.5.91	3	*Acacia polyacantha*	Nyeri	3.97
Loruk	27.4.90	1	*Acacia mellifera*	Wamba	4.60
	27.4.90	1	*Acacia polyacantha*	Kitui	4.60
Lodwar riverine	4.6.90	1	*Acacia tortilis*	Turkana	4.68[b]
	4.6.90	1	*Acacia polyacantha*	Nyeri	>5.38[d]
	17.8.92	2	*Acacia tortilis*	Turkana	<0.55[e]
	17.8.92	2	*Acacia tortilis*	Mwatate	<0.55[e]
	17.8.92	2	*Acacia polyacantha*	Nyeri	<0.55[e]
	17.8.92	2	*Faidherbia albida*	Kainuk	<0.55[e]
Gede white sand	23.6.90	1	*Acacia polyacantha*	Nyeri	<0.55[e]
Jilore red soil	2.3.90	1	*Acacia Polyacantha*	Nyeri	<0.55[e]

Table 3.2. Continued

[a]Soil inocula were used for plant infection count as follows:

1. composite soil from the entire sampling site
2. composite soil from under legume trees; 1-2 m from the boles and under the canopies
3. composite soil away from legume trees; 1-2 m away from canopies
4. composite soil from sampling site after incidental fire razed the undergrowth

[b]Code considered as unreliable after a Range of Transition (ROT) test
[c]Unreliable code that was adjusted to estimate the MPN
[d]> Indicates that dilution range of the MPN assay was exceeded by population
[e]< Indicates that population was not detectable by the MPN assay

3.3.2 Indigenous rhizobial populations

The numbers of indigenous rhizobia for various trap host species at 10 sites are presented in Table 3.2. *Acacia polyacantha*, used in all these sites, detected no rhizobia at Gede and Jilore sites. The maximum size detectable by the serial dilution employed (5.38 $\log_{10}$ cells g^{-1} soil) on at least one sampling occassion was at Lodwar riverine, Bura riverine and Bura savanna. These estimates with *A. polyacantha* provided comparisons with those of the site native test species as follows: *Sesbania sesban*, a native species at Yala swamp estimated higher counts; *Acacia zanzibarica* (on one sampling occasion using composite soil from under legume trees) and *Acacia mellifera*, native to Bura savanna and Loruk respectively, gave similar estimates; *A. zanzibarica* (on another sampling occasion using composite soil from the entire site), *Acacia tortilis* native to Kibwezi savanna, Marigat and Lodwar riverine and *Acacia xanthophloea*, native to Kibwezi riverine gave rhizobial estimates that were less than those estimated by *A. polyacantha*.

There appeared to be temporal (date of collection) and method of soil collection influences on the number of rhizobia estimated by native test species where comparable data were available. These influences were observed at Bura savanna and Lodwar riverine although it was difficult to discern which of the two factors (or both) were causal. At Marigat, it was apparent that method of collection influenced rhizobial counts in the second soil collection with the native *A. tortilis* but less so with *A. polyacantha*. However, some of the codes (see section 3.2.2) derived from the nodulation patterns failed the ROT test. The test species which gave unreliable codes

were *A. polyacantha* at Yala swamp and Marigat, *A. xanthophloea* at Yala swamp, *Faidherbia albida* at Kibwezi savanna and *A. tortilis* at Lodwar riverine.

Although no quantitative determination was made to assess N-fixation, such as N content and ARA, qualitative assessments showed that nodulated plants were greener and healthier in the transition between the lowest and highest dilution of nodulated plants compared to uninoculated control plants. All uninoculated control plants were without nodules.

3.3.3 Nodulation of woody legumes

Results in Table 3.3 show that indigenous rhizobia capable of eliciting nodules on at least one or more woody legume species tested were present, either transiently or permanently, in the site soils. However, one species not shown in the Table, *Acacia brevispica*, never nodulated in any of the soils and further confirmatory experiments indicated that this species has probably lost its nodulating ability (Odee and Sprent, 1992). *Faidherbia albida*, *A. xanthophloea*, *S. sesban*, *A. seyal*, *A. polyacantha* and *A. tortilis* showed the highest natural nodulation ability across the site in descending order as shown in the proportion of nodulated plants in all sites. Other species eg. *A. elatior*, *A. mellifera*, *A. nilotica*, *A. reficiens*, *A. senegal*, *A. zanzibarica*, *Sesbania grandiflora* and *Prosopis juliflora* had moderate to low proportions of nodulated plants. *Sesbania rostrata* and *P. chilensis* were tested in soils from less than half of the sites.

TABLE 3.3. Nodulation status of various woody legume species and provenances in soils from 12 Kenyan sites (see footnotes)

Trap host species[a]	No of prove-nances tested	Site Nyamonye	Yala swamp	Marigat	Loruk	Lodwar shrubland	Lodwar riverine	Kibwezi savanna	Kibwezi riverine	Bura savanna	Bura riverine	Gede white sand	Jilore red soil	Proportion of nodulated plants in all sites
Acacia elatior	1	+(0.50)[b]	+(0.55)	+(1.00)	+(1.00)	-(0)	-(0)	+(0.60)	+(0.27)	+(0.93)	+(**0.50**)[c]	+(0.42)	-(0)	0.44
Acacia mearnsii	2	NT[d]	+(0.93)	+(0.70)	+(0.33)	NT	NT	NT	NT	NT	-(0)	NT	NT	0.50
Acacia mellifera	4	-(0)	+(0.24)	+(0.42)	+(**0.82**)	-(0)	-(0)	+(0.43)	-(0)	+(0.84)	+(0.33)	+(0.03)	-(0)	0.30
Acacia nilotica	3	+(0.47)	+(0.27)	+(0.72)	+(0.47)	+(0.14)	-(0)	+(0.71)	+(0.75)	+(0.73)	+(0.86)	+(0.50)	-(0)	0.45
Acacia nubica	3	+(0.38)	+(0.20)	+(0.40)	+(**1.00**)	+(**0.43**)	NT	NT	NT	+(1.00)	+(0.13)	NT	NT	0.44
Acacia polyacantha	2	+(**1.00**)	+(0.76)	+(0.92)	+(0.72)	+(0.51)	-(0)	+(0.66)	+(0.06)	+(0.87)	+(0.72)	+(0.25)	-(0)	0.60
Acacia reficiens	1	+(0.04)	+(0.27)	+(0.26)	+(**0.47**)	-(0)	-(0)	+(0.4)	NT	+(0.67)	-(0)	-(0)	-(0)	0.22
Acacia senegal	3	NT	(0.17)	+(20)	+(0.64)	+(0.38)	-(0)	+(0.91)	NT	NT	+(0.2)	NT	+(0.08)	0.30
Acacia seyal	3	+(**0.96**)	+(0.59)	+(0.57)	+(0.80)	+(0.69)	-(0)	+(1.00)	+(0.13)	+(0.90)	+(1.00)	+(0.38)	+(0.38)	0.61
Acacia tortilis	6	+(0.24)	+(0.46)	+(**0.59**)	+(0.84)	+(0.70)	+(**0.32**)	+(**0.78**)	+(0.57)	+(0.93)	+(0.78)	+(0.46)	-(0)	0.57
Acacia xanthophloea	4	+(0.87)	+(0.56)	+(0.90)	+(1.00)	+(0.33)	-(0)	+(1.00)	+(**0.80**)	+(1.00)	+(0.63)	+(0.33)	+(0.5)	0.72

Table 3.3. Continued

Trap host species[a]	No of prove-nances tested	Site Nyamonye	Yala swamp	Marigat	Loruk	Lodwar shrubland	Lodwar riverine	Kibwezi savanna	Kibwezi riverine	Bura savanna	Bura riverine	Gede white sand	Jilore red soil	Proportion of nodulated plants in all sites
Acacia zanzibarica	1	+(0.10)	+(0.35)	+(1.00)	+(1.00)	-(0)	-(0)	+(0.75)	+(0.13)	**+(0.93)**	+(0.71)	+(0.13)	-(0)	0.41
Faidherbia albida	5	+(0.67)	+(1.00)	+(0.85)	+(0.95)	+(0.63)	-(0)	+(1.00)	+(0.88)	+(1.00)	+(0.83)	+(1.00)	+(0.90)	0.79
Prosopis chilensis	2	NT	-(0)	NT	NT	+(0.50)	-(0)	NT	+(0.83)	+(0.63)	-(0)	NT	NT	0.30
Prosopis juliflora	3	+(0.21)	+(0.03)	+(0.66)	+(0.67)	+(0.39)	-(0)	+(1.00)	+(0.29)	+(0.91)	+(0.74)	-(0)	-(0)	0.39
Sesbania grandiflora	1	+(1.00)	+(0.76)	+(0.08)	+(0.75)	+(0.43)	-(0)	NT	NT	NT	-(0)	NT	+(0.57)	0.52
Sesbania rostrata	1	NT	-(0)	-(0)	+(0.33)	NT	NT	NT	NT	NT	NT	NT	NT	0.15
Sesbania sesban	2	+(1.00)	**+(1.00)**	+(0.70)	+(0.85)	+(0.47)	+(0.17)	+(0.75)	+(0.04)	+(0.50)	+(0.23)	+(0.33)	+(0.58)	0.62
Proportion of nodulated plants in each soil		0.56	0.57	0.62	0.75	0.40	0.06	0.77	0.39	0.85	0.48	0.30	0.25	

[a] Trap host species are indigenous to Kenya except *Acacia mearnsii*, *Prosopis juliflora* and *Sesbania grandiflora* which are introduced species. The sources of *Sesbania rostrata* and *Prosopis chilensis* were Senegal and Chile provenances respectively

[b] Data are derived from 4-5 nodulation experiments. Values in parentheses represent the proportion of seedlings that were nodulated. Any seedling which had at least one nodule was scored as positive (+) and those which did not have any nodule were scored as (-).

[c] Bold highlights data for the native trap host species that grows at the site where the soil was sampled.

[d] NT = not tested.

TABLE 3.4. Variability in nodulation of native trap host species and provenances in the various Kenyan experimental sites

Site	Native species[a]	Provenance[b] used in tests	No. of nodulation[c] tests	Mean no No. of nodules ± s.e.	Min-max no. of nodules	Coefficient of variation %
Nyamonye	*Acacia polyacantha*	Nyeri	2	30 ± 6.2	2 - 76	78.8
Nyamonye	*Acacia polyacantha*	Kitui	2	20 ± 4.0	4 - 65	76.0
Nyamonye	*Acacia seyal*	Mukaa	1	20 ± 6.6	8 - 65	91.9
Nyamonye	*Acacia seyal*	Esageri	2	11 ± 2.6	0 - 41	93.5
Yala swamp	*Sesbania sesban*	**Yala swamp**	1	220 ± 17.0	105 - 347	31.0
Yala swamp	*Sesbania sesban*	Kakamega	1	244 ± 35.0	63 - 533	57.4
Marigat	*Acacia tortilis*	**Marigat**	4	3 ± 1.0	0 - 24	169.2
Marigat	*Acacia tortilis*	Mwatate	1	10 ± 1.8	1 - 23	72.3
Marigat	*Acacia tortilis*	Turkana	1	2 ± 0.8	0 - 5	118.0
Marigat	*Acacia tortilis*	Wamba	1	5 ± 3.6	0 - 27	176.3
Marigat	*Acacia tortilis*	Katilu	1	2 ± 1.0	0 - 7	158.0
Kibwezi savanna	*Acacia tortilis*	Marigat	1	40 ± 8.8	10 - 80	61.7
Kibwezi savanna	*Acacia tortilis*	Mwatate	1	17 ± 4.1	0 - 63	99.4
Kibwezi savanna	*Acacia tortilis*	**Kibwezi**	1	29 ± 5.4	8 - 73	74.4
Lodwar riverine	*Acacia tortilis*	Mwatate	1	11 ± 3.6	0 - 58	127.6
Lodwar riverine	*Acacia tortilis*	**Turkana**	1	0	0	NA[d]
Loruk	*Acacia mellifera*	Kibwezi	1	5 ± 1.1	1 - 10	59.6
Loruk	*Acacia mellifera*	**Loruk**	1	3 ± 1.3	0 - 20	175.0
Loruk	*Acacia mellifera*	Isiolo	2	14 ± 2.7	3 - 54	85.5
Lodwar Shrubland	*Acacia nubica*	Marigat	1	6 ± 1.5	0 - 16	102.0
Lodwar Shrubland	*Acacia nubica*	Wamba	1	6 ± 2.3	0 - 27	144.8
Loruk	*Acacia reficiens*	Katilu	1	8 ± 4.3	0 - 37	148.8
Kibwezi riverine	*Acacia xanthophloea*	Athi river	2	14 ± 4.7	0 - 64	135.7
Kibwezi riverine	*Acacia xanthophloea*	Mai Mahiu	1	106 ± 19.9	22 - 375	75.5
Kibwezi riverine	*Acacia xanthophloea*	**Kibwezi**	1	90 ± 14.8	0 - 206	65.9
Bura savanna	*Acacia zanzibarica*	Garissa	1	66 ± 12.1	0 - 167	74.0
Bura riverine	*Acacia elatior*	Turkana	2	3 ± 1.3	0 - 15	161.2

[a] Native species implies that the host trap species grows at the site.

[b] Bold highlights the provenance of the trap host species that grows at the site where the soil was collected.

[c] Nodulation test refers to the number of soil collections from which the nodulation data are represented.

[d] Not applicable

Most species had high proportions of nodulated plants in soils from their native sites namely *S. sesban* at Yala swamp, *A. polyacantha* and *A. seyal* at Nyamonye, *A. mellifera* and *A. nubica* at Loruk, *A. tortilis* at Kibwezi savanna *A. xanthophloea* at Kibwezi riverine and *A. zanzibarica* at Bura savanna. Species with moderate to low proportion values in soils from their native sites were *A. tortilis* at Marigat and Lodwar riverine, *A. reficiens* at Loruk, *A. nubica* at Lodwar shrubland and *A. elatior* at Bura riverine.

Bura savanna, Kibwezi savanna and Loruk sites had high proportions of nodulated plants; Marigat, Yala swamp, Nyamonye and Bura riverine had moderate, and Lodwar shrubland, Kibwezi riverine Gede white sand, Jilore red soil and Lodwar riverine had low proportions of nodulated plants (in descending order): it should be noted that nodulation at Lodwar riverine was in only one soil collection with *A. tortilis* and *S. sesban*.

Sesbania sesban was the most prolific nodulating species and with the least coefficient of variation tested in soil from a native site (Table 3.4). *Acacia xanthophloea* was variably prolific, with Kibwezi (native) and Mai Mahiu provenances showing prolific attributes and less variability vis-a-vis the less prolific and more variable Athi river provenance. *Acacia zanzibarica* was also a prolific nodulator though only one provenance was tested. Two moderately nodulating species namely *A. polyacantha* and *A. seyal* and their respective provenances showed less interplant and intra-specific variability. *Acacia tortilis* was the most erratic nodulating species tested. Because it is native to 3 of the sites from which soils were collected,

several provenances were tested. One of the irregular nodulation results was at Marigat with the native provenance (Marigat) that was poorly nodulated and had a high variability while one non-native provenance (Mwatate) was moderately nodulated. At Kibwezi, nodulation was moderate to near prolific with all the 3 provenances (native provenance included) tested, while at Lodwar riverine only the Mwatate provenance nodulated only on one occasion. The native provenance (Turkana) did not nodulate yet it trapped a large rhizobial population on one occasion. *Acacia elatior* and *A. reficiens* were in general poorly nodulated in soils from their respective native sites.

In an attempt to determine the relationship between soil properties and nodulation parameters, simple linear correlation analyses were made using the various soil properties as independent variables. Rhizobial populations trapped with *A. polyacantha* ($\log_{10}$ g^{-1} soil) were significantly correlated with soil pH ($r=0.68$, $P=0.05$), P ($r=0.77$, $P=0.01$), K^+ ($r=0.78$, $P=0.01$), Na^+ ($r=0.66$, $P=0.05$) Ca^{2+} ($r=0.82$, $P=0.01$) and Mg^{2+} ($r=0.75$, $P=0.01$). However, too much significance should not be placed on correlations with pH as the soil pH values were not evenly spread over the range. Electrical conductivity was not significantly correlated with rhizobial population. Maximum number of nodules per plant was negatively correlated with pH at $r=-0.58$, $P=0.05$. There were no significant correlations between mean number of nodules and proportion of nodulated plants with the independent soil variables.

3.4 DISCUSSION

The pH appeared to be associated with soil texture. Low pH values (<6.0) were found in loamy-clay to clay soils at Yala swamp, Nyamonye and Jilore red soil, higher values (> 7.0) were found in sandy-loam to sandy soils at Marigat, Gede, Lodwar riverine, Lodwar shrubland and Bura riverine. The exceptions were soils at Kibwezi savanna and Kibwezi riverine, both developed on an underlying volcanic lava, Bura savanna with clay but poorly drained and strongly sodic (Sombroek *et al*, 1982); and Loruk which had an underlying hard pan. The relatively higher availability of P at both Kibwezi sites may be due to the locally underlying volcanic lava: this parent material is important in the formation of P-rich soils (Wild, 1988). Although vegetation cover was not quantified, visual observations ascribed to the fact that C and N, which were highly correlated, reflected the amount of organic matter as a direct function of litterfall from the vegetation. The narrow range of C:N ratio (10-13) indicates that decomposition of litter was less variable among sites despite having different conditions. In general, although data for each parameter were pooled over the number of sample collections, there were no marked deviations from the mean values, suggesting little, if any, temporal or seasonal effect.

Because of the lack of locally (experimental sites) isolated rhizobial cultures associated with native leguminous tree species at the start of this work, there was no preliminary systematic cross-inoculation study to determine a suitable or appropriate trap host species for enumerating soil resident rhizobial populations. Instead *A. polyacantha* was selected for this

purpose because it was one of the two species, the other being *S. sesban*, which were found to have nodules at their respective sites (Odee and Sprent, 1992), and it was also taxonomically more related (generic level) to most of the species used in this study.

The MPN results assayed by *A. polyacantha* gave in most cases, higher or comparable results with the native species/provenance except at Yala swamp. This is an indication of the presence of a large rhizobial population that is homologous to *S. sesban* but with less affinity for *A. polyacantha* at Yala swamp. Because *A. polyacantha* detected soil resident rhizobia in 8 out of the 10 sites tested this suggests that various *Acacia spp* native to the sites may belong to the same cross-inoculation group. Rhizobia were not detected by the MPN method at Gede and Jilore yet some test plant species nodulated when grown in whole soils from the two sites. Gede and Jilore are the only sites that did not have natural stands of any of the test plant species though a woody papilionoid legume shrub, *Mundulea sericea* (Willd.) A. Chev. was growing at the latter site . The nodulation status of this legume at the site was not determined although it has been reported to nodulate in Hawaii and Zimbabwe (Allen and Allen,1981). However, the poor nodulation recorded at Gede with most species (Table 3.3) indicate that the sensitivity of the MPN assay employed was not high enough to detect the low population of rhizobia compatible with *A. polyacantha*: its lower limit of detection was 3.57 cells g^{-1} of soil.

At Marigat, the use of a native *A. tortilis* provenance showed that there was no difference in rhizobial populations between the 2 sampling

occasions despite having been sampled by different methods. However, in the second soil collection, comparisons of MPN estimates in soil samples collected beneath the legumimous trees versus those collected away from the canopies gave a nearly 15-fold increase with *A. tortilis* and nearly 3-fold increase with *A. polyacantha* in favour of the latter method of collection. Several factors, both biotic and abiotic, are known to influence the abundance of rhizobia in soils (Lawson *et al*, 1987; Woomer *et al*, 1990; Woomer *et al*, 1988a; Yousef *et al*, 1987; Chaudri *et al*, 1993), these may act either singly or concertedly. Lawson *et al* (1987) showed that the abundance of *Rhizobium leguminosarum* bv. *trifolii* was positively related to solar radiation, while other workers (Woomer *et al*, 1990; Woomer *et al*, 1988a) showed positive correlations of soil resident rhizobia with legume and non-legume % cover. Although vegetation cover was not determined, a similar interaction may have taken place: because the shrub and herb layers could get more natural light outside the tree canopies their increased photosynthetic activity would have indirectly influenced their multiplication in the rhizospheres through increased root activities such as exudation, hence higher populations compared with the rhizospheres of the partially shaded shrub and herb layers beneath the trees. The *A. tortilis* trees themselves contributed little, if any, rhizosphere effect because they were taprooted with no laterals within 30 cm of the surface and a radius of 1 m from the boles. Lodwar riverine site on the other hand, with virtually surface sand dotted with 15-25 m high *A. tortilis* trees bearing thin crowns, was the most stressful site for rhizobial survival in which MPN was assayed. This site,

along with its sister site Lodwar shrubland which was not assayed, has a mean maximum temperature of 39.8 °C on the hottest month, a variation of annual rainfall totals of 19 to 498 mm and an average annual potential evaporation of over 2,100 mm (Amanyunzu, 1988). Therefore under these conditions, rhizobial populations would be expected to fluctuate if not transiently aggregating in pockets of favourable microclimate conditions. This is reflected in the high MPN estimate with *A. polyacantha* in the first soil collection and no detection at all in the subsequent collections.

Most probable number by plant infection test is based on the assumption that organisms are randomly distributed and that one or more rhizobia are capable of causing nodulation on an appropriate host (Woomer *et al,* 1988b). If the right dilution series is selected, then an orderly transition between nodulated and non-nodulated test plants is derived to give a 'code' with which the MPN is estimated from tables containing common possible codes for various dilution factors and numbers of replicates (Brockwell, 1963; Brockwell, 1980; Brockwell *et al*, 1975; Vincent, 1970) or as a computer software (Bennett *et al*, 1990). However, occasionally there is an extended transition between the positive and the negative results, or 'skips' (Vincent, 1970; Brockwell, 1980) where a lower dilution gives a negative result and a higher dilution gives a positive result thus rendering the MPN data unreliable. This phenomenon may occur as a result of the following factors: rooting pattern of test plant (Turk and Keyser, 1992), inherent inability to nodulate (Boonkerd and Weaver, 1982), single or mixed culture and unhealthy state of test plant (Scott and Porter, 1986), and

cotyledons of large seeded plants and growth substrate composition (Toomsan *et al*, 1984). In this study, species which gave 'unreliable' results were categorized according to the likely causal factors as follows: *A. polyacantha* and *A. xanthophloea* in Yala swamp soil, because of the low numbers of compatible rhizobia; *A. polyacantha* and *F. albida* in Marigat and Kibwezi savanna soils respectively, because of method of collection; and *A. tortilis* in Lodwar riverine because of a combination of inherent inability to nodulate well and adverse site effects on the spatial distribution of rhizobia.

Natural nodulation of most species at moderate to high proportions in their native sites is supportive of the view that the abundance of the soil resident rhizobial population is influenced by the occurrence of a compatible legume host (Vincent, 1981). Lie *et al* (1987) have also demonstrated the co-existence of pea genotypes and their specific *Rhizobium* strains within the same locality. The low proportion of nodulated seedlings at Gede and Jilore may thus be attributed to the paucity of rhizobial types that were compatible with the test species used in this study.

The varying proportions of nodulated seedlings in each site therefore allude to the nature of its rhizobial composition and affinities of the legume(s) that the site supports. Because species such as *A. xanthophloea*, *F. albida*, *S. sesban*, *A. seyal*, *A. polyacantha* and *A. tortilis* gave high proportions of nodulated seedlings, their compatibilities with the indigenous rhizobial types need to be elucidated. However, some of the species have well documented rhizobial affinities. Dreyfus and Dommergues (1981)

demonstrated that *F. albida* nodulated exclusively with the slow-growing type (*Bradyrhizobium* sp.) while *A. seyal* was capable of nodulating with both the slow-growing and the fast-growing (*Rhizobium* sp.) types. *A. polyacantha*, *A. tortilis* and *S. sesban* have been shown to nodulate with fast-growing rhizobia (Habish and Khairi, 1970; Duhoux and Dommergues, 1985). *A. xanthophloea* also appears to nodulate only with fast growing rhizobia (this study). It is therefore apparent that the natural rhizobial populations are of mixed types irrespective of the type (taxonomic) of native legume species at a site.

Nodulation data of site native species presented a different picture in their actual nodulation characteristics. The fact that a nodule score with a range of 0 - 206 was recorded in such a prolific nodulator as *A. xanthophloea* in its native site indicated large interplant variability. The phenomenon was commonplace in all the test species but to a greater extent for some species than others. Large variations, either interplant or intraspecific, in woody legumes in symbiotic traits are not uncommon. Sniezko (1987) reported a range-wide provenance (as defined by different African countries seed sources) in nodulation of *F. albida* in two Zimbabwean soils; and in another work with 25 provenances of *Gliricidia sepium* by Sanginga *et al* (1991) a range of 10 - 113 nodules per plant (NB. both extremes representing the least and most nodulating provenances respectively) was reported. A plausible explanation for variations in my results may be attributed to the manner in which the seeds were collected. Except for *S. sesban* Yala provenance seeds which were collected on site

from a single tree, and unknown collection methods for exotics or introduced species, the rest of the indigenous species were general collections from natural stands in a locality or provenance. Each provenance thus comprised pooled seeds from a sufficient number of trees which were within a distance of one another that minimised inbreeding (W. Omondi; KEFRI Seed Centre Curator, personal communication). However, interplant and specific variation may persist even in single tree (families) seed sources (Gwaze *et al,* 1988), perhaps as a result of cross-pollination, as has been deduced for interplant differences in nodulation and rhizobial affinities in *Medicago sativa* cultivars (Bromfield, 1984; Bromfield *et al,* 1986).

The availability of comparable data for *A. tortilis* provenances over three native sites showed how the species is erratic in its symbiotic attributes, notwithstanding its nodulation ability in 11 out of 12 sites. It is a typical example of the various forms of variability: interplant, intraspecific and intersite. Because at Marigat, the native provenance was just as variable as other provenances except Mwatate provenance, while at Kibwezi savanna all the three provenances tested gave comparable results, the differences between the two sites could therefore have been influenced by the differences in the sizes of rhizobial populations compatible with *A. tortilis*: 0.87 - 2.04 $\log_{10}$ cells g^{-1} soil at Marigat and 3.97 $\log_{10}$ cells g^{-1} soil at Kibwezi. Lodwar riverine native provenance (Turkana) was the most erratic of the 3 main provenances (ie Marigat, Lodwar and Mwatate). It did not nodulate in Lodwar riverine soil native site while Mwatate provenance did. Its MPN estimate on one occasion was 4.68 $\log_{10}$ cells g^{-1} soil and on another no

rhizobia were detected with it in Lodwar riverine soil. The explanation offered for the inconsistency in MPN, that of poor spatial distribution due to adverse conditions, is applicable in this instance too. Brockwell *et al* (1991) showed similar inconsistencies in the symbiotic attributes of *Cajanus cajan* (L.) Millsp. in New South Wales (Australia) soils. The Mwatate provenance, despite being non-native in any of the sites studied but originating from the coastal region of Kenya, had better symbiotic attributes than the other two. It would therefore be a favourable candidate for biological nitrogen fixation potential screening among the Kenyan *A. tortilis* provenances.

Positive correlations between rhizobial abundance and cations (K^+, Na^+, Ca^{2+}, Mg^{2+}); and negative correlation between maximum number per plant and pH of the soils suggest that these soil properties influenced rhizobial survival. Like any heterotrophic micro-organism in soil, rhizobia would require these elements to synthesize all the necessary enzymes and cell constituents for growth; the pH of the soil solution determining the availability of these nutrients to rhizobia by the process of desorption from exchange sites in soil. The maximum number of nodules per plant recorded at any site irrespective of the species or provenance would hypothetically be an indication of the number of successful infection process attempts. Thus, assuming that each nodule was initiated by a single rhizobial cell, and that no other factor was limiting except pH, then the negative correlations between these two variables suggest that infection processes were favoured at pH around neutral or below. The influence of pH may also have been

indirect by, as discussed above, changing the soil chemical (cations) composition in the rhizosphere and root walls conducive to infection process (Sprent, 1989).

This study has therefore shown that there are varying sizes of natural populations of rhizobia in the different ecological zones of Kenya, and that these populations may fluctuate depending on the predominant environmental factor(s) at any given time. Because one trap host (*A. polyacantha*) detected rhizobia in most sites indicate that these populations contain rhizobial types with broad host ranges but that the abundance of a particular type at a site is influenced by the the rhizobial affinities of the native host species. It was also evident that introduced species were variably compatible with indigenous rhizobia in some sites. Variations in symbiotic attributes at all taxonomic levels of woody legumes studied and sizes and composition of indigenous rhizobia have important practical implications in ensuing and future work. It will be necessary to test the symbiotic properties of indigenous rhizobial isolates vis-a-vis 'exotic' ones against selected superior provenances to identify the most efficient matches of micro-and macro-symbionts before they can be re-introduced into the sites.

CHAPTER 4

4.0 ROOT NODULE MORPHOLOGY AND STRUCTURE IN TWO WOODY LEGUME SPECIES: *ACACIA POLYACANTHA* AND *FAIDHERBIA ALBIDA*.

4.1 INTRODUCTION

In Kenya, the two species are reported to have similar habitat requirements; riverine or alluvial valleys with groundwater (Brenan, 1959; Bogdan and Pratt, 1974). They belong to the tribe Acacieae in the family Leguminosae (Lock, 1989). *Faidherbia albida*, formerly *Acacia albida*, was classified in the subgenus *Acacia* and *A. polyacantha* in the subgenus *Aculeiferum* (Ross, 1981, Coe and Beentje, 1991).

Nodule morphology has been applied in legume systematics (Corby,1981), and recently Naisbitt *et al* (1992) have shown the usefulness of nodule anatomy even at specific level in the genus *Chamaecrista* (Caesalpinioideae).

In this chapter, nodule morphology, structure and distribution on roots of seedlings are compared in *A. polyacantha* and *F. albida*.

4.2 MATERIALS AND METHODS

4.2.1 Source of nodules for shape and distribution description

Descriptions were based on nodules harvested from the nodulation assessment experiments in chapter 2. For light microscopy, at least 3 active

and well developed nodules were selected from 8-week old plants grown in Leonard jars. Each of the species was grown in triplicate under Dundee University greenhouse conditions (see chapter 2) and inoculated with an effective strain. *Acacia polyacantha* was inoculated with its homologous strain ARN1/S1/P1/1. *Faidherbia albida* was inoculated with a slow-growing strain BR2/S1/P3/3B, originally isolated from *A. polyacantha* but effective on the former host. Effectiveness of the strains was confirmed on their respective hosts with the acetylene reduction assay (ARA) as described in chapter 2. Specific activity (acetylene reduction rates) of nodules of *A. polyacantha* was 75.9 $\pm$ 58.1 ($\pm$ SE, n=3) μmol g^{-1} fresh wt h^{-1} and that of *F. albida* was 57.0 $\pm$ 30.6 μmol g^{-1} fresh wt h^{-1}.

4.3 RESULTS AND DISCUSSION

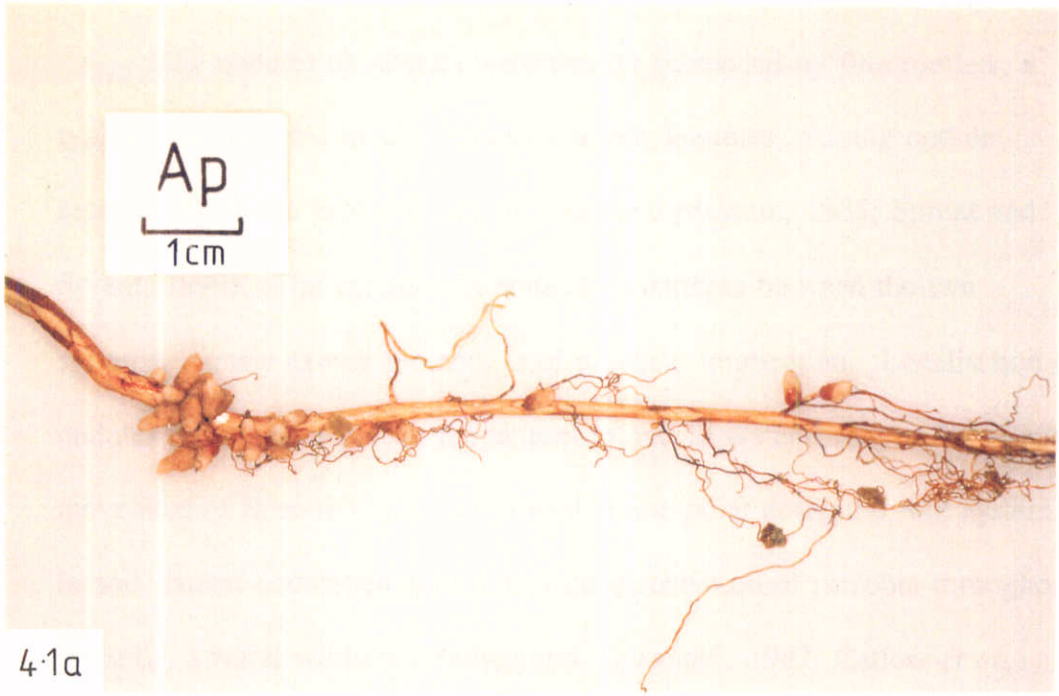
4.3.1 Nodule description and distribution

Data on nodule morphology were collated from nodulation experiments done in soils in which nodulation occurred. In general, nodule shapes obtained were similar for both species irrespective of source of soil. The shapes found on a root system were mainly elongate, lobed or coralloid or both, with occasional smaller globose types. The consistency in nodule shape in soils from various sites where it is expected that there would be different soil resident rhizobial types indicates that nodule morphology is a host determined characteristic in these species. Lim and Ng (1977) also found that nodule shapes were characteristic of legume species and independent of rhizobial type, supporting the application of nodule shape in

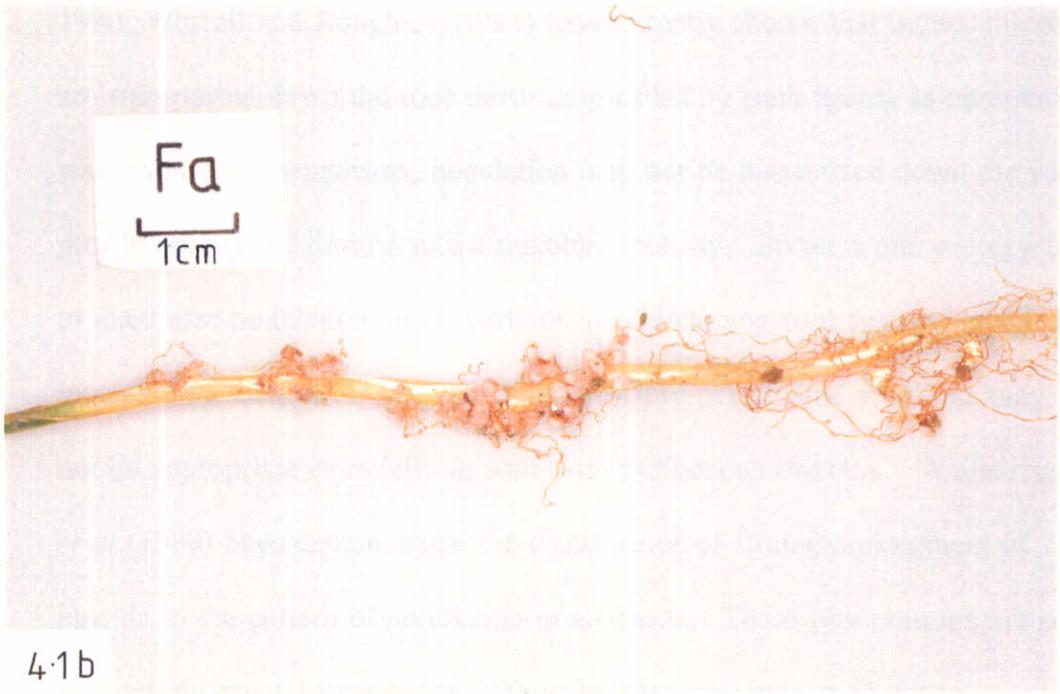
ranged from 0.15 cm x 0.40 cm (width x length) to 0.70 cm x 1.15 cm for *A. polyacantha* and 0.05 cm x 0.10 cm to 0.72 cm x 1.42 cm for *F. albida*. These sizes are comparable to those reported for other mimosoid woody legume species (Lim and Ng, 1977; Venkateswarlu and Singh, 1988; Johnson and Mayeux, 1990), and the shapes conform to the astragaloid type of Corby (1981). The nodules had woody-like proximal region and meristematic distal region indicating that they were of indeterminate type (Sprent, 1986). The occasional globose nodules represented intermediate stages of nodule morphogenesis as was discerned by the occurrence of a distal meristematic zone when cut longitudinally. However, Baird *et al* (1985), reported the lack of a meristematic zone in globose nodules from *Prosopis glandulosa* in plants inoculated with a slow-growing strain, whereas nodules induced by a fast-growing strain were of the normal indeterminate type. That observation suggested that the slow growing strain was the causative agent for the reversion of the normal nodule type of the host.

Nodule distribution on the root systems was mainly on the laterals and occasionally on the taproot in soil grown plants. On the other hand, axenically grown plants showed a predominantly contiguous taproot nodulation (Figs. 4.1a and b). The nodules that appeared to be on taproot were either attached at the end of short fine rootlets or at the axils, a feature more common in the aechynomenoid nodule types than astragaloid (Corby, 1981). The 'atypical' nodulation pattern of these mimosoids suggests that the mode of infection through root hair as reported in other woody mimosoids (Baird *et al*, 1985, James *et al*, 1993) may not be exclusive.

Figure 4.1a and b Nodulated root systems of *Acacia polyacantha* (Ap) and *Faidherbia albida* (Fa) plants grown in Leonard jars.



4·1a



4·1b

Other modes such as epidermal and wound entry (Sprent and Faria *et al*, 1988) may be possibilities.

The nodules on laterals were weakly subtended by fine rootlets, a typical characteristic of the mimosoid woody legumes, making nodule recovery from the field difficult (Johnson and Mayeux, 1985; Sprent and Sprent, 1990). The variation in nodulation patterns between the two systems, namely axenic and soil, is of practical implication. Localisation of nodules in the upper part of the taproot in axenic systems may reflect limited movement of rhizobial inoculum placed at one point down the root system. In soil, lateral nodulation may reflect the distribution of rhizobia throughout the soil. Several workers (Madsen and Alexander, 1982; Catlow *et al*, 1990; Worrall and Roughley, 1991) have recently shown that unless rhizobia are transported down the root substrate profiles by such agents as percolating water and other organisms, nodulation may not be maximized down the root profile because of limited active rhizobial motility. Because our strategy is to maximize nodulation on all parts of the developing root system, localized inoculations such as on seeds or radicles of pre-germinated seedlings may not be appropriate especially in soils with indigenous rhizobia. Wadisirisuk *et al* (1989) have demonstrated the significance of strategic placement of inocula in the pattern of nodulation in soybeans. These observations are of particularly crucial importance with woody legume species in which inoculants are applied on seed at nursery stage to be outplanted in sites with 'unwanted' soil resident rhizobia.

4.3.2 Nodule internal structure

Figures 4.2a and 4.3a show light micrographs of transverse (TS) sections of a mature *A. polyacantha* nodule from the active region just behind the meristem. The infected cells are extra-ordinarily large compared with the uninfected interstitial cells. A considerable proportion of the infected cells are not contiguous with one another. The transmission electron micrograph (TEM) of infected cells (Fig. 4.4a) shows how the host cell is densely packed with bacteroids, and cytoplasmic volume is limited. However, when viewed by scanning electron microscopy (Fig. 4.5a) relatively few rod-shaped bacteroids are visible. Figures 4.2b and 4.3b show light micrographs of *F. albida* in TS section of a comparable region of nodule. Infected cells are vacuolate and much smaller, but more aggregated than in *A. polyacantha*. There are fewer and smaller interstitial cells. The (TEM) of infected cells of *F. albida* shows that they are less densely packed with bacteroids (Fig. 4.4b). The density of bacteroids in an infected cell of *F. albida* as seen in the scanning micrograph (Fig. 4.5b) may suggest the opposite of what has been shown in the light and and transmission electron micrographs. However, Streeter and Salminen (1993) have recently established that bacteroids of certain *Bradyrhizobium japonicum* strains synthesize and deposit polysaccharides in the symbiosomes of infected cells of soybean (*Glycine max* L. Merr.) nodules. Thus the apparently greater density of bacteroids visible in the SEM of *F. albida* may result from greater retention of bacteroids during processing due to the surrounding polysaccharide visible in the TEM but less abundant in the TEM of

Figure 4.2a Light micrograph of transverse section of mature *Acacia polyacantha* nodule.

Figure 4.2b Light micrograph of transverse section of mature *Faidherbia albida* nodule.

Abbreviations

C Cortex

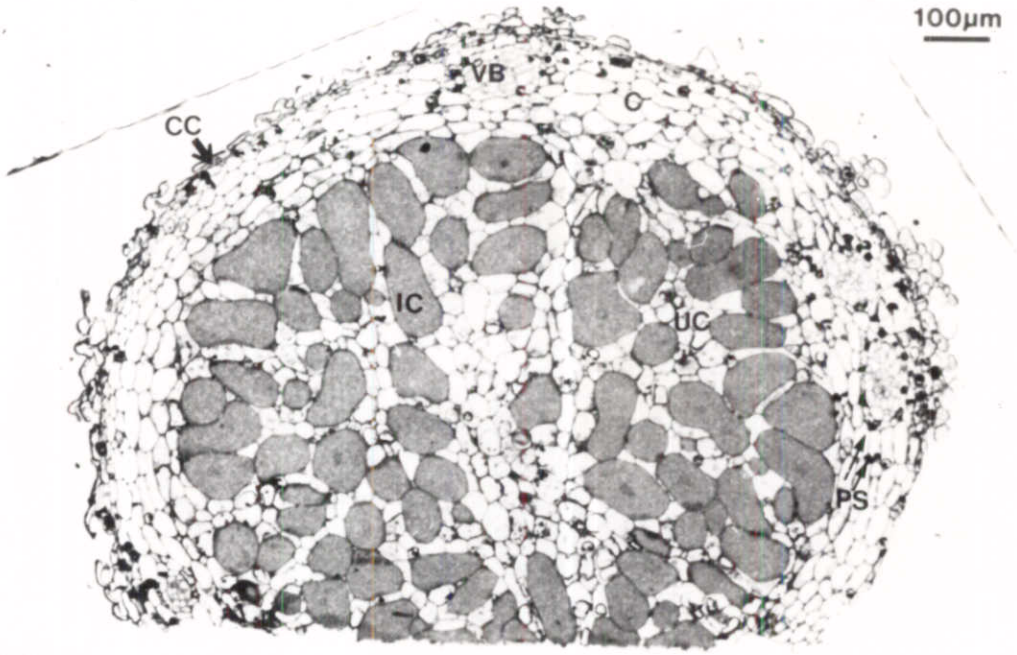
CC Cork cambium

IC Infected cell

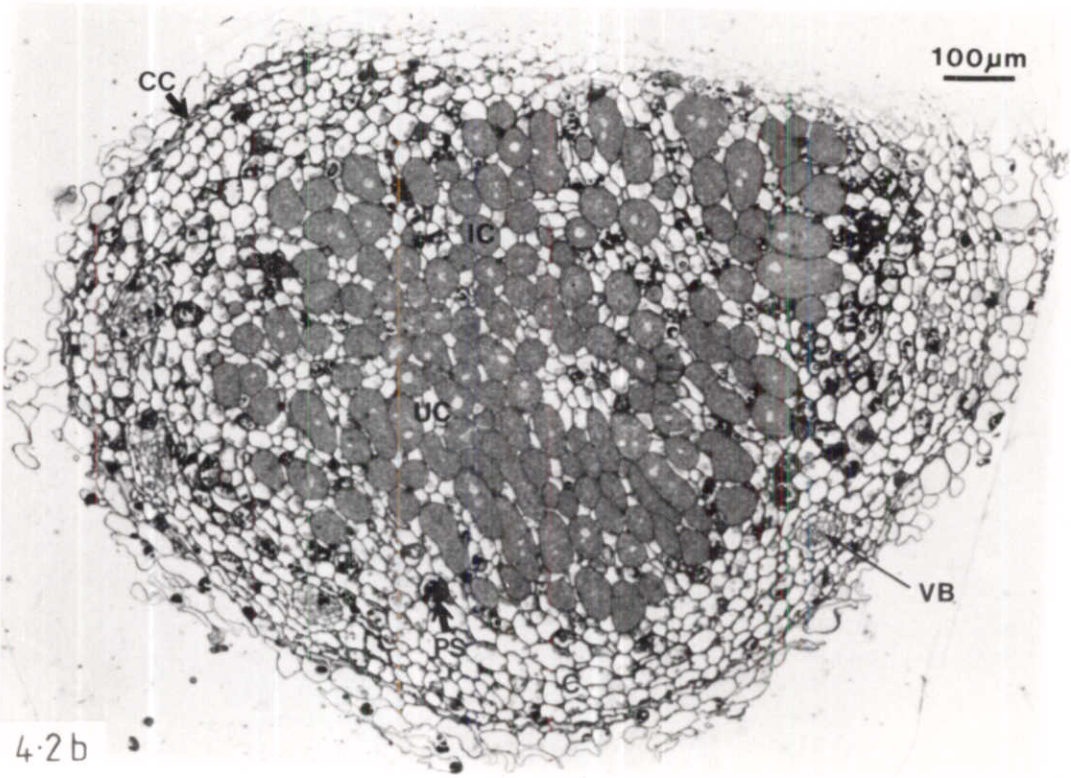
PS Phenolic substance

UC Uninfected cell

VB Vascular bundle



4·2a



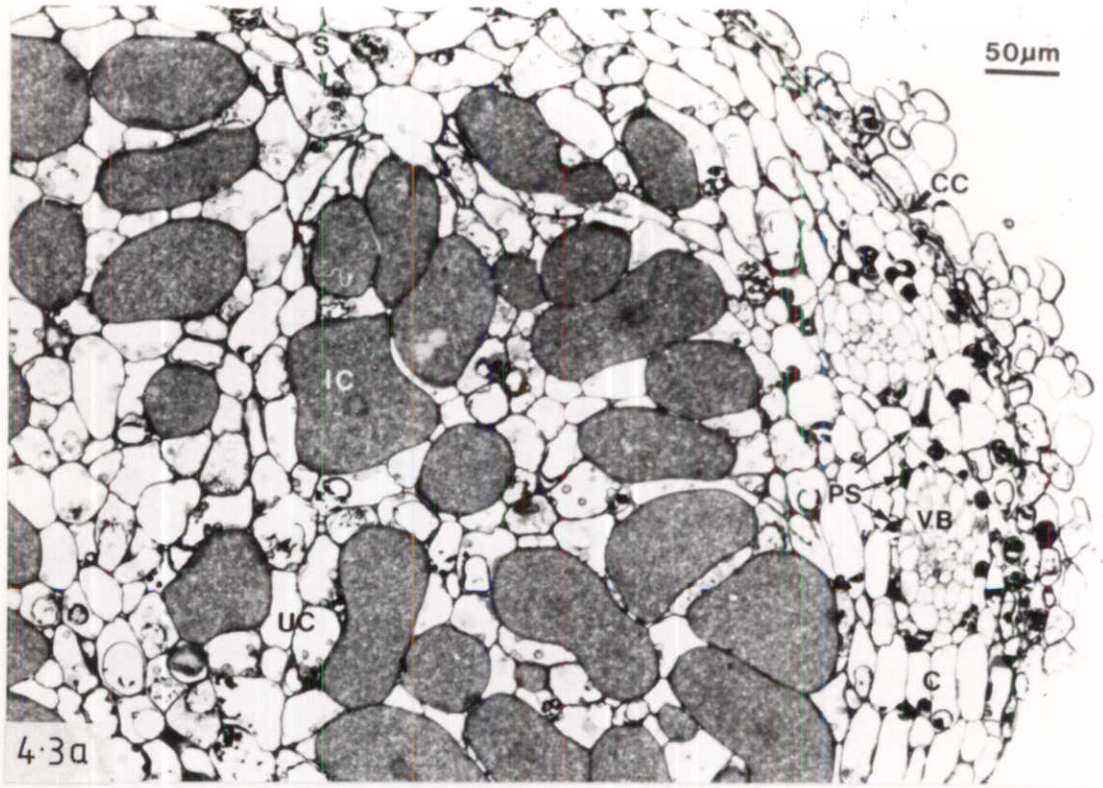
4·2b

Figure 4.3a Light micrograph of transverse section of mature *Acacia polyacantha* nodule, at a higher magnification than in 4.2a.

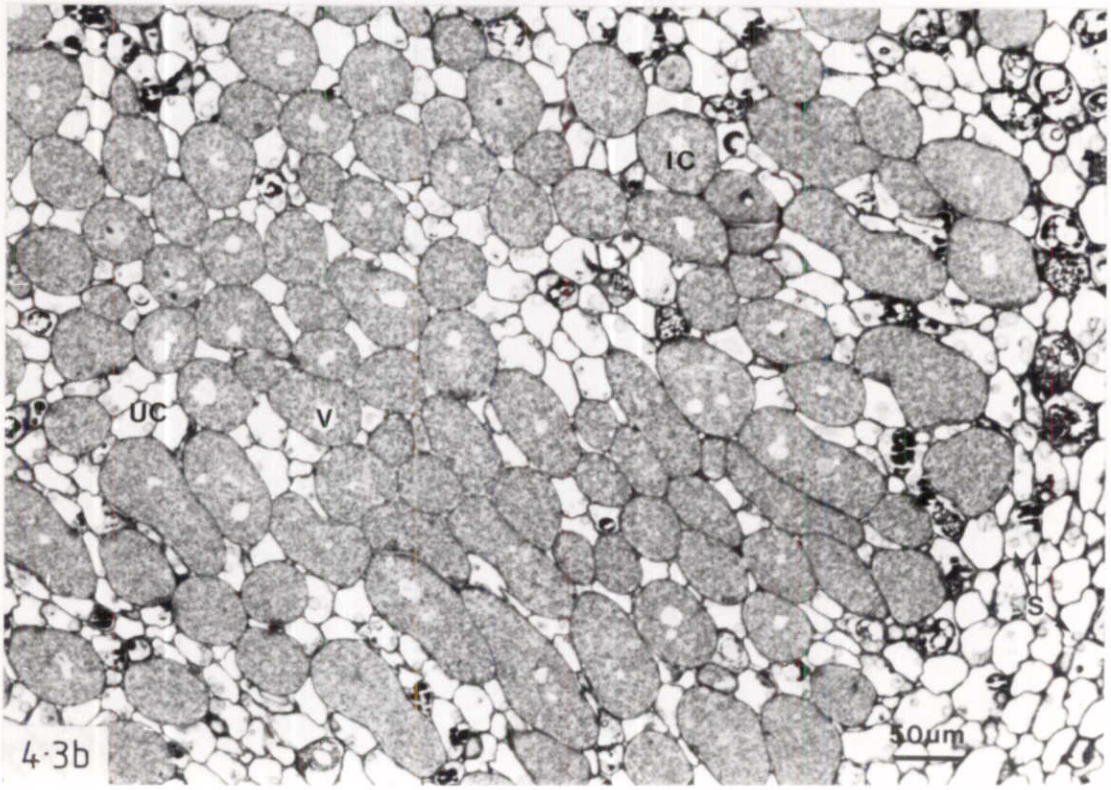
Figure 4.3b Light micrograph of transverse section of mature *Faidherbia albida* nodule, at a higher magnification than in 4.2b.

Abbreviations

C	Cortex
CC	Cork cambium
IC	Infected cell
PS	Phenolic substance
S	Starch
UC	Uninfected cell
V	Vacuole
VB	Vascular bundle



4·3a



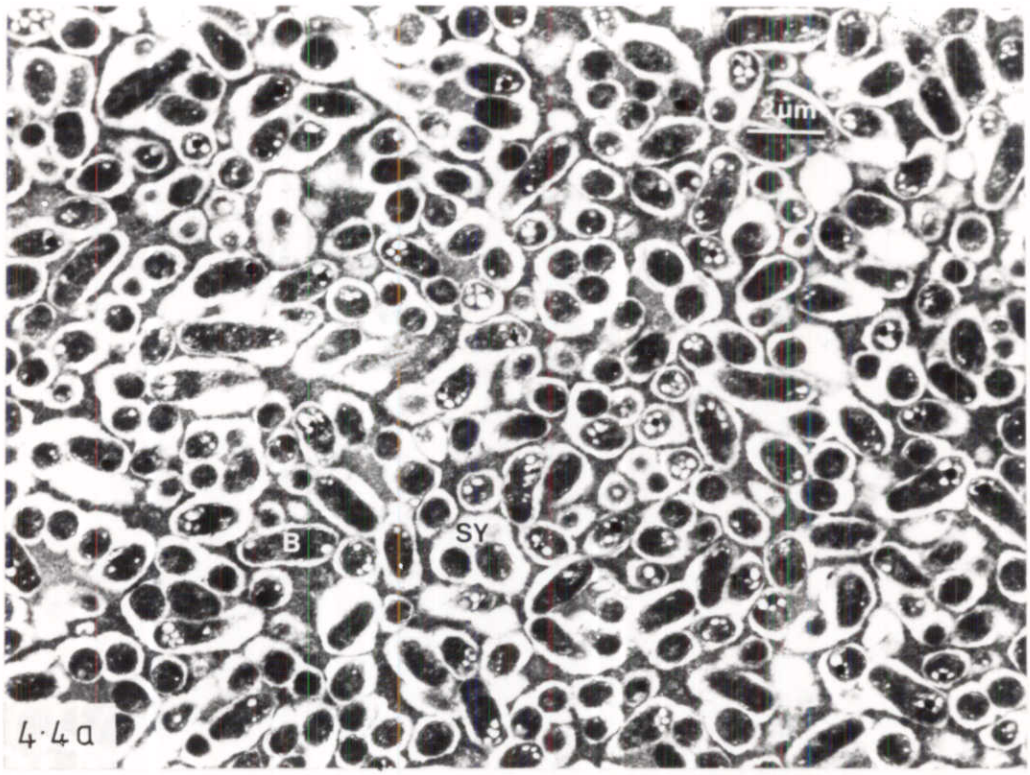
4·3b

Figure 4.4a Transmission electron micrograph of transverse section of mature *Acacia polyacantha* nodule.

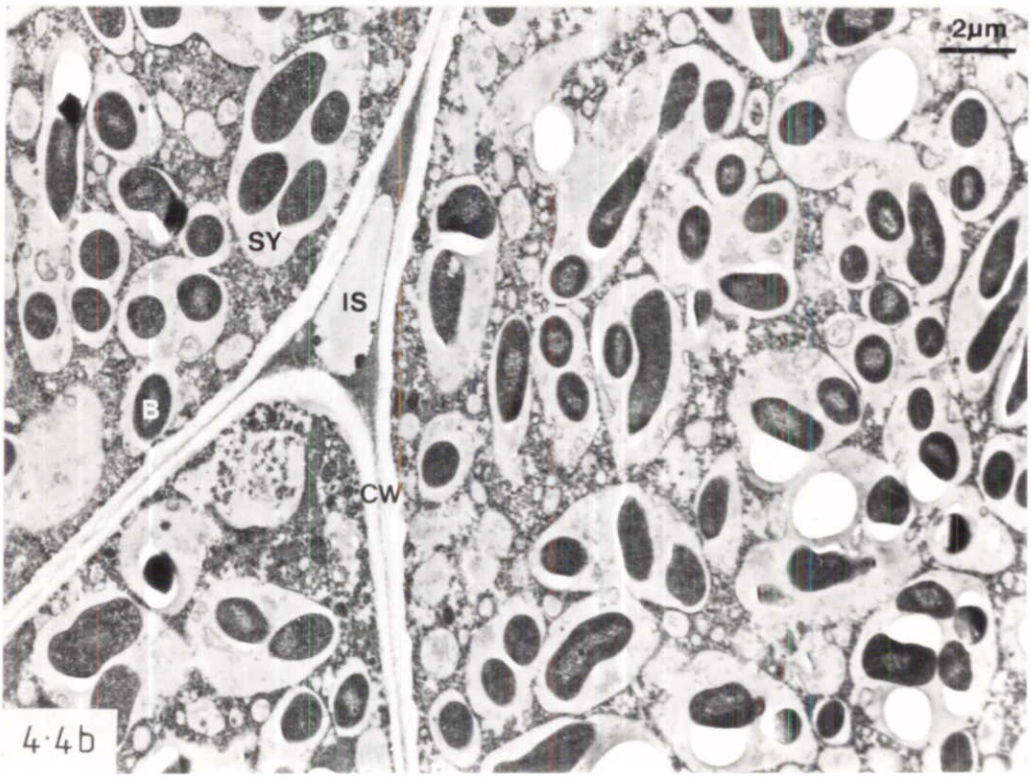
Figure 4.4b Transmission electron micrograph of transverse section of *Faidherbia albida* nodule.

Abbreviations

B	Bacteroid
CW	Cell wall
IS	Intercellular space
SY	Symbiosome



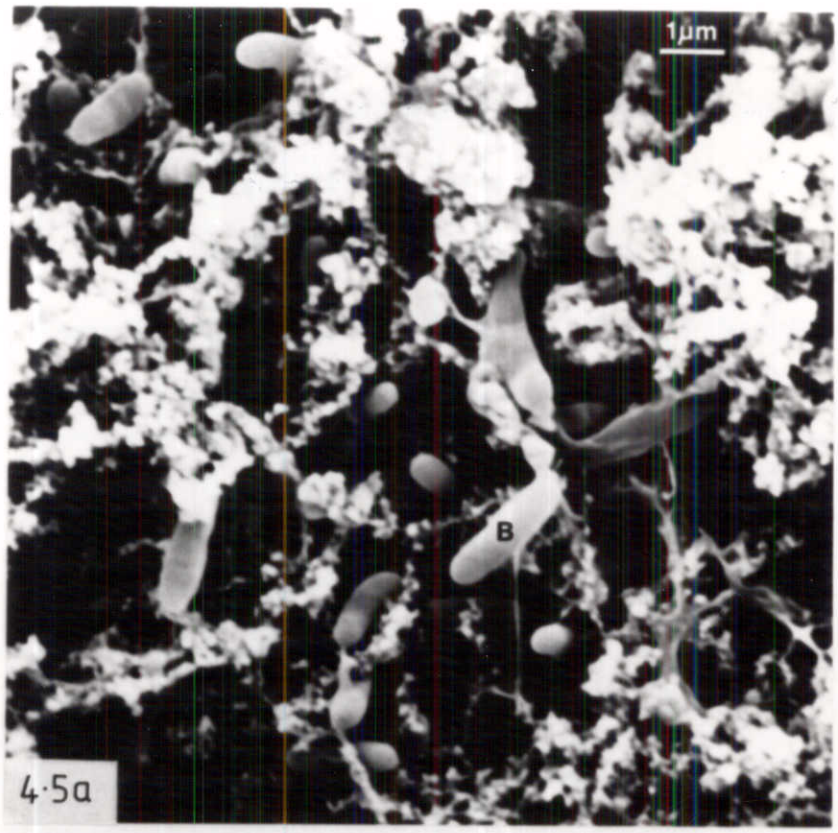
4·4a



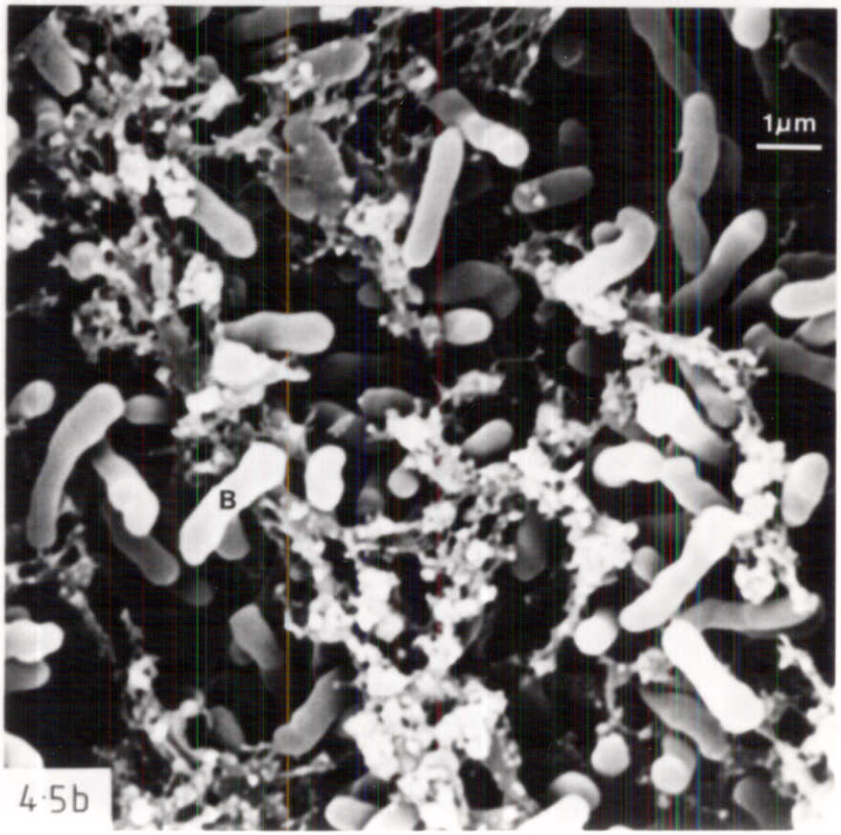
4·4b

Figure 4.5a Scanning electron micrograph showing bacteroids (B) in a mature *Acacia polyacantha* nodule.

Figure 4.5b Scanning electron micrograph showing bacteroids (B) in a mature *Faidherbia albida* nodule.



4·5a



4·5b

A. polyacantha. The major differences between the nodule structures of the two species are shown in Table 4.1.

TABLE 4.1. Comparison of some of the major differentiating features of the nodule structures

	Species	
	A. polyacantha	*F. albida*
Vacuoles in infected cells	absent	present
Phenolic substances in cortex and interstitial cells	less abundant	more abundant
Starch in cortex and interstitial cells	less abundant	more abundant
infected cells	fewer, very large less contiguous	more but smaller and mainly contiguous
Uninfected cells	more abundant, evidence of rays of uninfected cells therefore more contact with infected cells	less abundant therefore less contact with infected cells
Bacteroids	densely packed in infected cells. Bacteroids are enveloped in diffuse peribacteroid membrane (PBM)	less densely packed. Bacteroids in well defined PBM.

The two species appear to have different strategies in accomplishing nitrogen fixation as seen in their spatial distribution of infected and uninfected cells. *Acacia polyacantha* invests more in large cells with a greater number of bacteroids per unit volume, and the relatively fewer starch

granules in the surrounding and adjacent infected cells may be testimony to the energy requirements of this system. On the other hand *F. albida* shows a spatial distribution more akin to those observed in other mimosoid woody legumes eg. *Prosopis glandulosa* (Baird *et al*, 1985). If there is indeed a mechanism that ensures that a certain number of cells remain uninfected as suggested by Selker and Newcomb (1985), then that mechanism operating in *A. polyacantha* which targets only a few cells to be infected, but so densely as to be parasitic, may be regarded as being more primitive.

The conclusion from root nodule morphology is that both species showed similar characteristics, which appear to be universal in mimosoids reported so far. Differences shown in internal structures indicate that there is a potential in nodule structure as tool to aid in the taxonomy of acacias in general. However, further exhaustive work, looking at the infection and nodule morphogenesis using different rhizobial strains or species, will be required to determine whether the structural differences are consistent.

CHAPTER 5

5.0 CHARACTERISTICS AND COMPOSITION OF RHIZOBIA IN SOILS FROM DIVERSE SITES OF KENYA

5.1 INTRODUCTION

The genus *Acacia* is one of the most important taxonomic groups of legumes in the country. *Acacia* species are widely distributed, growing from low to high altitude (Bogdan and Pratt, 1974), and represented in nearly all the eco-climatic zones of Kenya. They are particularly prominent in the arid and semi-arid lands (ASALs), where they form a unique floral landscape with other forms of perennial vegetation almost totally excluded due to prevalent harsher environmental conditions. Other important woody legumes have a relatively restricted distribution; for example *Sesbania sesban* occurs mainly in the more humid upland areas of Kenya (Agnew, 1974).

Legumes are strongly represented in natural habitats of the various eco-climatic zones, and constitute potential sources of diverse indigenous rhizobial populations. However, there is no known exhaustive work concerning rhizobial types associated with woody legumes in the country. Early reports of rhizobia associated with woody legumes in tropical regions described them as slow-growing cowpea type or miscellany (Lange, 1961; Basak and Goyal, 1975, 1980a, 1980b; Habish and Khairi, 1968, 1970) largely because they nodulated *Vigna* spp. More recent reports (Dreyfus and Dommergues, 1981; Lawrie, 1983; Barnet *et al*, 1985; Barnet and Catt,

1991) have shown that they actually represent the two major genera: *Rhizobium* and *Bradyrhizobium* (Jordan, 1984). Zang *et al* (1991) showed that rhizobia from woody legumes in the genus *Rhizobium* are so phenotypically diverse as to suggest taxonomic differences.

The objectives of this study were therefore to:

(a) isolate indigenous rhizobia associated with native woody legumes,

(b) delineate the isolates using cultural, morphological and physiological characteristics and

(c) tentatively relate them to the recognized rhizobial types.

5.2 MATERIALS AND METHODS

5.2.1 Isolation and delineation of rhizobia by growth characteristics

Nodules were obtained from the experiments described in chapter 3. In addition, some herbaceous legumes namely, *Phaseolus vulgaris*, *Macroptilium atropurpureum* and *Vigna unguiculata* were used to trap rhizobia in two of the several soil collections from Kibwezi savanna site. In instances where there was abundant nodulation, at least 10 nodules were randomly selected from roots of plants of each treatment (soil x species/provenance x sampling occasion combination) for isolation attempts. Every nodule was used for isolation in the event of scanty nodulation ($\leq$ 10 nodules).

Nodules were surface sterilized in 1 % NaOC1 for 6 min and rinsed in several changes of sterile water on a sterile petri-dish and then crushed with a flame sterilized blunt-tipped pair of forceps. A loopful of the crushed

nodule was then streaked across the surface of YMA plate. Typical well isolated colonies were re-isolated on diagnostic media namely

(a) YMA plates containing 25 ppm (w/v) bromothymol blue (BTB) as pH reaction indicator,

(b) YMA plates containing 25 ppm (w/v) congo red and

(c) peptone glucose agar plates (see appendix 3) containing 100 ppm (w/v) bromocresol purple (BCP).

Media (b) and (c) are used to determine the coexistence of contaminants with rhizobia. Rhizobia absorb little or no congo red in the dark and grow poorly in peptone glucose agar (Somasegaran and Hoben, 1985) with little pH change (Brockwell, 1980). However, there are exceptions to this rule (see Jordan, 1984). Colonies were reisolated on fresh plates where there was more than one type of colony from a single nodule.

The rhizobial isolates were characterized by colony appearance and colour, extracellular polysaccharide slime (EPS) production, ability to change pH of the media and growth size attainment of the first discrete colonies to appear away from confluent growth on YMA plates incubated at 28°C. The growth sizes were determined by measuring to the nearest mm the diameter of the colonies using a x10 magnifier with graticule, at 3, 5, 7 and 10 d of incubation. This set of data was used to categorize the isolates according to growth rates as described by Jordan (1984) with some modifications to fit isolates whose growth rate were not falling within the 'recognized' divisions. The categories were as follows:

(a) Very fast growers - colonies $\geq$ 5 mm in diameter within 3 d of

incubation, acid producers;

(b) Fast growers - colonies ≥ 2 mm in diameter within 5 d of incubation, acid producers;

(c) Intermediates - colonies 1 - 2 mm in diameter within 5 - 7 d of incubation, two subgroups (i) acid producers (ii) alkali producers; and

(d) Slow growers colonies ≤ 1 mm within 7 d of incubation, within this category were those isolates with colonies appearing at 10 d and which were considered very slow. All isolates in this category were alkali producers.

Cultures representing members of the different colony types and categories described above were authenticated on homologous or appropriate host species. All isolates, both authenticated and presumptive were transferred to YMA slants in universals or Mcartney bottles and stored at 4 °C for further work. There was no evidence of either colony variance (Kuykendall, 1987) or dimorphism (Sylvester-Bradley *et al*, 1988) between subculturing and after storage.

5.2.2 Determination of mean generation times (MGT)

Representative isolates of the 4 categories above were selected to determine their MGT in YMB. Each isolate was grown as a starter culture in sterilized 10 cm^3 of YMB in a Mcartney bottle to late exponential phase ($\sim 10^9$ cells cm^{-3}). Thirty μl of the starter culture was then used to inoculate 100 cm^3 of sterilized YMB in a 250 cm^3 conical flask. The inoculum was dispersed and mixed thoroughly into the broth by twirling the flask clockwise

and counter-clockwise for 10 s. One cm^3 was removed and serially diluted (10-fold) in quarter strength YMB and then plated by the drop-plate method (Somasegaran and Hoben, 1985) to perform a zero-time viable count. The inoculated flask was incubated on a rotary shaker (100 rev min^{-1}) at 28°C. Viable counts were performed every 24 h until 7 d. Each isolate was assayed in duplicate. Viable counts data were transformed to $\log_{10}$ and a growth curve was plotted against time. Mean generation time was computed using values from the exponential phase of the curve.

5.2.3 Rhizobial affinities among selected test plant species

A cross-inoculation experiment of indigenous rhizobial isolates with test plant species was performed to determine the rhizobial affinities between or among some of the test plants in this study. Five species and cultures originally isolated from them were used namely (strain no. in brackets) *Faidherbia albida* (S2, fast), *Acacia tortilis* (S10, fast), *Acacia polyacantha* (S1, fast), *Sesbania sesban* (S13, fast) and *Macroptilium atropurpureum* (S24, slow). *Leucaena leucocephala* was also included with its homologous strain KFR 818A (formerly CIAT 1967). Strain S2 was initially authenticated on *A. polyacantha*.

Three axenically germinated seedlings were transplanted to Leonard jars and inoculated 3 d later with 1 cm^3 aliquot of yeast extract mannitol broth (YMB)-grown cultures containing $\sim 10^9$ cells cm^{-3} in late exponential phase. Three jars were established for each strain x test plant species combination, 3 jars +N (70 ppm KNO_3) and 3 jars - N uninoculated plants

were included as controls. Plants were thinned to two per jar after 2 weeks. All plants were grown in the same conditions and duration as for MPN experiments (chapter 3). At harvest plants were subjectively categorized as follows:

(a) vigorous growth and green colour, better than +N control = very effective (VE),

(b) growth and colour comparable with +N control = effective (E),

(c) growth stunted and yellow = ineffective (e), and

(d) no nodulation, growth stunted and yellow = non-infective (i).

All uninoculated controls were free of nodules. *Sesbania sesban* cross-inoculation with S24 produced isolates not identical with the inoculant strain and was therefore considered as exogenous contamination.

5.2.4 Determination of intrinsic antibiotic resistance (IAR) and salt tolerance level

Preparation of media containing various concentrations of antibiotics namely streptomycin (str) kanamycin (kan) and ampicillin (amp), and NaC1 are described in chapter 2. Each plate contained approximately 30 cm^3 of agar media. Poured plates were surface dried in a laminar flow cabinet (Bassaire, Southampton, U.K.) for 20 min before use.

Each isolate was grown to late exponential phase and diluted in quarter strength YMB that gave an inoculum of 10^4 viable cells of rhizobia at the point of inoculation on the agar surface. The size of inoculum was therefore within the recommended range known to give reliable results while

avoiding the problems of spontaneous mutations (Josey *et al*, 1979). The pins of the multipoint inoculator were sterilized by dipping in 95% ethanol and then flaming with a bunsen burner at each change of treatment (i.e. antibiotics/NaC1 concentration and fresh load of isolates).

A batch of 20-40 isolates was assayed at any single time. There were 15 such batches that constituted a run. As a check on the stability and reproducibility of data, a second run was assayed in the same sequence as was in the first run. The two runs were carried out over a period of 9 months (1992). Reproduciblity was as follows:

(a) comparison of replicates within a batch was > 99% (str) and 100% (kan and amp),

(b) comparison of consecutive batches by inclusion of 10 randomly selected isolates from a previous batch in the subsequent batch numbering to 14 such comparisons per run was 99% (str) >99% (kan) and 98% (amp).

(c) Isolate to isolate comparison in a batch in the first run with a corresponding batch in the second run was 90% (str), 97% (kan) and 90% (amp). In this category it was observed that lower reproducibility was as a result of the instability of some isolates from Nyamonye and Loruk sites.

For NaC1, reproducibility was > 99% in similar comparisons described above.

Minimum inhibitory concentrations (MIC) of the 3 antibiotics were determined on 38 randomly selected isolates that had IAR on at least one of

the antibiotics at 40 μg cm^{-3} and were different from one another in IAR patterns. The isolates were assayed on YMA plates containing 50, 100, 200, 300, 400, 500 and 1000 μg cm^{-3} each of the 3 antibiotics. Each concentration was assayed in duplicate. Two natural mutants selected at str 200 μg cm^{-3} (J. Shaw, personal communication) of *Rhizobium* stains DUS 408 and DUS 390 (formerly B556 and NHRlegD respectively, from John Innes Institute) were included as reference strains. Inoculated plates were incubated and read as above.

5.2.5 Growth curve characteristic of a typical EPS producer in YMB

Because of the observation in IAR results that a considerable proportion of some EPS producers (31%, 33% and 43% for str, kan and amp respectively) appeared to increase in size when subjected to antibiotics at concentrations below their MIC, a typical isolate code-numbered GW5/S10/P2/2 was used to determine whether the increase in size was due to para-normal EPS production or increase in cells numbers (see also Figure 5.2). The MIC of this isolate was relatively high: 500, 50 and $>$ 1000 μg cm^{-3} of str, kan and amp respectively.

The isolate was grown in 10 cm^3 of YMB in a Mcartney bottle as a starter culture before being used to inoculate 100 cm^3 of YMB in 250 cm^3 conical flasks bearing one of the following treatments:

(a) antibiotic-free (control)

(b) 200 μg cm^{-3} of str

(c) 40 μg cm^{-3} of kan

(d) 400 μg cm^{-3} of amp.

All treatments were duplicated. Optical density (OD) at 540 nm was determined for each treatment over 48 h, in addition to viable count assays as for MGT. Growth curves were plotted of viable counts (log_{10} transformed) and OD against time.

5.2.6 Numerical analyses of IAR and salt tolerance data

Cluster analyses were performed with Genstat 5.0 using IAR and salt tolerance data of the isolates. The isolates were defined as entities and antibiotics/NaCl concentration levels as character states (Sneath, 1984). For computational purposes the character states were coded and entered as 1 for resistance and 0 for sensitivity. Data were analyzed on an individual or combined site basis depending on the number of isolates. Hierarchial clustering analysis was done using unweighted average linkage method. Results were presented as % similarity or relatedness of isolates as phenograms. Randomly selected isolates from major phenons across the sites were re-analyzed in two separate groups as *Rhizobium* and *Bradyrhizobium* (see later groupings in discussion). Dundee University cultures were included as reference strains namely (host of isolation or rhizobial species in brackets):

(a) *Rhizobium*; DUS 8 (*Acacia tortilis*), DUS 13 (*Faidherbia albida*),

DUS 203 (*A. seyal*) and DUS 255 (*A. polyacantha*),

(b) *Bradyrhizobium*; DUS 18 (*Millettia usaramensis*), DUS 19 (*Indigofera tinctoria*). DUS 88 (*A. auriculiformis*), DUS 195 (*A. decurrens*), DUS 89 (*Bradyrhizobium japonicum* WPBS 3407) and DUS 283 (*Parasponia* sp.). Results were presented as phenograms and in 3-D perspective drawings derived from the principal coordinate analysis tables.

5.3 RESULTS

5.3.1 Characterization of isolates by growth morphology and growth rates

Over 480 isolates were recovered from the nodules of the trap host species grown in soils from various sites. There were 3 major colony types: Watery and milky translucent and curdled milk type. These types were circular, shiny, raised and produced moderate to copious EPS. They constituted the bulk of the total number of isolates (91%). The rest were categorized as creamy and white opaque and others with none or moderate EPS production (Table 5.1).

Categorization by growth rate showed that 47.4% were fast growing and 10% were slow-growing. The other categories outwith the recognized types were very fast (34.3%) and intermediates (8.4%), 63.4% of the latter groups had similar growth habit attributes to slow-growers e.g alkali production in YMA, except that they had a slightly different growth rate: 1 - 2 mm in colony diameter within 5 - 7 d and MGT of 5.6 - 5.7 h (see Tables 5.2 and 5.3).

TABLE 5.1. Proportion of colony characteristics of rhizobial isolates on YMA

Colony type	Extracellular polysaccharide slime production category	Growth rate type represented[a]	Proportion (%)
Watery translucent	moderate to copious	**Very Fast**, Fast	39.4
Milky translucent	moderate to copious	Very Fast, **Fast**, Slow	35.3
Curdled milky type	moderate to copious	Very Fast, Fast **Intermediate, Slow**	17.0
Creamy opaque	None	Fast, **Slow**	4.9
White opaque	None to moderate	Fast, **Slow**	3.3
Others	None to moderate	Fast	0.8

[a] Bold indicates growth rate type contributing the highest proportion to the colony type category.

TABLE 5.2. Generation times in YMB of representative isolates for the various colony types

Growth rate category	Colony type represented	No. tested	Mean generation time (h)	Range
Very fast	Watery translucent, Milky translucent	8	2.1	1.6 - 2.5
Fast	Watery translucent, Milky translucent	4	3.8	2.8 - 4.8
Intermediate	Curdled milky type, Milky translucent	2	5.7	5.6 - 5.7
Slow	Curdled milk type Creamy opaque, White opaque	3	7.6	6.4 - 8.8

TABLE 5.3. Growth rate characteristics of rhizobial isolates in soils from various sites

characteristics / Source of soil	Number of isolates with growth rate			
	Very fast	Fast	Intermediate	Slow
Nyamonye	26	51	6,3[a]	1
Yala swamp	25	49	3,1[a]	1
Marigat	20	46	2[a]	8
Loruk	13	8	1	3
Lodwar shrubland	36	7	0	6
Lodwar riverine	5	1	0	0
Kibwezi savanna	9	20	18[a]	15
Kibwezi riverine	4	5	2	0
Bura savanna	3	5	0	2,2[b]
Bura riverine	3	5	3,1[a]	5
Gede white sand	9	22	0	2
Jilore red soil	14	12	1[a]	3

[a]Isolates with intermediate growth that produce alkali in YMA

[b]Very slow, colonies appear in 10 d.

Results in Table 5.3 also indicate that among the sites contributing large numbers of isolates, Lodwar shrubland was predominantly represented by very fast-growing rhizobia while Kibwezi savanna was predominantly intermediates (alkali producers) and slow-growing types. Table 5.4 showed that only 4 woody legume species had nodules occupied by the slow-growing and alkali producing intermediate types namely: *F. albida* (50%), *A. tortilis* (8.1%), *A. nubica* (5.4%) and *A. polyacantha* (1.4%). The bulk of the alkali producing intermediates were trapped by herbaceous legumes (*M. atropurpureum* and *V. unguiculata*) at Kibwezi savanna site.

Nodules were predominantly occupied by a single type of rhizobia. However about 6.6% of isolates were recovered as dual occupants of different types from a single nodule; there were no multiple occupants (Table 5.5). Results on the dual occupancy also showed that in some instances only one of the cohabitants was able to cause nodulation on the host of isolation; this did not imply that the other was non-infective on another host. Nearly all the possible combinations of cohabitants in terms of growth rate and cultural characteristics were retrieved indicating that it was not a specific occurrence. However, results presented in Table 5.6 of the resistance patterns comparing each pair showed that the response to the antibiotics and NaC1 concentration levels between each pair was in most cases different; one being more tolerant or sensitive than the other.

TABLE 5.4. Growth rate characteristics of rhizobial isolates trapped with various legumes

Host of Isolation		No of isolates with growth rate characteristics			
		Very Fast	Fast	Intermediate	Slow
(a)	Woody Legumes				
	Acacia mellifera	2	6	0	0
	Acacia nilotica	7	6	1	0
	Acacia nubica	24	12	1	4
	Acacia polyacantha	31	22	3,1[a]	0
	Acacia reficiens	0	1	0	0
	Acacia senegal	0	7	0	0
	Acacia seyal	1	6	0	0
	Acacia tortilis	33	48	4,3[a]	3
	Acacia xanthophloea	9	28	0	0
	Faidherbia albida	29	22	3,5[a]	30,2[b]
	Prosopis juliflora	0	2	0	0
	Sesbania grandiflora	14	16	1	0
	Sesbania sesban	17	41	2	0
(b)	Herbaceous Legumes				
	Phaseolus vulgaris	0	8	0	0
	Macroptilium atropurpureum	0	6	11[a]	5
	Vigna unguiculata	0	0	6[a]	4

[a] Isolates with intermediate growth rate that produce alkali in YMA
[b] Very slow, colonies appear in 10 d

TABLE 5.5. Occurrence of dual occupancy of different types of rhizobia in a single nodule (see footnotes)

Site	No.	Host of isolation	Types of isolate[a] 1	2	Type infective on host of isolation[b]
Nyamonye	1	*Acacia polyacantha*	Milky translucent (F, + +)	Watery translucent (VF, + +)[c]	1
Yala swamp	2	*Acacia xanthophloea*	Milky opaque (F, + +)	Watery translucent (VF, + + +)	1,2
Yala swamp	3	*Faidherbia albida*	White opaque (F, +)	Creamy opaque (S,O)	2
Marigat	4	*Faidherbia albida*	Curdled milk type (I/al, + +)	Creamy opaque (S,O)	1,2
Marigat	5	*Faidherbia albida*	Curdled milk type (F, + +)	Curdled milk type (S, + +)	2
Loruk	6	*Faidherbia albida*	Milky translucent (F, + +)	Curdled milk type (S, + +)	2
Lodwar shrubland	7	*Acacia nubica*	Watery translucent (VF, + +)	Curdled milk type (S, + +)	1
Lodwar shrubland	8	*Acacia nubica*	Milky translucent (VF, + + +)	Curdled milk type (S, + +)	1
Kibwezi savanna	9	*Macroptilium atropurpureum*	Milky translucent (I/al, + +)	Curdled milk type (S, + +)	1,2
Kibwezi riverine	10	*Acacia xanthophloea*	Milky translucent (VF, + +)	Watery translucent (VF, + +)[c]	1
Bura savanna	11	*Faidherbia albida*	Watery translucent (VF, + + +)[c]	Creamy opaque (S,O)	2

Table 5.5. Continued

[a] VF = very fast, F = fast, I/al = intermediate and alkali producer, S = slow, +++ = copious EPS, ++ = moderate EPS, + = little EPS, 0 = no EPS, dry.

[b] ability of one or both types to cause nodulation on host of isolation, data based on authentications.

[c] isolates which were not authenticated but considered as presumptive rhizobia.

TABLE 5.6. Resistance patterns to antibiotics and NaCl of dual occupants in a single nodule

Isolate pair No.[a]	Isolate type[b]	Str ($\mu g\ cm^{-3}$) 5	10	20	40	Kan ($\mu g\ cm^{-3}$) 5	10	20	40	Amp ($\mu g\ cm^{-3}$) 5	10	20	40	% NaCl (w/v) 0.1	0.5	1.0	2.0	3.0
1 ARN1/S1/P1 (1)	1	+[c]	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-
ARN1/S1/P1 (2)	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2 RY2/S11/P1/8 (2)	1	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-	-
RY2/S11/P1/8 (1)	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
3 RY5/S2/P1/10 (1)	1	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-	-
RY5/S2/P1/10 (2)	2	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	-	-
4 MB5/S2/P2/1 (1)	1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
MB5/S2/P2/1 (2)	2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
5 MB5/S2/P1/6 (2)	1	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	-	-
MB5/S2/P1/6 (1)	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
6 BL5/S2/P1/7 (2)	1	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-
BL5/S2/P1/7 (1)	2	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-	-
7 LD5/S7/P1/7A (2)	1	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+
LD5/S7/P1/7 (1)	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8 LD5/S7/P3/5 (1)	1	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-
LD5/S7/P3/5 (2)	2	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-	-
9 KD6u/S24/P1/8 (1)	1	+	+	+	-	+	+	+	-	+	-	-	-	+	-	-	-	-
KD6u/S24/P1/8 (2)	2	+	+	+	-	+	+	+	-	+	+	+	-	+	-	-	-	-
10 KR5/S11/P4/ (1)	1	+	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-
KR5/S11/P4/ (2)	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
11 BD5/S2/P1/7 (2)	1	+	-	-	-	+	+	-	-	+	+	+	-	+	-	-	-	-
BD5/S2/P1/7 (1)	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-

[a] isolate pair no. is the same as host of isolation number in Table 5.5

[b] isolate type as in table 5.5

[c] + = growth, - = no growth

Results of the cross inoculation experiment (Table 5.7) showed that *M. atropurpureum*, *A. polyacantha* and *S. sesban* were effectively nodulated by their homologous strains. *Leucaena leucocephala* was ineffectively nodulated with its homologous strain KFR 818. *Faidherbia albida* was effectively nodulated by strain S24 while it did not nodulate with other strains. *Acacia tortilis* did not nodulate at all. Strains S2 and S10 effectively nodulated *A. polyacantha* and *S. Sesban* while they failed to nodulate their homologous hosts.

5.3.2 Tolerance to antibiotics and NaC1

More than 50% of isolates on a site basis from (in descending order) Lodwar riverine, Jilore, Lodwar shrubland, Marigat and Bura savanna were tolerant to str 40μg cm^{-3}; and Lodwar riverine, Lodwar shrubland, Jilore, Yala swamp, Loruk and Gede were tolerant to kan 40 μg cm^{-3}. In general isolates across the sites were tolerant to amp 40 μg cm^{-3}; only 3 sites namely: Kibwezi riverine, Kibwezi savanna and Nyamonye had less than 50% tolerant isolates.

Salt tolerance was greatest at Lodwar sites and Loruk site. The least tolerant isolates were from Jilore and Yala swamp sites (Table 5.8).

Fig 5.1 illustrates the response to the antibiotics and NaC1. In general, str was the most potent antibiotic while kan and amp were comparable. The alkali-producers were very sensitive to higher concentrations of antibiotics (40 μg cm^{-3}) and NaC1 concentration $\geq$ 0.5% (see also Figures 5.2 and 5.3).

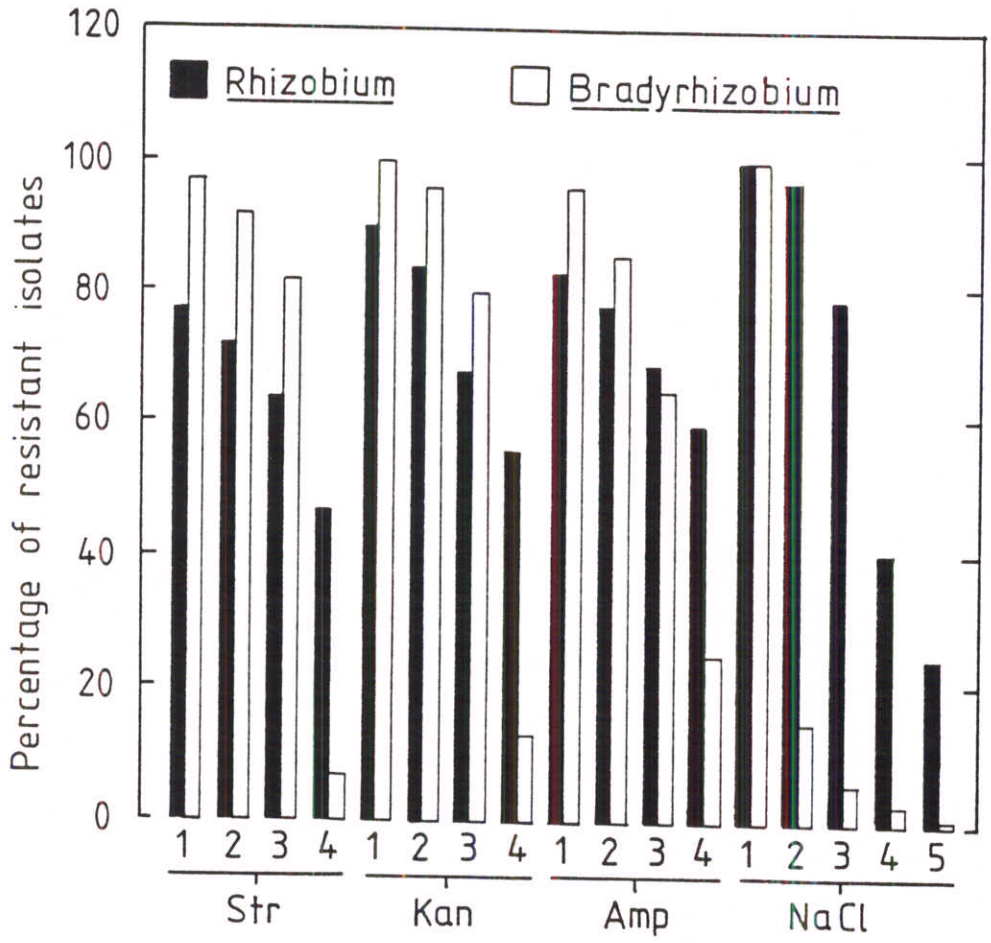


Figure 5.1 Rhizobial tolerance to antibiotics and NaCl in YMA (yeast extract mannitol agar).

Str, Kan, Amp (antibiotics) level 1 = 5 μg cm^{-3}, 2 = 10 μg cm^{-3}, 3 = 20 μg cm^{-3}, 4 = 40 μg cm^{-3}.

NaCl level 1 = 0.1%, 2 = 0.5%, 3 = 1.0%, 4 = 2.0% (w/v).

Figure 5.2 Growth of isolates on YMA plates (containing antibiotics) inoculated with a multipoint inoculator.

(a) Control plate (no antibiotics)

(b) Schematic diagram showing orientation of isolates (OP = orientation point)

isolates numbered 1-7 = *Bradyrhizobium*

isolates numbered 8-12, 18-19 = *Rhizobium*

isolates numbered 13-17 = *Rhizobium*, EPS producer that appear to increase in size when subjected to antibiotic concentrations below their MIC.

Number 20 is non-rhizobia (a 'contaminant')

(c)-(h) Growth of the above isolates in different concentrations of antibiotics.

All plates are same size, reduced to 2/3 of the original size.

STR = Streptomycin

KAN = Kanamycin

AMP = Ampicillin

Numbers after antibiotic labels represent concentrations in $\mu g\ cm^{-3}$.

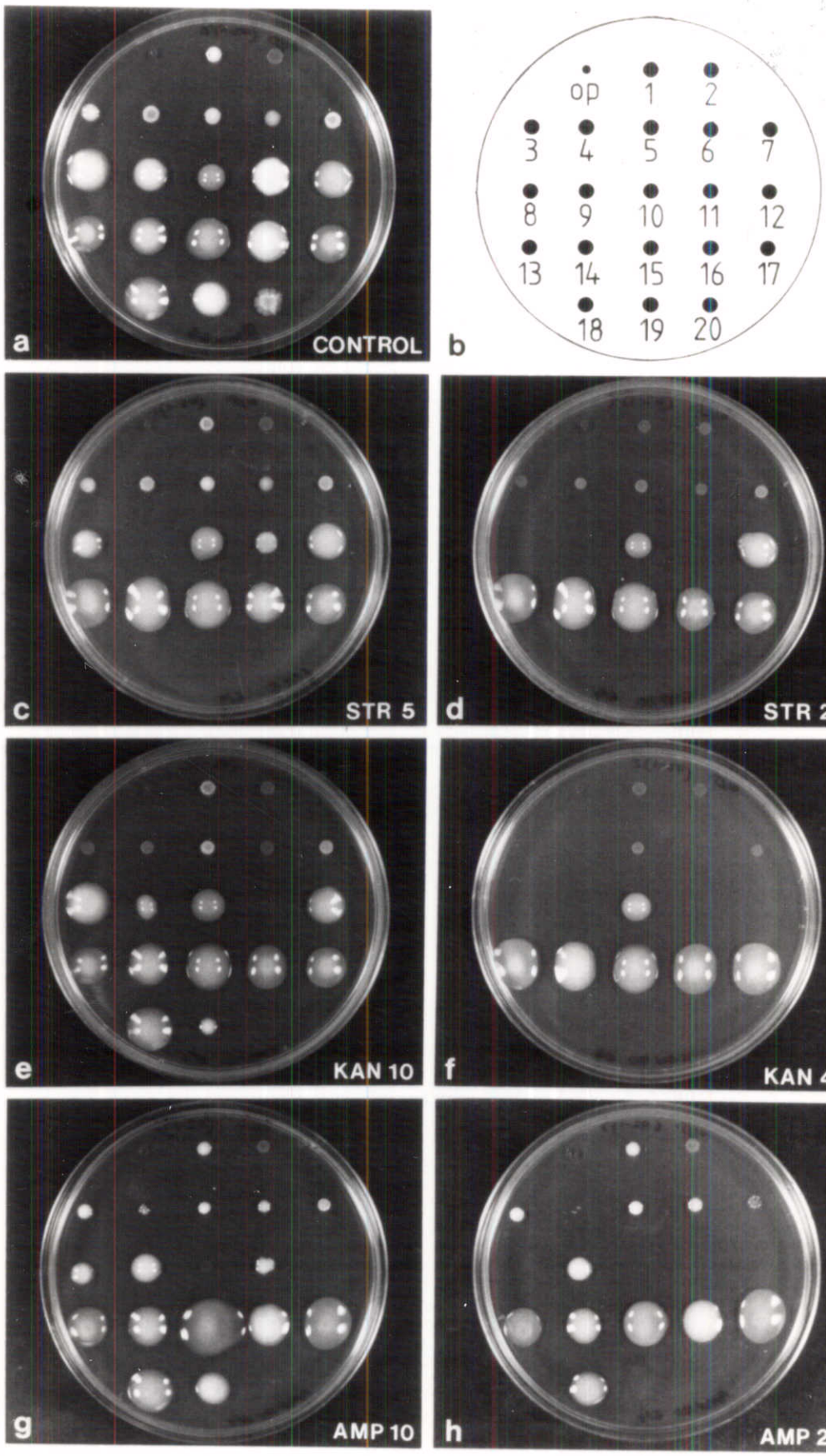

a
CONTROL
b
op
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
c
STR 5
d
STR 20
e
KAN 10
f
KAN 40
g
AMP 10
h
AMP 20

Figure 5.3 Growth of isolates on YMA plates (containing increasing NaCl concentrations) inoculated with a multipoint inoculator.

(a) Control plate (normal NaCl concentration, 0.01%)

(b)-(f) Increasing NaCl concentrations: 0.1, 0.5, 1.0, 2.0 and 3.0%.

Isolates and order of arrangement, and plate size are as in Figure 5.2.

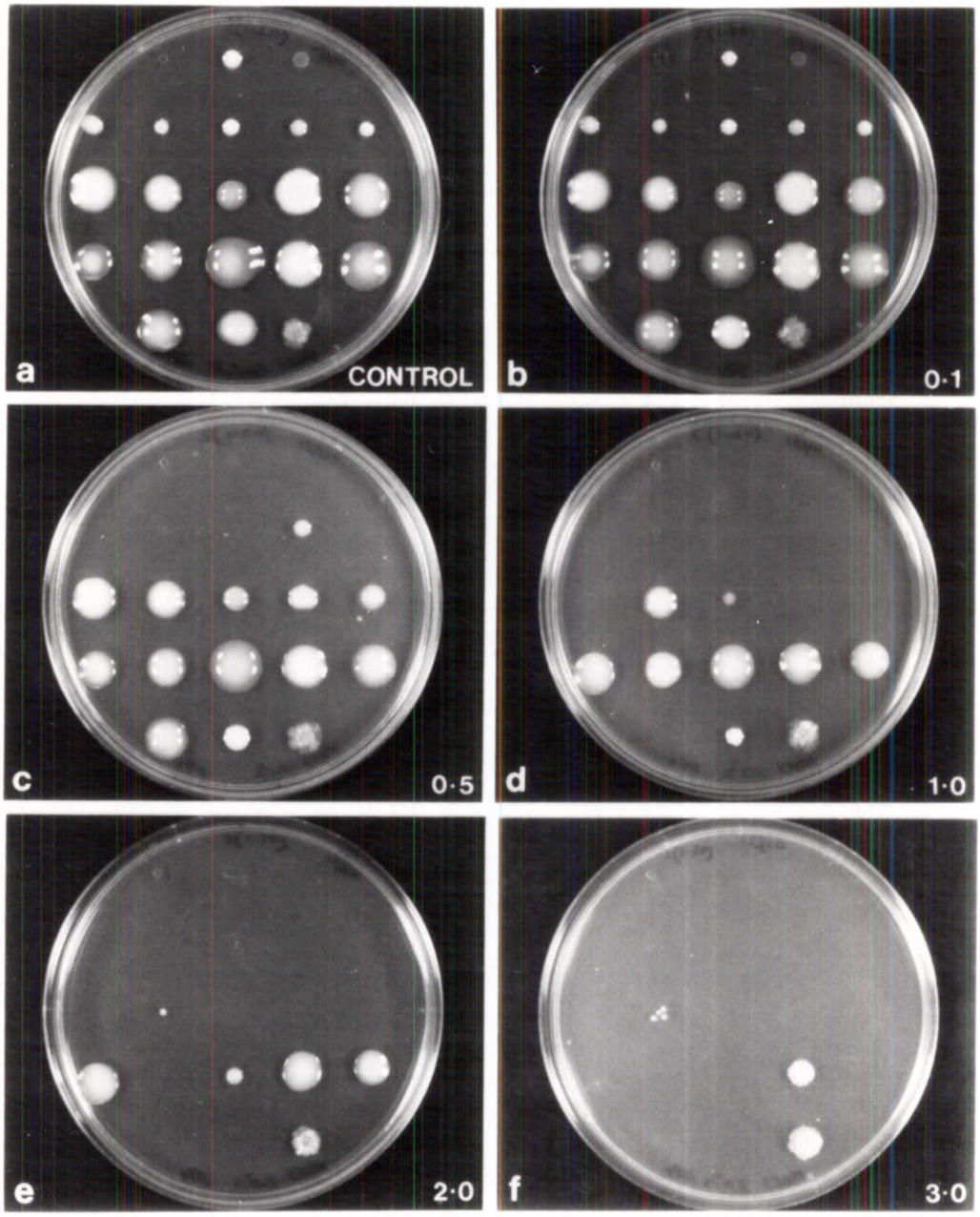

a
CONTROL
b
0·1
c
0·5
d
1·0
e
2·0
f
3·0

TABLE 5.7. Rhizobial affinities among selected test plant species

Test plants species		Strain no. (host of isolation in brackets)					
		S24 (Ma)	818A (Ll)	S2 (Fa)	S1 (Ap)	S13 (Ss)	S10 (At)
Macroptilium atropurpureum	(Ma)	VE[a]	i	i	i	i	i
Leucaena leucocephala	(Ll)	e	e	i	e	e	i
Faidherbia albida	(Fa)	E	i	i	i	i	i
Acacia polyacantha	(Ap)	e	i	E	VE	i	E
Sesbania sesban	(Ss)	E[b]	E	E	E	VE	E
Acacia tortilis	(At)	i	i	i	i	i	i

[a] VE = very effective, E = effective, e = ineffective, i = non-infective

[b] Suspected exogenous contamination on the *S.sesban* x strain S24 combination because re-isolates of this treatment had characteristics not akin to S24.

TABLE 5.8. Resistance/tolerance of rhizobial isolates to various concentrations of antibiotics and NaCl in YMA

Site	No. of isolates tested	Proportion of resistant isolates (%) at:																	
		Str ($\mu g\ cm^{-3}$)				Kan ($\mu g\ cm^{-3}$)				Amp ($\mu g\ cm^{-3}$)				NaCl% (w/v)					
		5	10	20	40	5	10	20	40	5	10	20	40	0.1	0.5	1.0	2.0	3.0	
Nyamonye	86	60	56	51	28	100	97	83	41	71	62	44	30	100	92	56	23	23	
Yala swamp	82	74	72	51	32	78	77	71	65	74	72	66	52	100	96	78	26	4	
Marigat	56	86	84	82	55	96	84	57	45	95	93	93	70	100	82	73	46	25	
Loruk	20	100	85	85	70	90	90	80	65	100	100	90	85	100	90	75	75	55	
Lodwar shrubland	43	98	86	86	74	100	100	74	67	100	100	88	81	100	93	84	77	53	
Lodwar riverine	5	100	80	80	80	100	100	100	80	100	100	80	80	100	100	80	60	60	
Kibwezi savanna	63	84	81	79	19	81	75	67	21	81	67	40	27	100	40	35	24	14	
Kibwezi riverine	13	69	54	46	38	77	69	38	38	100	77	38	31	100	100	85	38	31	
Bura savanna	18	67	56	44	28	94	61	39	28	100	100	94	61	100	72	67	44	22	
Bura riverine	18	83	67	61	50	78	72	50	44	100	100	94	83	100	94	83	28	28	
Gede white sand	33	100	94	64	18	100	100	79	64	100	94	91	70	100	94	61	15	15	
Jilore red sand	37	84	84	84	76	100	97	81	65	70	70	70	70	100	100	89	30	5	

The MIC of the two *Rhizobium* mutants DUS 408 and 390 were str > 1000 and 1000, kan 50 and 100, and amp 50 μg cm^{-3} each, respectively. This indicates that natural mutants can extend their tolerance to concentrations beyond the level used for selection. Table 5.9 showed that kan was most potent to the strains at concentrations higher than 400 μg cm^{-3}. Although numbers in different categories varied greatly, there was no discernable trend in MIC between *Rhizobium* and *Bradyrhizobium*. There was also no difference betweens EPS$^+$ and EPS$^-$ among *Bradyrhizobium*.

5.3.3 Growth curve characteristic of EPS producer in YMB containing antibiotics.

Inoculum size was > 5.1 $\log_{10}$ number of viable cells. Fig. 5.4a shows that growth of cells in control and amp 400 μg cm^{-3} treatments were similar, both having an exponential phase of less than 2 d and reaching the stationary phase within 3 d. Growth in the str 200 μg cm^{-3} treatment was reduced by nearly 3-fold and then recovered to stationary phases within 3 d. Kan 40 μg cm^{-3} inhibited growth for 5 d but then showed some growth in 6 d.

Measurement of growth by OD was relatively insensitive, failing to detect population sizes of < 6 $\log_{10}$ cells (Fig. 5.4b). Amp 400 μg cm^{-3} treatment appeared to allow greater OD compared to the control from late exponential phase. Str 200 μg cm^{-3} culture lagged for 2 d and grew to a stationary phase with lower OD than control and amp 400 μg cm^{-3}. The OD for kan 40 μg cm^{-3} was never detected within the duration of the experiment.

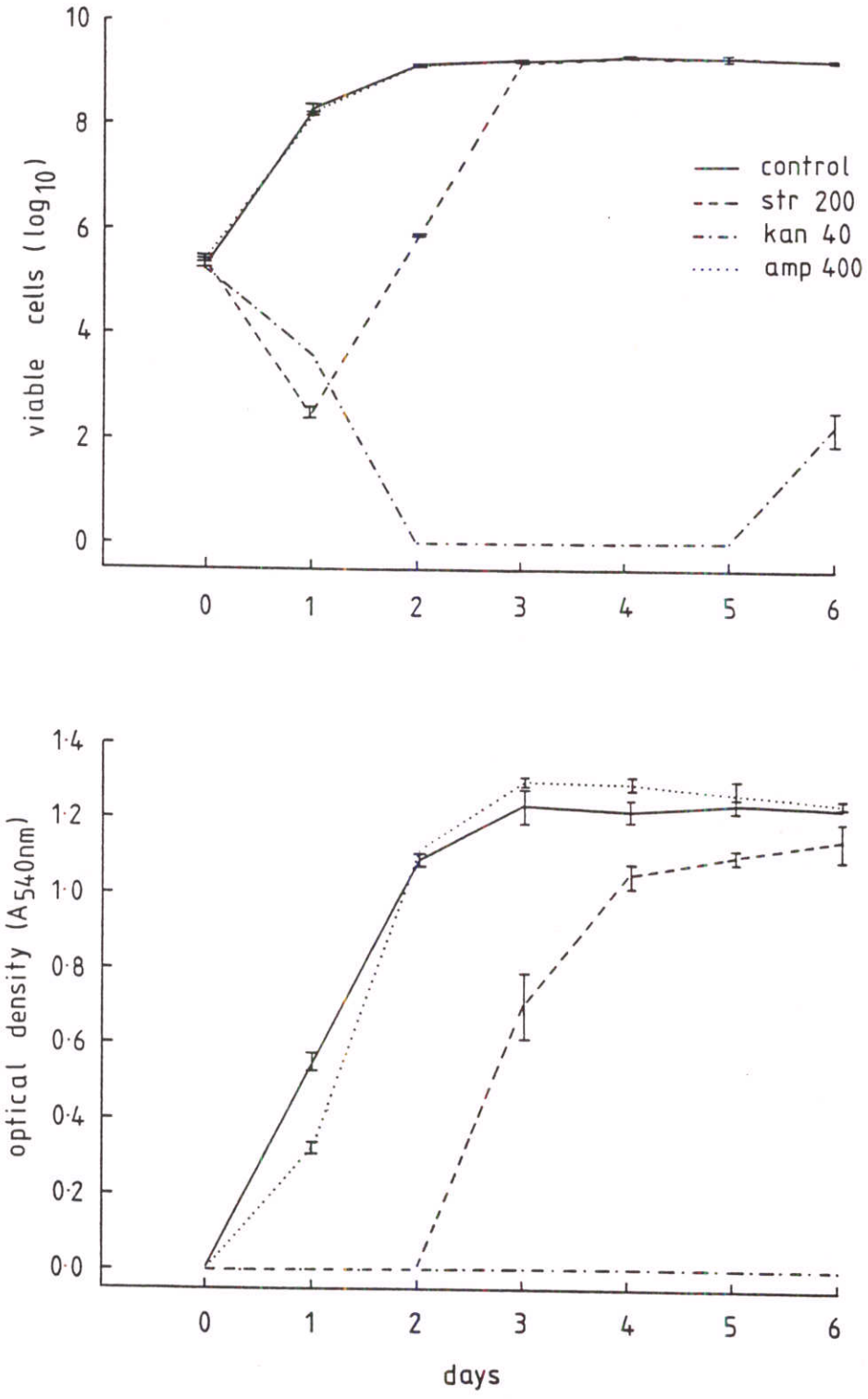


Figure 5.4 Growth response curves of extracellular polysaccharide (EPS) producing strain in yeast extract mannitol broth containing antibiotics. a) viable cells b) optical density (OD). Bars represent ± SE.

TABLE 5.9. Minimum inhibitory concentrations of 3 antibiotics with 40 strains (33 *Rhizobium*, 7 *Bradyrhizobium*)

Antibiotic	Concentration ($\mu g\ cm^{-3}$)	No of rhizobial strains inhibited		
		Rhizobium	*Bradyrhizobium*	
		All	EPS^+	EPS^+	EPS^-
Str	50	10	2	1
	100	16	4	2
	200	24	4	2
	300	27	4	2
	400	28	4	3
	500	28	4	3
	1000	31	4	3
Kan	50	6	2	1
	100	21	4	1
	200	32	4	3
	300	32	4	3
	400	33	4	3
Amp	50	9	2	1
	100	20	3	2
	200	21	3	2
	300	21	3	2
	400	21	3	2
	500	21	4	3
	1000	24	4	3

5.3.4 Cluster analyses.

Results of the various cluster analyses are given in Fig 5.5a - h. Individually analyzed sites were Nyamonye, Yala swamp, Gede and Jilore.

Nyamonye isolates segrated into 7 phenons (Fig. 5.5a). The two largest phenons 1 and 7 clustered into isolates with similar growth characteristics. Phenon 1 had very fast growers with watery translucent colony types and copious to very copious EPS. Phenon 7 comprised fast growers with milky translucent colony types and copious EPS. Clustering within phenon ranged from 95% to 65% similarity.

Yala swamp had 6 phenons, 4 of which were evenly distributed (Fig.5.5b) Phenon 1 comprised isolates with mixed characteristics. The majority in phenon 2 were very fast growers, watery translucent types with very copious EPS. Phenon 4 had fast growing, watery-milky translucent types and copious EPS, and nearly all the isolates (20:1) were trapped by *S. sesban*. Phenon 5 was represented by isolates with similar characteristics to those clustered in phenons 2 and 4. The range of clustering within each phenon was between 90% to 72.5% similarity.

Gede and Jilore had fewer isolates. Gede had 5 phenons (Fig. 5.5c), the largest of which (phenon 4) comprised 2 types of isolates, very fast growers and fast growers with the former being predominant (12:6). All were the watery to milky translucent type with copious to very copious EPS. The smaller phenons clustered into one or mixed types, and within phenon similarity was between 97.5% and 85%. There were 5 phenons in Jilore site (Fig. 5.5d). The largest phenons 2 and 3 comprised mainly the very fast

Figure 5.5 Phenograms showing rhizobial types: composition and diversity.

(a) Phenons 1 = VF, WT (19); 2 = F, WT-MT:WT (7:3);
3 = I/al:VF, WT (2:1); 4 = VF, WT:F, MT (1:1); 5 = I/al and S (3);
6 = VF:F (3:4); and 7 = F, MT:I/ac (38:1)

(b) Phenons 1 = mixed; 2 VF (25); 3 = I/al and S (2);
4 = F, *S. sesban* : *S. grandiflora* hosts (13:7); 5 = VF:F (11:8); and
6 = F:CO (4:1)

(c) Phenons 1 = F (5); 2 = S (2), 3 = F, MT:WT (3:2),
4 = VF:F (12:6) and 5 = F,MT (3)

(d) Phenons 1 = S, I/al:F (4:2); 2 = VF:F (12:1); 3 = VF:F (6:1);
4 = F, *S. sesban* host (6), and 5 = F, *S. grandiflora* host (5).

Numbers down the phenogram profiles represent phenons.
VF = very fast, F = fast, I/ac = Intermediate acid producer, I/al - Intermediate alkali producer.
WT = watery translucent, MT = milky translucent, CO = creamy opaque, WO = white opaque.
Numbers in parentheses represent the proportion of isolate types (and host of isolation where indicated) in the phenon.
• represents unclustered isolate(s) in the phenogram.

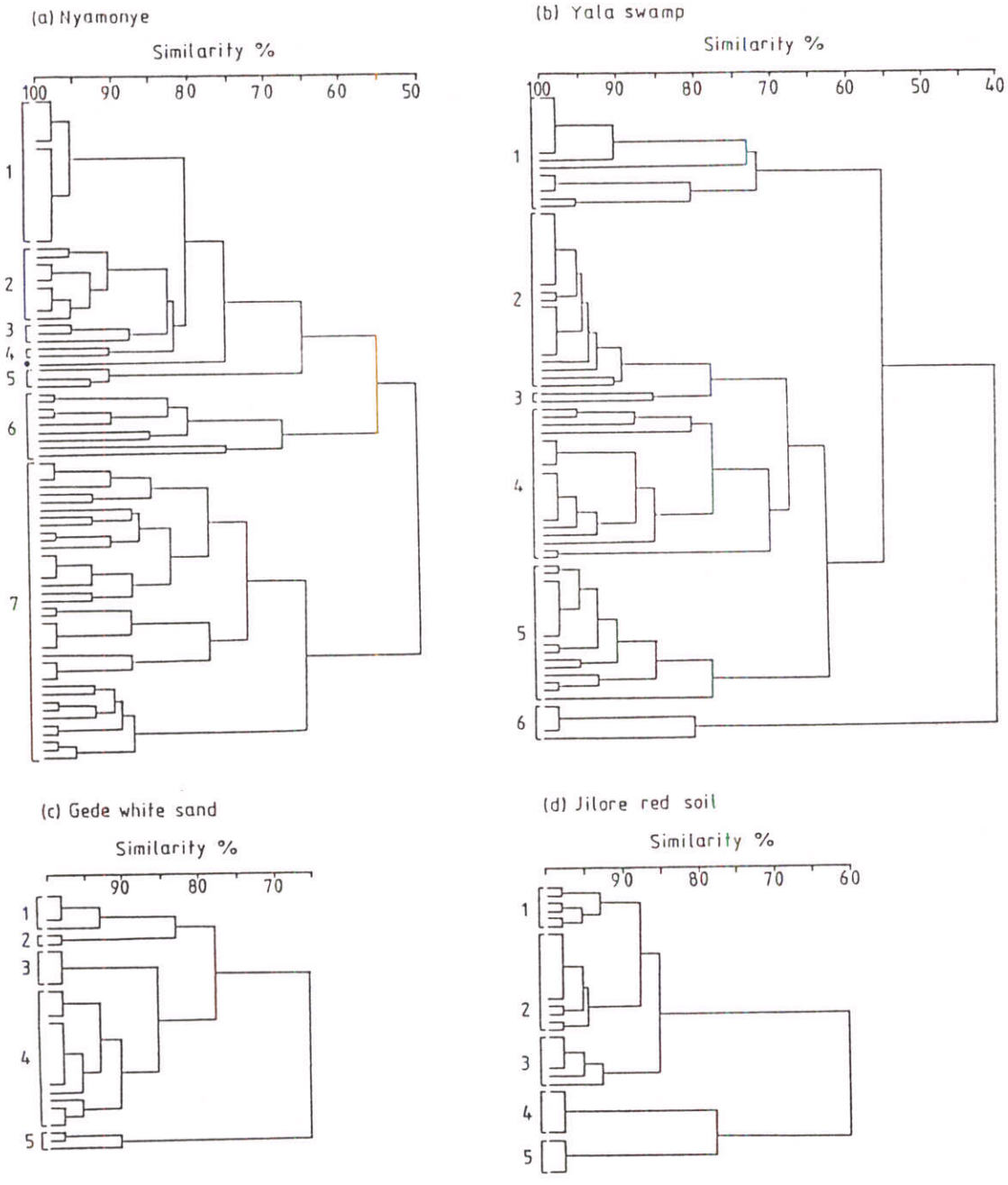

(a) Nyamonye
Similarity %
100
90
80
70
60
50
1
2
3
4
5
6
7
(b) Yala swamp
Similarity %
100
90
80
70
60
50
40
1
2
3
4
5
6
(c) Gede white sand
Similarity %
90
80
70
1
2
3
4
5
(d) Jilore red soil
Similarity %
90
80
70
60
1
2
3
4
5

Figure 5.5

Figure 5.5 continued.

(e) Phenons 1 = F, *P. vulgaris* : *A. tortilis* hosts (6:1); 2 = mixed (14)
3 = S, I/al: F (18:4) mainly *M. atropurpureum* host;
4 = S, I/al (9); 5 = VF/F: I/al (14:1);
6 = I/al (4), and 7 = F, CO (4).

(f) Phenons 1 = VF:F:S (26:4:1); 2 = VF, MT (6);
3 = mixed, F, S, I/al (4); 4 = mixed, F and S (2), and
5 = VF, MT (5).

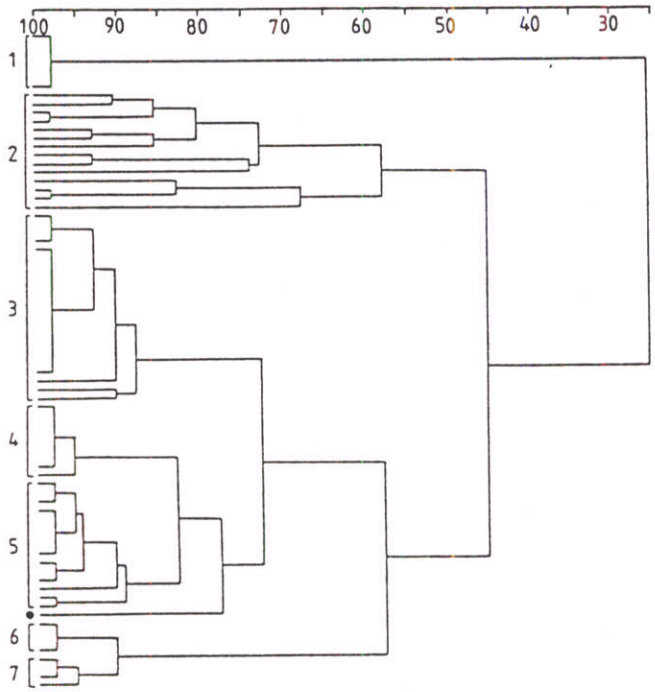
(g) Phenons 1 = VF:F (24:8); 2 = S (2); 3 = F, *S. sesban* host (3)
4 = S: I/al (8:1); 5 = F, mixed WT, MT and WO (5);
6 = F:VF (12:5), and 7 = F, mixed MT and WO (3).

(h) Phenons 1 = mixed, F, I/ac, S (8); 2 = F, MT:CO (5:2);
3 = I/al, S (2:2); 4 = VF, WT: F, MT (10:1);
5 = S:VF (2:1), and 6 = S, CO (2).

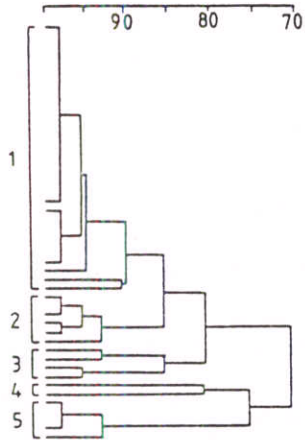
Numbers down the phenogram profiles represent phenons.
VF = very fast, F = fast, I/ac = Intermediate acid producer, I/al - Intermediate alkali producer.
WT = watery translucent, MT = milky translucent, CO = creamy opaque, WO = white opaque.
Numbers in parentheses represent the proportion of isolate types (and host of isolation where indicated) in the phenon.

• represents unclustered isolate(s) in the phenogram.

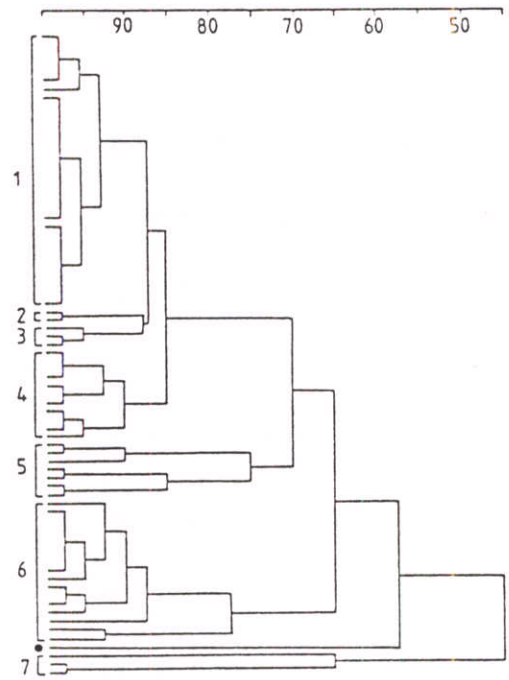
(e) Kibwezi savanna and riverine



(f) Lodwar shrubland and riverine



(g) Marigat and Loruk



(h) Bura savanna and riverine

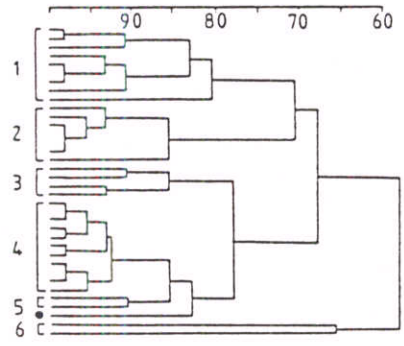


Figure 5.5 cont

growing and watery-milky translucent types with copious to very copious EPS. Other smaller phenons separated into single or mixed rhizobial types. The range of within phenon clustering was same as in Gede.

In general, in the single site analysis the slow growing and intermediate types with alkaline reaction in most cases formed separate clusters at $\geq$ 85% and were separated from the very fast and fast growing types from 92.5% at Jilore to 65% similarity at Nyamonye. At Jilore a predominantly slow/intermediate cluster also contained some fast growers (4:2). There was no apparent relationship between season of collection and clustering patterns in the above sites, host influence was however observed within some clusters at Yala swamp with *S. sesban* and at Jilore with *S.sesban* and *S. grandiflora.*

The other sites were analyzed in combinations as follows: Marigat and Loruk, Lodwar shrubland and riverine, Kibwezi savanna and riverine, and Bura savanna and riverine. Marigat and Loruk are 25 km apart and are in the same ecological zone. The other combinations are virtually differentiated by localized variations due to sitings by or away from a 'river' (see also chapter 2 for site description and location).

Seven phenons were formed with isolates from Marigat and Loruk sites (Fig.5.5g). Phenon 1 was the largest consisting of 42% of the total number of isolates between the 2 sites. It comprised predominantly a very fast growing and watery translucent types, and there was evidence of subclustering within the phenon according to EPS: copious segregated from very copious EPS producers. There was no apparent separation due to

season or site. A cluster (phenon 3) of 3 isolates was trapped by *S. sesban*. Within phenon similarity ranged from 97.5% to 65%. A single isolate (fast growing and milky translucent type) from *A. tortilis* at the Marigat site was separated from the closest phenon at 57.5%.

The Lodwar sites had 1 large and 4 small phenons (Fig.5.5f). The largest phenon had 67% of total number of isolates and was predominantly comprised of very fast growers with copious to very copious EPS and a similarity of > 90%. Within phenon similarity was between 92.5% and 80%. There was no relationship between clustering pattern and site although the riverine site was only represented by 10.9% of the total number of isolates.

Forty two % of isolates from Kibwezi sites (Fig 5.5e) were slow/intermediate (alkaline) types, because of the use of the herbaceous species for trapping in the savanna site. There were 7 phenons of which phenon 1 comprised isolates trapped with *P. vulgaris* : *A. tortilis* (6:1) and were separated from the closest phenons at 25% similarity suggesting a very distinct group from the rest of the isolates. These were fast growing, milky translucent types and produced copious EPS. The largest cluster, phenon 3, consisted predominantly of isolates trapped with *M. atropurpureum* and were mainly intermediate (alkaline) types. Other phenons were of mixed types. One isolate was separated by 77.5% similarity from phenon 5. Similarity within phenon ranged from 97.5% to 57.5%.

Bura sites yielded a very diverse and heterogeneous population: 36 isolates in 6 phenons (Fig 5.5h). Phenon 2 comprised mainly fast growing

milky translucent types while phenon 6 were slow growing creamy opaque types. Phenon 4 comprised mainly very fast growing watery translucent types. Other phenons were of mixed growth and cultural characteristics. Within phenon similarity ranged from 92.5% to 65%.

In general, as in the single site analysis, there was no apparent clustering due to season of soil collection, neither was site seen to influence the phenon separations. Slow and intermediate types tended to cluster together in sites where they were the minority except Lodwar riverine where they were distributed within the phenons of the predominantly very fast to fast growing types.

Results of the analysis for site representative isolates of *Rhizobium* and *Bradyrhizobium* are presented in Fig 5.6a-b.

The *Rhizobium* group analysis gave two major phenons. The largest one, phenon 1, comprised 65% of the total number of isolates analyzed. The phenon displayed a characteristic hierarchial stepwise pattern. The isolates clustered in subgroups in a manner that appeared to be a transition in growth rate and cultural characteristics. Very fast watery translucent types grouped with high similarity end (97.5%) while the fast milky translucent mainly showed lower similarity (67.5%). Reference strain DUS 8 (*A. tortilis)*, a very fast milky translucent type, was separated from this phenon at 57.5% similarity. Phenon 2 was less ordered and with lower similarity; the two main subgroups separated at 65%. It was predominantly fast and milky translucent type. Phenons 1 and 2 were separated at 47.5%. Phenon 3 consisted of an isolate from *P. vulgaris* and a reference strain DUS 255

Figure 5.6a Phenogram of *Rhizobium* isolates from major phenons across the sites.

Phenon 1 = VF, mainly WT:F (15:13)
Phenon 2 = F, MT:VF,WT (10:2)
Phenon 3 = 2 isolates, both F,MT; one from *P. vulgaris* host, the other from *A. polyacantha* host.

Figure 5.6b Phenogram of *Bradyrhizobium* isolates from major phenons across the sites.

Phenon 1 = subclusters of mixed cultural and morphological types (39).
Phenon 2 = CO:WO (3:1)

Numbers down the phenogram profiles represent phenons within a phenon.
VF = very fast, F = fast
WT = watery translucent, MT = milky translucent, CO = creamy opaque, WO = white opaque.
Numbers in parentheses represent the proportion of isolate types (and host of isolation where indicated) in the phenon.
• represents unclustered isolate(s) in the phenogram.

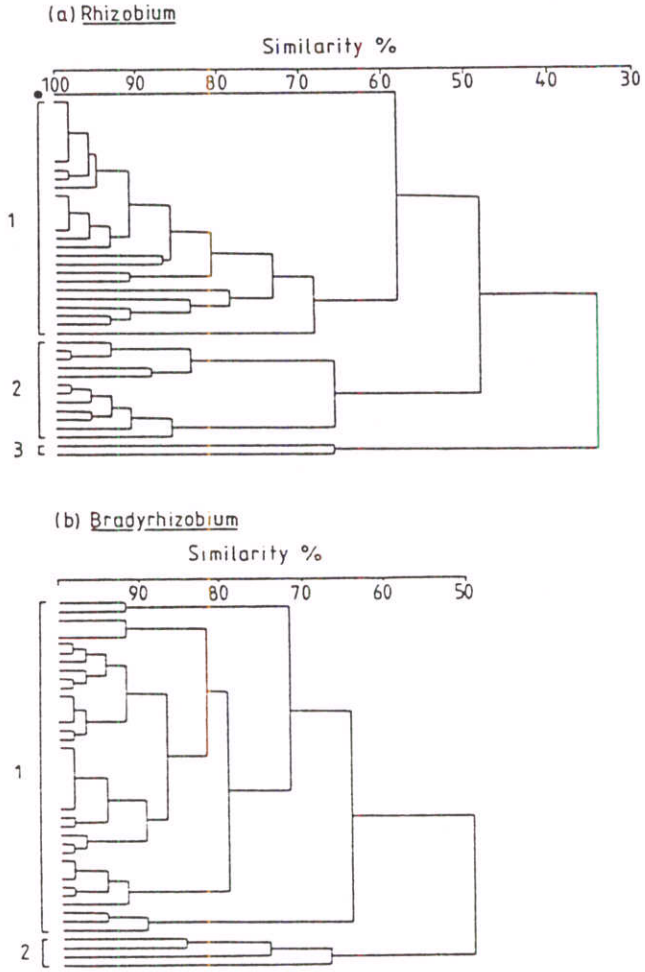

(a) Rhizobium
Similarity %
100
90
80
70
60
50
40
30
1
2
3
(b) Bradyrhizobium
Similarity %
90
80
70
60
50
1
2

Fig 5.6

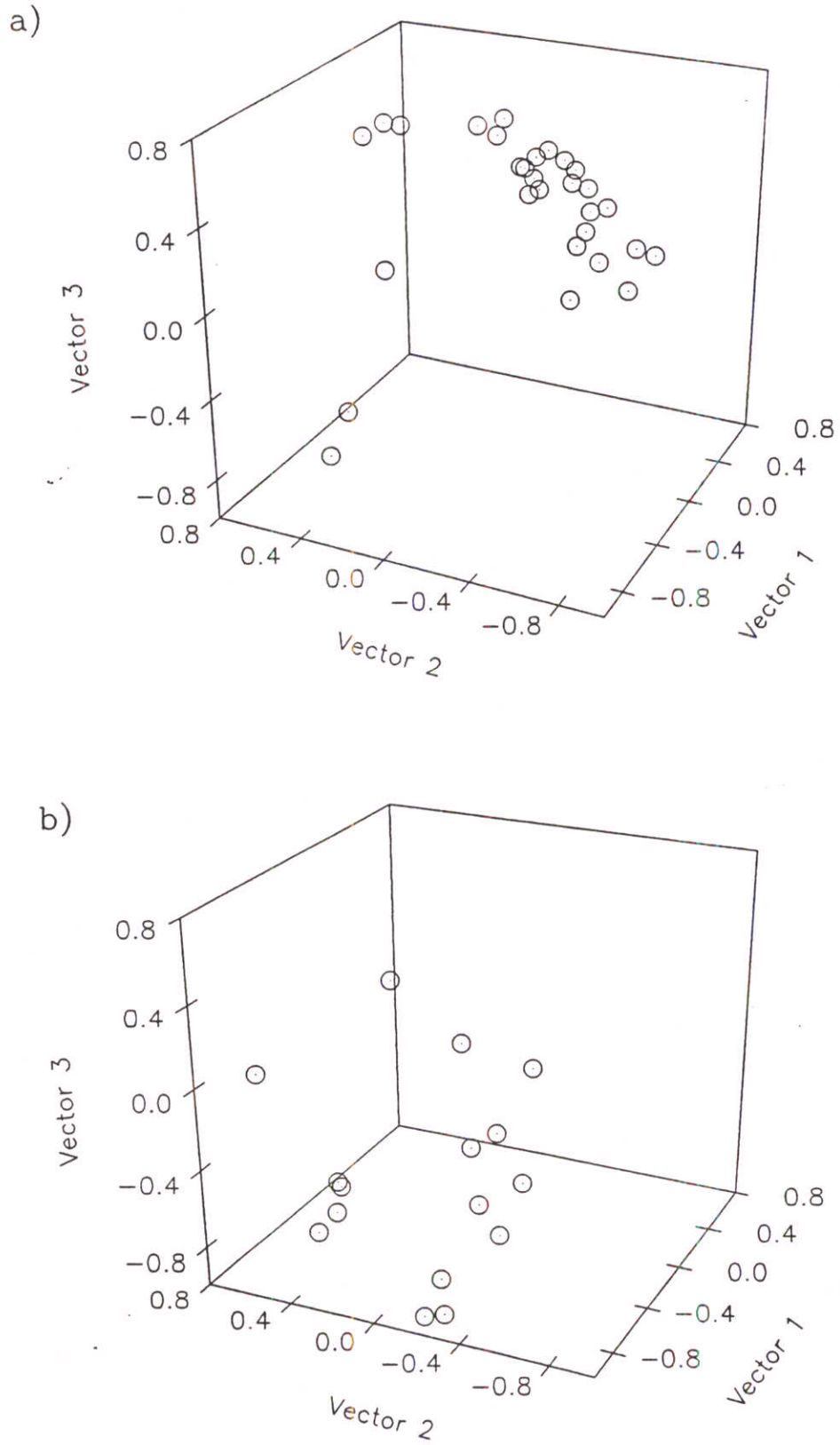


Figure 5.7 3-D spatial distribution of (a) *Rhizobium* and (b) *Bradyrhizobium* using vectors 1, 2 and 3 of principal coordinate analysis.

(*A. polyacantha*), both clustered at 65% and separated from the 2 main phenons at 35% similarity.

The *Bradyrhizobium* group had one major phenon consisting of 90% of the total number of isolates. The subclusters in this phenon showed a dichotomous pattern with similarities from 97.5% that converged at 72.5%. The isolates in each subcluster did not show any commonality in host of isolation, site or cultural morphology type. Clustering according to cultural characteristic was observed in the small phenon 2 with 3 isolates of creamy opaque type and 1 reference strain DUS 195 (*A. decurrens*) that was white opaque. All the 4 isolates were dry type with no EPS production. The two phenons were separated at 50% similarity.

Figs 5.7a and b show the 3-D scatter distribution of the *Rhizobium* and *Bradyrhizobium* groups respectively. They lend support to the trends already observed in the respective phenograms in which the *Rhizobium* group has a main core of clusters closely linked together with a few outliers, while the *Bradyrhizobium* has smaller, spatially distributed clusters.

5.4 DISCUSSION

According to Bergey's Manual of Systematic Bacteriology classification of the family Rhizobiaceae (Jordan, 1984), the two main genera of the root nodule bacteria are *Rhizobium* and *Bradyrhizobium*. *Rhizobium* is characterized by fast growth on YMA, colonies attain 2 - 4 mm in diameter within 3 - 5 d incubation, produce acid reaction and growth is usually accompanied by copious EPS at optimum temperature (25-30 °C) and pH (6-

7). *Bradyrhizobium* is characterized by slow growth, colonies do not exceed 1 mm within 5 -7 days incubation, produce an alkaline reaction and growth is usually accompanied by EPS under similar growth conditions. A recent addition to the rhizobial genera is *Azorhizobium,* fast-growing and capable of inducing nodules on both root and stem on the legume *Sesbania rostrata* (Dreyfus *et al*, 1988). Other proposed genera; *Sinorhizobium* (Chen *et al*, 1988) and *Photorhizobium* (Eaglesham *et al*, 1990) are disputed because ribosomal analysis show that the former is closely related to some species of *Rhizobium* (Jarvis *et al*, 1986) and the latter to *Bradyrhizobium* (Young *et al,* 1991).

It is clear from the above classification of Jordan (1984) that there are limitations even with the basic phenotypic characteristics which are supposed to differentiate the two main genera of *Rhizobium* and *Bradyrhizobium* when dealing with isolates from tropical woody legumes. For example, Barnet and Catt (1991) included two extra categories: intermediate and very slow to accommodate isolates from Australian *Acacia* spp. that did not conform to the traditional fast-and slow-growing rhizobial types. There also have been variations of the classification schemes according to growth rates. Roughley (1987) classified root nodule bacteria that nodulate acacias as belonging to *Rhizobium* (fast-growers) with colonies of 3 - 4 mm within 3 - 4 d and *Bradyrhizobium* (slow-growers) with colonies less than 1 mm after 5 d; while Habish and Khairi (1970) described isolates from African acacias (*A. seyal* and *A. mellifera)* with colonies which attained a growth of 1 - 2 mm in diameter after 5 d as slow-growing.

In view of the above variations and inconsistencies in the preliminary placement of rhizobial types at the genus level, and with consideration of the fact that in this study, only a few phenotypic characteristics were used, the Kenyan isolates can tentatively be assigned to: *Rhizobium,* to include very fast, fast and intermediate (acid-producing) types, and *Bradyrhizobium* to include very slow, slow and intermediate (alkali-producing). Although the dividing factor among intermediate was pH reaction, it is known that the general rule of *Rhizobium*-acid-producers and *Bradyrhizobium* - alkali-producers (Norris, 1965) is not universal: there have been reports of alkali-producing *Rhizobium* strains (Hernandez and Focht, 1984) though the latter may be due to the type of growth medium used (Sylvester-Bradley *et al*, 1988). Two points may justify the placement of Kenyan isolates with intermediate growth rate into the two genera:

1. The alkali-producing intermediates were isolated and authenticated on host species known to nodulate with *Bradyrhizobium* e.g. *F. albida* (Dreyfus and Dommergues, 1981; Dupuy and Dreyfus, 1992), *Vigna unguiculata* and *Macroptilium atropurpureum* (Jordan, 1984).

2. The acid-producing intermediates were isolated and authenticated on host species known to nodulate with *Rhizobium* e.g. *S. sesban* (Duhoux and Dommergues, 1985; Odee, 1990).

However, the fact that the generation times of the intermediates, and in particular the predominant alkali-producing types were outside the ranges

reported in the literature for *Rhizobium* and *Bradyrhizobium* (Brockwell, 1980; Vincent, 1981; Sylvester-Bradley *et al*, 1988; Giller and Wilson, 1991; Graham *et al*, 1991), is a point to note without excluding the possibility of variations due to different growth conditions.

The incidence of recovery of *Bradyrhizobium* isolates was relatively low compared with *Rhizobium* isolates. Only 15% of the total number of isolates were *Bradyrhizobium* because *F. albida* was the main trap host among the woody species across the sites. Therefore, this proportion should not be regarded as being commensurate with the actual sizes of *Rhizobium* and *Bradyrhizobium* but rather the rhizobial affinities of the trap host species used. Inclusion of *V. unguiculata* and *M. atropurpureum* which usually nodulate with *Bradyrhizobium* (Jordan, 1984) but not always (Trinick, 1980; Barnet *et al*, 1985; Barnet and Catt, 1991) in trapping rhizobia at Kibwezi savanna produced results to suggest that the *Bradyrhizobium* population may be as abundant as that of *Rhizobium*. In fact the size of *Bradyrhizobium* population as determined by MPN technique on one occasion at Kibwezi savanna with *F. albida* as trap host was comparable to that obtained with *A. polyacantha* (See Table 3.1, chapter 3). This is an indication of the existence of mixed populations of rhizobial types and may be interpreted to imply that in nature, various types may co-exist irrespective of their affinities to the native legume(s).

Bradyrhizobium isolates were also trapped to a lesser extent by *A. tortilis*, *A. nubica* and *A. polyacantha*, but they were always recovered as dual occupants with *Rhizobium*. Dual occupancy was not confined to

combinations between genera but also between isolates within a genus differentiated by colony characteristics. The incidence of dual occupancy was low (6.6%) and probably an underestimation of what is likely to be a prevalent occurrence with native woody species growing in relatively undisturbed or natural sites. The low proportion of isolates recovered as dual occupants may be attributed to the fact that it was solely based on physical recovery of isolates with obvious differences and also few nodule samples for isolation. Dual occupancy has also been reported in Australian legumes between *Rhizobium* and *Bradyrhizobium* (Barnet *et al*, 1985). Other workers (May and Bohlool, 1983; Moawad and Schmidt, 1987) also have reported as high as 38% incidence of dual occupancy in nodules of herbaceous legumes in monocultured field soils using fluorescent antibody (FA) technique.

Various points of ecological significance can be made on the results of dual occupancy. The differential response, especially to antibiotics, of cohabitants may imply that the resistant strain passively affords protection of some kind, eg as a physical barrier, to the sensitive strain against antagonistic rhizosphere organisms. Antibiotics are known to be produced in plant rhizosphere by some organisms (e.g. actinomycetes) as a competitive strategy (Whaley and Boyle, 1967), some of which are known to be inhibitory to rhizobia (van Schreven, 1964). Another point is the fact that either type (*Rhizobium* or *Bradyrhizobium*) may occupy a nodule, in conjunction with an infective type, in plants which they normally would not cause nodulation. Under these circumstances, and at any stage of the

symbiotic process, is the non-infective rhizobia with that particular host helping the infective type (hence 'helper' strains) or is it merely a 'freeloader' ?. This phenomenon is exemplified in the cross-inoculation results where for instance a fast-growing *Rhizobium* strain S2 originally isolated from *F. albida* and non-infective to this host effectively nodulated *A. polyacantha* and *S. sesban*; it is presumed that the nodule from which the fast-growing strain was isolated was induced by a slow-growing strain although the latter was not recovered. The 'helper' strain effect can occur even between rhizobia and non-rhizobial bacteria partnership (Burns *et al*, 1981; Nishijima *et al*, 1988, Halverson and Handelson, 1991) in which nodulation of the host plant is enhanced presumably due to improved infection process but lacking in information as to whether both bacteria occupy the nodule. Partnership between rhizobial strains have also been shown in which for instance, a non-infective strain inoculated together with an infective but ineffective strain, cause effective nodulation on a host (Rolfe *et al*, 1980; Kapp *et al*, 1990). A partnership nearly analogous to the *F. albida* nodule example cited above and probably commonplace in sites with mixed rhizobial populations is that of 'freeloader'. Under field conditions, the non-infective strain, in this context lacking an appropriate host, chances on the path of the infective strain and once inside, may multiply and differentiate alongside the infective strain without necessarily, influencing the symbiosis. A nodule can contain a large number of viable rhizobial cells, and although the viability may be reduced at senescence (Sprent and Sprent, 1990), this mode of propagation should be ecologically efficient because it is

not subject to environmental stress conditions as in saprophytic state in the soil. Therefore this is probably the main means by which rhizobial population of a particular type may be sustained timelessly in relatively undisturbed sites in the absence of an appropriate or compatible host.

Results of the cross-inoculation experiment have reaffirmed the recalcitrant nature of *A. tortilis* (Marigat provenance) to nodulation. This was one of the provenances discussed in chapter 3 as being an erratic nodulator. It is typical of a group of African acacias which would normally require mixed inoculant ('helper' strain) to nodulate (Sutherland and Sprent, 1993) especially under axenic conditions. Incompatibility of *M. atropurpureum* with strains isolated from woody legumes indicates how its use as a promiscuous host for authentications (Vincent, 1970; Dakora and Vincent, 1984;) may be unreliable for isolates from African woody legumes. It also provides more evidence as to the mixed populations and existence of rhizobial types that are not necessarily infective to the site native legumes.

In general, tolerance to antibiotics (at 40 μg cm^{-3}) was greatest with isolates from Lodwar sites. Although the occurrence of antibiotic producing organisms was not assessed in the soil, it is known that arid or desert soils favour their growth (Miller and Pepper, 1988). Therefore, rhizobia in Lodwar soils may have developed resistance due to co-existence with the antibiotic producing organisms. Similarly, tolerance to high levels of NaC1 ($\geq$ 3.0%) appeared to reflect the salinity of the soils as given by electrical conductivity, with the exception of Loruk site, which had comparable tolerance but with a relatively lower electrical conductivity.

Bradyrhizobium isolates were mostly sensitive to antibiotics at 40 μg cm^{-3}. This is contrary to the general opinion which holds that they are markedly resistant to a number of antibiotics (Jordan, 1984; Eaglesham, 1987). For instance Borges *et al* (1990) reported resistance of >100 μg cm^{-3} of str, kan, amp among others, of *B. japonicum* (soybeans) isolated from Brazilian soils. On the other hand sensitivity to NaC1 was low compared with *Rhizobium* (see Plate 5.3), and generally within the range reported by others (Jordan, 1984; Elsheik and Wood, 1990).

The levels of concentrations of antibiotics (5, 10, 20 and 40 μg cm^{-3}) were useful in distinguishing isolates within sites irrespective of rhizobial genus; whereas NaC1 would only distinguish between *Rhizobium* and *Bradyrhizobium*. The MIC of antibiotics of some isolates were $\geq$ 1000 μg cm^{-3} for str and amp. Such high intrinsic resistance appear to be common in tropical soils. Roughley *et al* (1992) have also reported very highly resistant rhizobia from Malaysian soils. This implies that ecological studies in soils favouring the development of highly resistant indigenous rhizobia would not be amenable to the use of antibiotic markers.

The higher OD of strain GW5/S10/P2/2 grown in YMB containing amp 400 μg cm^{-3} compared to control treatment while maintaining normal growth rate, imply that the bacteria produced more EPS than normal as a direct response to the antibiotic. Therefore, it also means that increases in colony size of resistant isolates on YMA plates containing antibiotics were not due to increase in cell numbers but EPS production. Lack of a similar response with other treatments (kan 40 μg cm^{-3} and Str 200 μg cm^{-3}) is

probably due to these concentrations being nearer the MIC. They would also be more susceptible in liquid than in agar media.

Results of the cluster analyses generally agreed with categorization according to colony appearance and growth rate by colony size thereby indicating the reliability of such categorizations. The diversity and composition of rhizobial isolates within a site would therefore be shown by the number of phenons and the number of isolates within them. Isolates belonging to the genus *Rhizobium* were more diverse than members of *Bradyrhizobium*, probably due to a small representation of the latter genus. Zang *et al* (1991), using more phenotypic characteristics than in this study, obtained a high level of diversity among fast-growing rhizobia mostly isolated from the nodules of *A. senegal* and *P. chilensis* grown in Sudanese soils.

Rhizobia isolated from Yala swamp were heterogeneous as shown by the even distribution of isolates within the various phenons. Clustering of some isolates from *S. sesban* into one phenon underlines the occurrence of rhizobia in the site which are predominantly specific to this host species; a point also mentioned in the discussion in chapter 3. There were also smaller *Sesbania* clusters of isolates from Jilore and Marigat sites.

The most dissimilar phenon from a single analysis was that obtained with isolates from *P. vulgaris* at Kibwezi sites. This phenon included a single isolate from *A. tortilis*. These isolates were very sensitive to low concentrations of antibiotics ($\leq$ 10 μg cm^{-3}) and NaCl (0.5 %). Other workers (Josey *et al*, 1979; Beynon and Josey, 1980) also reported low

resistance to antibiotics by *R. leguminosarum* strains. Although specific names have been assigned to *Rhizobium* isolated from *P. vulgaris* in some Kenyan soils (Karanja and Wood, 1988a,b), the use of host of isolation may not be accurate especially now that there are two species, namely *R. leguminosarum* bv. *phaseoli* and *R. tropici*, that can effectively nodulate it (Martinez - Romero *et al*, 1991).

Lodwar sites had the least diverse and most homogeneous rhizobial population with a minimum similarity of 80%; and *Bradyrhizobium* did not separate into distinct clusters. This is not surprising because diversity tends to be low in stressful conditions (Pielou, 1975). The desert-like conditions in Lodwar would require highly specialized and adapted populations in order to survive.

The *Rhizobium* and *Bradyrhizobium* group cluster analyses were based on isolates selected from major phenons within each site and were therefore not entirely representative. This notwithstanding, the results shown in the respective phenograms and 3-D presentations indicate differences in diversity and spatial distribution of rhizobia associated with woody legumes growing in various Kenyan climatic conditions. The *Rhizobium* group is more versatile comprising two major types: very fast growing watery translucent and fast growing milky translucent types, with the former being less diverse than the latter. It is plausible to interpret the phenotypic transition observed between the two types as reflecting their ability to change or adapt to the unstable and fluctuating conditions in the surface soil; with the very fast growing occurring in the least favourable conditions and the

fast growing occurring in the more favourable and stable conditions. On the other hand, the dispersed spatial distribution of *Bradyrhizobium* may suggest low versatility because they are localized in favourable microsites within the surface soil and are therefore likely to retain their original phenotypes for a long time. Jenkins *et al* (1987) reported a shift from a mixed population of fast growing and slow growing isolates from the surface soil to a population dominated by slow growing in the phreatic soil in a mesquite (*Prosopis glandulosa*) woodland in the Sonoran Desert of southern California. The distinct distribution between the two was attributed to the differential physiological adaptations, with the slow growing preferring the stable phreatic environment. However, it should be noted that unlike the mesquite-rhizobia association, the symbiotic preferences of *Rhizobium* and *Bradyrhizobium* with the native woody legumes have not been fully established, although the low incidence of the latter may suggest that they are not as symbiotically significant as the former.

It can be concluded that the physiological tests (IAR and salt tolerance) used in this study were a useful adjunct to the cultural and morphological characterization of the isolates because the two methods were, in most cases, in accord as was evident in the cluster analyses. Most isolates conformed to either *Rhizobium* or *Bradyrhizobium* with the exception of the intermediate types. However, further phenotypic and phylogenetic studies as proposed by Graham *et al* (1991) will be necessary in order to determine the taxonomic positions of the Kenyan rhizobial isolates. Occurrence of isolates which appear to be non-infective to site native woody

legume species yet infective on other test species reaffirms the existence of mixed rhizobial populations suggested in chapter 3. These results showed that the native species have more affinity to *Rhizobium* than *Bradyrhizobium*, although it could also be a question of competitive ability rather than compatibility.

CHAPTER 6

6.0 NITROGEN-FIXING POTENTIAL AND MYCORRHIZAL STATUS OF WOODY LEGUMES IN KENYAN SOILS.

6.1 INTRODUCTION

It is apparent in the preceding chapters that all the soils used in this study bore indigenous rhizobial populations, and that they variably nodulated the test woody legume species with the exception of *Acacia brevispica* (Odee and Sprent, 1992). However, other than visual observations on growth and colour of active nitrogen fixing nodule tissue of nodules, there was no quantitative measurement ascribed to the symbioses. In order for the data accumulated so far to be of practical use, it is important to quantify the nitrogen-fixing potential of the test species under glasshouse conditions. It is expected that such assessments would reflect nitrogen-fixing potential in the field at optimum moisture availability and temperature.

The acetylene reduction assay (ARA) can be used to monitor nitrogen-fixing activity of nodules (Hardy *et al*, 1968; Bergersen, 1980). Despite the limitations of the technique under field conditions (Giller and Wilson, 1991) , and its inaccuracy in measurement of nitrogen-fixation by the conventional closed system assay (Minchin *et al*, 1986), the instantaneous measurements produce quantitative data that can be compared with one another if the procedure is standardized.

Nitrogen-fixing plants have a high phosphorus (P) requirement

(Abbott and Robson, 1984; Sprent and Sprent, 1990). However, many tropical soils are low in P because it occurs in 'fixed' or poorly soluble inorganic and organic forms (Alexander, 1989; Hetrick, 1989). Because mycorrhizas have developed specialized mechanisms which enable them to obtain P from low P soils, many land plants associate with them to guarantee adequate supply (Gianinazzi-Pearson and Gianinazzi, 1989; Marks, 1991). The extensive external mycelium of mycorrhizas enable them to grow into the undepleted soil zone thus efficiently exploiting larger soil volumes than would the host plant roots (Allen, 1991; Giller and Wilson, 1991; Sieverding, 1991). In general mycorrhizas are ubiquitous on tropical trees, but the majority specifically form symbiotic association with vesicular-arbuscular mycorrhizal (VAM) fungi (Alexander, 1989), although ectomycorrhizal (ECM) fungi may sometimes occur singly or simultaneously with VAM on the same host plant (Högberg, 1989). Due to the large body of evidence that the legume - *Rhizobium* - VAM fungi association enhances nitrogen - fixation, P and N nutrition, and growth (Harley and Smith, 1983; Manjunath and Bagyaraj, 1984; Bayne and Bethenfalvay, 1987; de Lucena Costa and Paulino, 1990) it is of paramount importance that the mycorrhizal status of the test woody legumes is assessed and their role in plant nutrition determined.

I therefore present in this chapter, nitrogen-fixation data based on ARA, mycorrhizal status of test species assessed to date, and a detailed intraspecific comparison of *A. tortilis* provenances in Marigat soil in relation to growth, biomass and nutrient uptake.

6.2 MATERIALS AND METHODS

Plants used in this study were those grown for nodulation assessment in the 5th soil collection made between 18/1/91 - 27/1/91. For each species and provenance, 2 plants were grown per bag, replicated 8 times and grown under KEFRI glasshouse for 12 weeks. Harvests, root preparation for mycorrhizal infection assessment, ARA, plant concentrations of N and P were determined as described in Chapter 2. N and P uptake were calculated as follows: uptake = concentration in plant (shoot, root or whole plant) x dry weight of the same. ARA was performed on 4 randomly selected bags (8 seedlings). Nitrogen fixed by *A. tortilis* provenances Marigat and Mwatate was estimated by the N-difference method (Peoples and Herridge, 1990). The difference in total N accumulated by the nodulated legume (NI = *A. tortilis*) and non-nodulated legume control (Nnn = *A. brevispica*) seedlings grown under the same conditions was regarded as the contribution of nitrogen fixation to the nodulated calculated as follows: N_2 fixed = NI - Nnn. Two assumptions were made:

(a) The N contained in the Nnn was derived only from soil.

(b) Both nodulated and non-nodulated plants assimilated the same amount of mineral N.

6.2.1 Statistical analyses

ARA and VAM fungi infection data for species other than *A. tortilis* in Marigat soil were analyzed by mean and standard error of mean (SE).

For the two *A. tortilis* provenances Marigat and Mwatate, a single factor analysis of variance (ANOVA) was conducted on each variable and the least significant difference (LSD) was calculated for P = 0.05. Data for VAM root length infection (%) were transformed by arcsine $\sqrt{X}\%$ and nodule number by $\sqrt{X+0.5}$ (Zar, 1974) prior to ANOVA and transformed back to normal values after the analysis.

6.3 RESULTS AND DISCUSSION

6.3.1 Nitrogen fixation potential

Results in Table 6.1 indicate that site native species showed substantial rates of acetylene reduction (>1.0 μmoles C_2H_4 $plant^{-1}$ h^{-1}) with the exception of *A. tortilis* Marigat provenance at Marigat site, *A. zanzibarica* at Bura savanna site and *A. nubica* at Lodwar shrubland (<1.0 μmoles C_2H_4 $plant^{-1}$ h^{-1}). The low reduction rate of *A. tortilis* Marigat provenance reflects its poor nodulation ability mentioned in chapter 3.

In general, the acetylene reduction rates exhibited by these species are of the same magnitudes as those reported for other woody legume species seedlings grown under comparable conditions and ARA procedures (Eskew and Ting, 1978; Grove and Malajczuk, 1987; Hansen *et al*, 1987; Sutherland and Sprent, 1993). *Sesbania sesban* showed the highest total reduction rates while *F. albida* showed low total reduction rates but high specific activity rates. The acacia nodules had moderate specific activities. The high reduction rates but moderate specific activities of *S. sesban* is an indication of the higher nodule biomass compared with the other woody legumes.

Venkateswarlu and Singh (1988) also reported higher total and lower specific acetylene reduction rates in nodules of *Gliricidia sepium* and *Leucaena leucocephala* respectively. It thus appears that papilioniods invest more on nodule biomass than the mimosoids.

6.3.2 Natural mycorrhizal infection

All the woody legumes analyzed so far in this study were infected by VAM fungi, none of them was found to be ectomycorrhizal (Table 6.2). Legumes are normally VA-mycorrhizal with the exception of one legume genus *(Lupinus)* which appears generally to be non-VA-mycorrhizal (Abbott and Robson, 1991). Among the genus *Acacia*, all those species reported are VA-mycorrhizal (Bethlenfalvay *et al*, 1984; Alexander, 1989, Michelsen, 1992). Other reports indicate some Australian acacias may be both VA-mycorrhizal and ectomycorrhizal (Alexander, 1989; Osonubi *et al*, 1991).

By comparing the VAM fungi infection data at Yala swamp for *A. tortilis* and *S. sesban*, it is apparent that the former species is more mycorrhizal dependent than the latter. This is attributable to the superior root system of *S. sesban* which is fibrous with many root hairs, whereas *A. tortilis* has fewer laterals and root hairs. Graham *et al* (1991) suggested that species that have evolved root systems that are less dependent on mycorrhizas have also evolved mechanisms to regulate colonization and carbon expenditure on the fungus. The VAM infection data at Yala swamp and Marigat for *S. sesban* and *A. tortilis* respectively indicate that there were no intraspecific differences in root colonization.

ble 6.1. Acetylene reduction rates of 12-week old nodulated woody legume seedlings grown in Kenyan soils (± standard error of mean, n=4; also see footnotes)

e	Species/provenance[1]	TAR[2]	SAR[3]
amonye	***Acacia polyacantha***/Nyeri	2.4 ± 0.3	5.1 ± 0.7
	Faidherbia albida/Kainuk	2.9 ± 0.8	17.7 ± 7.5
la swamp	***Sesbania sesban*/Yala swamp**	11.6 ± 0.9	5.6 ± 4.4
	Sesbania sesban/Kakamega	19.1 ± 1.2	5.6 ± 2.7
	Faidherbia albida/Kainuk	3.9 ± 0.2	6.5 ± 0.8
	Acacia tortilis/Kibwezi	0.2 ± 0	5.0 ± 0.3 x 10^{-3}
rigat	***Acacia tortilis*/Marigat**	0.6 ± 0.2	2.9 ± 1.8
	Acacia tortilis/Mwatate	1.3 ± 0.4	4.0 ± 0.9
	Faidherbia albida/Kainuk	0.8 ± 0.2	30.6 ±15.5
ruk	***Acacia mellifera***/Isiolo	2.0 ± 0.5	9.7 ± 6.3
	Faidherbia albida/Kainuk	5.2 ± 0.7	37.9 ±12.0
	Acacia tortilis/Mwatate	1.0 ± 0.5	3.5 ± 2.3
lwar shrubland	***Acacia nubica***/Marigat	0.8 ± 0.7	1.0 ± 0.8
lwar riverine	***Acacia tortilis***/Mwatate	2.4 ± 0.4	6.5 ± 1.4

ble 6.1. Continued

wezi savanna	***Acacia tortilis*/Kibwezi**	1.7 ± 0.4	19.3 ±13.8
	***Acacia tortilis*/Mwatate**	1.7 ± 1.1	30.7 ±13.0
	Faidherbia albida/Kainuk	1.4 ± 0.6	17.2 ± 4.8
wezi riverine	***Acacia xanthophloea*/Kibwezi**	2.7 ± 0.9	2.8 ± 0.9
	***Acacia xanthophloea*/Mai Mahiu**	1.9 ± 0.6	2.8 ± 0.8
a savanna	***Acacia zanzibarica*/Garissa**	0.4 ± 0.08	1.1 ± 0.3
	Faidherbia albida/Kainuk	0.2 ± 0.01	0.8 ± 0.6
a riverine	*Faidherbia albida*/Kainuk	6.5 ± 0.2	15.9 ± 2.3
le white sand	*Faidherbia albida*/Kainuk	5.5 ± 2.1	49.9 ±16.5
re red soil	*Faidherbia albida*/Kainuk	1.1±0.9	23.2±16.5

Bold highlights site native species/provenance
Total acetylene reduction rate (μmoles C_2H_4 $plant^{-1}$ h^{-1})
Specific acetylene reduction rate (μmoles C_2H_4 g^{-1} nodule fresh weight h^{-1})

ɔle 6.2. VAM fungi infection of 12-week old woody legume seedlings grown in Kenyan soils

ɛ	Species/provenance [1]	No of root samples	Root length infection (%) ± standard error
la swamp	***Sesbania sesban*/Yala swamp**	15	6.2 ± 1.6
	Sesbania sesban/Kakamega	16	5.4 ± 1.2
	Acacia tortilis/Mwatate	16	41.8 ± 4.3
rigat	***Acacia tortilis*/Marigat**[2]	16	32.6 ± 3.6
	Acacia tortilis/Mwatate[2]	16	31.9 ± 4.3
	Faidherbia albida/Kainuk	4	6.7 ± 4.3
ruk	*Acacia tortilis*/Mwatate	15	29.9 ± 6.1
ɔwezi savanna	***Acacia tortilis***/Mwatate	2	4.9 ± 0.8
	Faidherbia albida/Kainuk	3	14.0 ± 3.7
ɔwezi riverine	***Acacia xanthophloea***/Mai Mahiu	4	17.5 ± 5.9
	Faidherbia albida/Kainuk	2	19.0 ±11.0

Bold highlights site native species/provenance
Also presented in Table 6.3

Table 6.3. Comparison of growth, biomass, nutrient uptake (N and P) and symbiotic status between two provenances of 12-week old *A. tortilis* seedlings grown in Marigat soil

Parameter (plant $^{-1}$)	Provenance		F-test[1]	LSD (P=0.05)
	Marigat	Mwatate		
Height (cm)	12.6	14.6	NS	2.4
Shoot dry weight (g)	1.09	1.03	NS	0.28
Root dry weight (g)	0.63	0.74	NS	0.21
Nodule dry weight (mg)	10.0	48.1	S**	24.3
Total dry weight (g)	1.72	1.83	NS	0.45
Root : shoot ratio	0.68	0.75	NS	0.28
Nodule number	3.2	8.9	S*	1.0
Shoot N (mg)	22.93	23.39	NS	7.56
Root N (mg)	10.15	12.98	NS	4.11
Nodule N (mg)	0.57	3.02	S**	1.60
Total N (mg)	31.03	36.79	NS	11.58
Fixed N (mg)	15.63	21.46	NS	11.55
VAM infection (%)	32.1	30.6	NS	2.1
Shoot P (mg)	0.40	0.22	S***	0.10
Root P (mg)	0.16	0.17	NS	0.10
Total P[2] (mg)	0.50	0.32	S*	0.16

1. NS = not significant, S* = significant at P = 0.05, S** = significant at P=0.01, S*** = significant at P = 0.001

2. Nodule P was not analyzed because of insufficient sample

6.3.3 An intraspecific comparison of *A. tortilis* in Marigat soil

There were no significant differences (P=0.05) in plant height, shoot, root and total dry weights between the Marigat and Mwatate *A. tortilis* provenances (Table 6.3). However, nodule number and biomass was significantly higher in favour of the Mwatate provenance at P=0.05 and P=0.01 respectively. A similar trend was shown in the N content in shoot, root and whole plant in which there were no significant differences except nodule N. Nodule N was the major source of differences observed between the the two provenances. These results show that *A. tortilis* provenances Marigat and Mwatate derived approximately 50 % and 58 % respectively of their total N from nitrogen fixation (also see Appendix 4).

It is beyond the scope of this experiment to ascertain whether the VAM fungi associated with *A. tortilis* provenances in Marigat soil were beneficial to their hosts or were merely parasitic, notwithstanding the fact that this host showed stronger evidence of mycorrhizal dependence than other species. Both provenances had similar extent of VAM fungi infection yet the site native Marigat provenance showed a significantly higher P uptake in shoot (P = 0.001) and whole plant (P = 0.05); root P was not significantly different. There may be two possible reasons why Marigat provenance appears to be superior in P uptake. The first one is that being a resident, it is either genotypically as a host or in partnership with the indigenous VAM fungi population more efficient at extracting P. Other workers (e.g. Azcon and Ocampo, 1981; Harley and Smith, 1983; Krishna *et al*, 1985; Estaùn *et al*, 1987; Pacovsky *et al*, 1991) have reported

intraspecific variations in VAM fungi root colonization and P uptake. The other reason is that because the Mwatate provenance had a higher number of nodules, higher nodule biomass, high N content and nitrogenase activity, it is plausible to suggest, on the assumption that differences in P uptake were due to nodular sink and not host or endophyte effect, that while the Marigat provenance invested its P assimilate in aerial growth, the Mwatate provenance diverted its P to nodule development and function at the expense of overall growth in the early seedling stage. Israel (1987) and Sprent *et al* (1988) have discussed the significance of P in the early stages of nodule development and function. It can therefore be suggested that at this stage of growth, P is more limiting than N during the making of the nodules. However, it would be required to establish whether *A. tortilis* Marigat povenance has inherently a higher P demand and if its inferior nitrogen fixation attributes are as a result of a strategy to circumvent the high P demand at the initiation of the nitrogen fixing system.

CHAPTER 7

7.0 GENERAL DISCUSSION AND CONCLUSIONS

The use of glasshouse-grown seedlings in studying the ecology of the microsymbionts of woody legumes in soils from various ecological zones of the country was a useful exercise. This study has shown that all the sites sampled contained indigenous rhizobial populations. The indigenous rhizobial populations were influenced by the presence of site native species/provenances but were not necessarily compatible with those hosts in terms of infectiveness of rhizobia or susceptibility of the host to nodulation.

In the MPN assays (chapter 3), the universal trap host, *Acacia polyacantha*, generally gave comparable rhizobial population estimates with that of the site native trap host with some exceptions. For instance at Yala swamp in which the site native legume was *Sesbania sesban*, the universal trap host gave lower estimates. At Lodwar and Kibwezi sites the universal trap host gave higher estimates than the native trap hosts, thus illustrating that good estimation of rhizobial populations is dependent on careful selection of trap hosts. The results have shown that most sites have mixed rhizobial populations. In general, it can be said that MPN and nodulation potentials showed more site specific than common trends. Because of the differential nodulation and rhizobial population estimates portrayed at intraspecific, interspecific and generic levels, it would be appropriate to determine the specificity or host ranges of the various indigenous rhizobial types within and among sites in order to evaluate their roles in the natural

symbioses observed.

Variation in nodulation pattern (chapter 4) showed the need to re-evaluate inoculation procedures in terms of inoculant placement. It showed that location of nodules on the upper part of the taproot was not always a feature of the host legume but that of the spatial distribution of the inoculant in the root substrate. Effective nodulation on woody legumes in unsterile soil was generally uniform throughout the root system, whereas nodules in axenic conditions were very localised. It is therefore essential to develop economically viable inoculation protocols to optimize nodulation throughout the root system in unsterile soil conditions.

This study has shown that the bulk of isolates from the various sites were *Rhizobium* sp. but within this genus there were various cultural and physiological phenotypes (chapter 5) that reflected the diversity of rhizobial types associated with woody legume species tested. *Bradyrhizobium* representation was relatively small but this was attributable to the fact that only one trap host *Faidherbia albida*, was used across the sites, although some were isolated as cohabitants of *Rhizobium* in nodules from legumes that they would not normally nodulate. Because of the occurrence of rhizobial types that were uncharacteristic of either genus, it is proposed that representatives of the various types are subjected to phylogenetic characterization in comparison with standard strains in order to determine their taxonomic positions. It is also essential to determine whether cohabitation of different rhizobial types in a single nodule is of symbiotic significance in terms of nitrogen fixation or merely chance occurrence.

The interplant and intraspecific variations in nodulation and nitrogen fixation observed with some of the woody legume species tested represent the first step towards the selection of superior nitrogen fixing phenotypes along with effective strains. It will also be necessary to isolate and identify VAM fungi types and the density of their propagules in soils in which root colonizations occurred, and to establish their significance in the nitrogen fixation symbioses.

This work has contributed towards the understanding of the rhizobial ecology of woody legumes in arid conditions. The findings are of direct relevance for the sites studied and have therefore provided a baseline from which strategies for improving nitrogen fixation in woody legumes under the Kenyan conditions can be developed.

REFERENCES

Abbott, L.K. and Robson, A.D. (1984). The effect of VA mycorrhizae on plant growth, p.113-130. In C. Ll. Powell and D.J. Bagyaraj (ed.), VA mycorrhiza. CRC Press, Inc., Boca Raton.

Abbott, L.K. and Robson, A.D. (1991). Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric. Ecosystems Environ. **35**: 121-150.

Agnew, A.D.Q. (1974). Upland Kenya wild flowers. William Claus and Sons, London.

Alexander, I.J. (1989). Systematics and ecology of ectomycorrhizal legumes, p.607-624. In C.H. Stirton and J.L. Zarucchi (ed.), Advances in legume biology. Monographs in Systematic Botany from the Missouri Botanical Garden Number 29, St Louis.

Alexander, I. (1989). Mycorrhizas in tropical forests, p.169-188. In J. Proctor (ed.), Mineral nutrients in tropical forest and savanna ecosystems. Blackwell Scientific Publications, Oxford.

Allen, M.F. (1991). The ecology of mycorrhizae. Cambridge University Press, Cambridge.

Allen, O.N. and Allen, E.K. (1981). The Leguminosae: A source book of characteristics, uses and nodulation. University of Wisconsin Press, Madison.

Amanyunzu, C.L. (1988). Vegetation resources and land use in central Turkana district, Kenya. TREMU technical report number D-1. United Nations Educational, Scientific and Cultural Organization (UNESCO), Nairobi.

Anderson, J.M. and Ingram, J.S.I. (1989). Tropical soil biology and fertility: A handbook of methods. C.A.B. International, Wallingford.

Azcon, R. and Ocampo, J.A. (1981). Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. New Phytol. **87**: 677-685.

Baird, L.M., Virginia, R.A. and Webster, B.D. (1985). Development of root nodules in a woody legume, *Prosopis glandulosa* Torr. Bot. Gaz. **146**: 39-43.

Barnet, Y.M. and Catt, P.C. (1991). Distribution and characteristics of root-nodule bacteria isolated from Australian *Acacia* sp. Plant Soil **135**: 109-120.

Barnet, Y.M., Catt, P.C. and Hearn, D.H. (1985). Biological nitrogen fixation and root-nodule bacteria. (*Rhizobium* sp. and *Bradyrhizobium* sp.) in two rehabilitating sand dune areas planted with *Acacia* spp. Aust. J. Bot. **33**: 595-610.

Basak, M.K. and Goyal, S.K. (1975). Studies on tree legumes I. Nodulation pattern and characterization of the symbiont. Ann. Arid Zone **14**: 367-370.

Basak, M.K. and Goyal, S.K. (1980a). Studies on the biology of tree legumes - *Rhizobium* symbiosis: Nodulation pattern and cross inoculation trials with the tree legumes and cultivated legumes. Ann. Arid Zone **19**: 427-431.

Basak, M.K. and Goyal, S.K. (1980b). Studies on tree legumes III. Characterization of the symbionts and direct and reciprocal cross inoculation studies with tree legumes and cultivated legumes. Plant Soil **56**: 39-51.

Bayne, H.G. and Bethlenfalvay, G.H. (1987). The *Glycine-Glomus-Rhizobium* symbiosis. IV. Interactions between the mycorrhizal and nitrogen-fixing endophytes. Plant Cell Environ. **10**: 607-612.

Bennett, J.E., Woomer, P.L. and Yost, R.S. (1990). Users manual for MPNES most-probable-number enumeration system ver.1.0. NifTAL project and University of Hawaii.

Bergersen, F.J. (1980). Methods for evaluating biological nitrogen fixation. John Wiley and Sons, Chichester.

Bethlenfalvay, G.J., Dakessian, S. and Pacovsky, R.S. (1984). Mycorrhizae in a southern California desert: ecological implications. Can. J. Bot. **62**: 519-524.

Beynon, J.L. and Josey, D.P. (1980). Demonstration of *Rhizobium phaseoli* using variation in intrinsic antibiotic resistance. J. Gen. Microbiol. **118**: 437-442.

Bogdan, A.V. and Pratt, D.J. (1974). Common acacias of Kenya. Ministry of Agriculture, animal husbandry and water resources grassland research stations, Kitale and Marigat, Kenya.

Boonkerd, N. and Weaver, R.W. (1982). Cowpea rhizobia: comparison of plant infection and plate counts. Soil Biol. Biochem. **14**: 305-307.

Borges, A.C., Guimarães, W.V., Muchovej, R.M.C. and Los Reyes Gonzalés, G. de (1990). Resistance of *Bradyrhizobium japonicum* strains to selected antibiotics. World J. Microbiol. Biotechnol. **6**: 340-342.

Brenan, J.P.M. (1959). Leguminosae subfamily Mimosoideae, p.1-173. In C.E. Hubbard and E. Milne-Redhead (ed.), Flora of tropical East Africa. Crown agents for overseas governments and administrations, London.

Brockwell, J. (1963). Accuracy of a plant-infection technique for counting populations of *Rhizobium trifolii*. Appl. Microbiol. **11**: 377-383.

Brockwell, J. (1980). Experiments with crop and pasture legumes - Principles and practice, p.417-488. In F.J. Bergersen (ed.), Methods for evaluating

biological nitrogen fixation. John Wiley and Sons, Chichester.

Brockwell, J., Andrews, J.A., Gault, R.R., Gemell, L.G., Griffith, G.W., Herridge, D.F., Holland, J.F., Karsono, S., Peoples, M.B., Roughley, R.J., Thompson, J.A., Thompson, J.A. and Troedson, R.J. (1991). Erratic nodulation and nitrogen fixation in field-grown pigeon pea [*Cajanus cajan* (L.) Millsp.]. Aust. J. Exp. Agric. **31**: 653-661.

Brockwell, J., Diatloff, A., Grassia, A. and Robinson, A.C. (1975). Use of wild soybean (*Glycine ussuriensis* Regel and Maack) as a test plant in dilution-nodulation frequency tests for counting *Rhizobium japonicum*. Soil Biol. Biochem. **7**: 305-311.

Bromfield, E.S.P. (1984). Variation in preference for *Rhizobium meliloti* within and between *Medicago sativa* cultivars grown in soil. Appl. Environ. Microbiol. **48**: 1231-1236.

Bromfield, E.S.P., Sinha, I.D. and Wolynetz, M.S. (1986). Influence of location, host cultivar and inoculation on the composition of naturalized population of *Rhizobium meliloti* in *Medicago sativa* nodules. Appl. Environ. Microbiol. **51**: 1077-1084.

Bumpus, E.D. (1957). Legume nodulation in Kenya. East Afr. Agric. For. J. **23**: 91-99.

Burns Jr., T.S., Bishop, P.E. and Israel, D.W. (1981). Enhanced nodulation of leguminous plant roots by mixed cultures of *Azotobacter vinelandii* and *Rhizobium*. Plant Soil **62**: 399-412.

Catlow, H.Y., Glenn, A.R. and Dilworth, M.J. (1990). Does rhizobial motility affect its ability to colonize along the legume root? Soil Biol. Biochem. **22**: 573-575.

Chaudri, A.M., McGrath, S.P., Giller, K.E., Rietz, E. and Sauerbeck, D.R. (1993). Enumeration of indigenous *Rhizobium leguminosarum* biovar *trifolii* in soils previously treated with metal-contaminated sewage-sludge. Soil Biol. Biochem. **25**: 301-309.

Chen, W.X., Han, G.H. and Li, J.L. (1988). Numerical taxonomy study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int. J. Syst. Bacteriol. **38**: 392-397.

Coe, M. and Beentje, H. (1991). A field guide to the acacias of Kenya. Oxford University Press, Oxford.

Corby, H.D.L. (1981). The systematic value of leguminous root nodules, p.657-670. In R.M. Polhill and P.H. Raven (ed.), Advances in legume systematics, part 2. Royal Botanic Gardens, Kew.

Dakora, F.D. and Vincent, J.M. (1984). Fast growing bacteria from nodules of cowpea [*Vigna unguiculata* (L.) Walp.]. J. Appl. Bacteriol. **56**: 327-330.

Doran, J.C., Turnbull, J.W., Boland, D.J. and Gunn, B.V. (1983). Handbook on seeds of dry-zone acacias. Food and Agriculture Organization of the United Nations, Rome.

Dreyfus, B., Garcia, J.L. and Gillis, M. (1988). Characterization of *Azorhizobium caulinodans* gen. nov., sp. nov., a stem-nodulating nitrogen fixing bacterium isolated from *Sesbania rostrata*. Int. J. Syst. Bacteriol. **38**: 89-98.

Dreyfus, B.L. and Dommergues, Y.R. (1981). Nodulation of *Acacia* species by fast- and slow-growing tropical strains of *Rhizobium*. Appl. Environ. Microbiol. **41**: 97-99.

Duhoux, E. and Dommergues, Y.R. (1985). The use of nitrogen fixing trees in

forest and soil restoration in the tropics, p.384-400. In H. Ssali and S.O. Keya (ed.), Biological nitrogen fixation in Africa: proceedings of the first conference of the African association for biological nitrogen fixation. Matianum Press Consultants, Nairobi.

Dupuy, N.C. and Dreyfus, B.L. (1992). *Bradyrhizobium* populations occur in deep soil under leguminous tree *Acacia albida*. Appl. Environ. Microbiol. **58**: 2415-2419.

Eaglesham, A.R.J. (1987). The use of intrinsic antibiotic resistance for *Rhizobium* study, p.185-204. In G.H. Elkan (ed.), Symbiotic nitrogen fixation technology. Marcel and Dekker Inc., New York and Basel.

Eaglesham, A.R.J., Ellis, J.M., Evans, W.R., Fleischman, D.E., Hungria, M. and Hardy, R.W.F. (1990). The first photosynthetic N_2-fixing *Rhizobium*: characteristics, p.805-811. In P.M. Gresshoff, L.E. Roth, G. Stacey and W.E. Newton (ed.), Nitrogen fixation: Achievements and Objectives. Chapman and Hall, New York.

Elsheikh, E.A.E. and Wood, M. (1990). Salt effects on survival and multiplication of chickpea and soybean rhizobia. Soil Biol. Biochem. **22**: 343-347.

Eskew, D.L. and Ting, I.P. (1978). Nitrogen fixation by legumes and blue-green algal-lichen crusts in a Colorado desert environment. Am. J. Bot. **65**: 850-856.

Estaún, V., Calvet, C. and Hayman, D.S. (1987). Influence of plant genotype on mycorrhizal infection: Response of three pea cultivars. Plant Soil **103**: 295-298.

Evans, D.O. (1990). What is *Sesbania*?, p.5-16. In B. Macklin and D.O. Evans

(ed.), Perennial *Sesbania* species in agroforestry systems. Nitrogen Fixing Tree Association, Hawaii.

Gianinazzi-Pearson, V. and Gianinazzi, S. (1989). Phosphorus metabolism in mycorrhizas, p.227-241. In L. Boddy, R. Marchant and D.J. Read (ed.), Nitrogen, phosphorus and sulphur utilization by fungi. Cambridge University Press, Cambridge.

Giller, K.E. and Wilson, K.J. (1991). Nitrogen fixation in tropical cropping systems. C.A.B. International, Wallingford.

Giovannetti, M. and Mosse, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. **84**: 489-500.

Graham, J.H., Eissenstat, D.M. and Drouillard, D.L. (1991). On the relationship between a plant's mycorrhizal dependency and rate of vesicular-arbuscular mycorrhizal colonization. Functional Ecol. **5**: 773-779.

Graham, P.H., Sadowsky, M.J., Keyser, H.H., Barnet, Y.M., Bradley, R.S., Cooper, J.E., Ley, D.J. de, Jarvis, B.D.W., Roslycky, E.B., Strijdom, B.W. and Young, J.P.W. (1991). Proposed minimal standards for the description of new genera and species of root- and stem-nodulating bacteria. Int. J. Syst. Bacteriol. **41**: 582-587.

Grove, T.S. and Malajczuk, N. (1987). Nitrogen fixation (acetylene reduction) by forest legumes: Sensitivity to pre-harvest and assay conditions. New Phytol. **106**: 115-127.

Gwaze, D.P., Stewart, H.T.L. and Sniezko, R.A. (1988). Variation in height, growth and nodulation of *Acacia albida* and *A. erioloba* seedlings from Zimbabwe seed sources. Nitrogen Fixing Tree Research Reports (NFTRR)

6: 34-36.

Habish, H.A. and Khairi, SH.M. (1968). Nodulation of legumes in the Sudan. Cross-inoculation groups and the associated *Rhizobium* strains. Expl. Agric. **4**: 227-234.

Habish, H.A. and Khairi, SH.M. (1970). Nodulation of legumes in the Sudan II. *Rhizobium* strains and cross-inoculations of *Acacia* spp. Expl. Agric. **6**: 171-176.

Halverson, L.J. and Handelsman, J. (1991). Enhancement of soybean nodulation by *Bacillus cereus* UW85 in the field and in growth chamber. Appl. Environ. Microbiol. **57**: 2767-2770.

Hansen, A.P., Pate, J.S. and Atkins, C.A. (1987). Relationship between acetylene reduction activity, hydrogen evolution and nitrogen fixation in nodules of *Acacia* spp.: Experimental background to assaying fixation by acetylene reduction under field conditions. J. Exp. Bot. **38**: 1-12.

Hardy, R.W.F., Holsten, R.D., Jackson, E.K. and Burns, R.C. (1968). The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. **43**: 1185-1207.

Harley, J.L. and Smith, S.E. (1983). Mycorrhizal symbiosis. Academic Press, London.

Hernandez, B.S. and Focht, D.D. (1984). Invalidity of the concept of slow growth and alkali production in cowpea rhizobia. Appl. Environ. Microbiol. **48**: 206-210.

Hetrick, B.A.D. (1989). Acquisition of phosphorus by VA mycorrhizal fungi and the growth responses of their host plants, p.205-226. In L. Boddy, R. Marchant and D.J. Read (ed.), Nitrogen, phosphorus and sulphur utilization

by fungi. Cambridge University Press, Cambridge.

Högberg, P. (1989). Root symbioses of trees in savannas, p.121-136. In J. Proctor (ed.), Mineral nutrients in tropical forest and savanna ecosystems. Blackwell Scientific Publications, Oxford.

Israel, D.W. (1987). Investigation of the role of phosphorus in symbiotic dinitrogen fixation. Plant Physiol. **84**: 835-840.

James, E.K., Shaw, J.E., Catellan, A.J., Faria, S.M. de and Sprent, J.I. (1993). The infection of aquatic and terrestrial *Neptunia* species by *Rhizobium*, In proceedings of the 9th International congress on nitrogen fixation, Cancun, December 16-12, 1992. In press.

Jarvis, B.D.W., Gillis, M. and de Ley, J. (1986). Intra- and intergeneric similarities between the ribosomal ribonucleic acid cistrons of *Rhizobium* and *Bradyrhizobium* species and some related bacteria. Int. J. Syst. Bacteriol. **36**: 129-138.

Jenkins, M.B., Virginia, R.A. and Jarrell, W.M. (1987). Rhizobial ecology of the woody legume mesquite (*Prosopis glandulosa*) in the Sonoran Desert. Appl. Environ. Microbiol. **53**: 36-40.

Johnson, H.B. and Mayeux, H.S. (1990). *Prosopis glandulosa* and the nitrogen balance of rangelands: extent and occurrence of nodulation. Oecologia **84**: 176-185.

Jordan, D.C. (1984). *Rhizobiaceae*, p.235-256. In N.R. Krieg and J.G. Hold (ed.), Bergey's manual of systematic bacteriology, vol.1. The Williams and Wilkins, Co., Baltimore.

Josey, D.P., Beynon, J.L., Johnston, A.W.B. and Beringer, J.E. (1979). Strain identification in *Rhizobium* using intrinsic antibiotic resistance. J. Appl.

Bacteriol. **46**: 343-350.

Kapp, D.K., Niehaus, K., Quandt, J., Müller, P. and Pühler, A. (1990). Cooperative action of *Rhizobium meliloti* nodulation and infection mutants during the process of forming mixed infected alfalfa nodules. Plant Cell **2**: 139-151.

Karanja, N.K. and Wood, M. (1988a). Selecting *Rhizobium phaseoli* strains for use with beans (*Phaseolus vulgaris* L.) in Kenya: Infectiveness and tolerance of acidity and aluminium. Plant Soil **112**: 7-13.

Karanja, N.K. and Wood, M. (1988b). Selecting *Rhizobium phaseoli* strains for use with beans (*Phaseolus vulgaris* L.) in Kenya: Tolerance of high temperature and antibiotic resistance. Plant Soil **112**: 15-22.

KEFRI. (1990). Kenya Forestry Research Institute (KEFRI) strategic plan 2000. A strategic plan for research and development 1989-2000. KEFRI, Nairobi.

Keya, N.C.O. and van Eijnatten, C.L.M. (1975). Studies on oversowing of natural grassland in Kenya. 2. Effects of seed inoculation and pelleting on the nodulation of *Desmodium uncinatum* (JACQ.) DC. East Afr. Agric. For. J. **40**: 351-358.

Keya, S.O. (1977). Nodulation and nitrogen fixation in legumes in East Africa, p.233-243. In A. Ayanaba and P.J. Dart (ed.), Biological nitrogen fixation in farming systems of the tropics. John Wiley and Sons, Chichester.

Koske, R.E. and Gemma, J.N. (1989). A modified procedure for staining roots to detect VA-mycorrhizas. Mycological Res. **92**: 486-488.

Krisha, K.R., Shetty, K.G., Dart, P.J. and Andrews, D.J. (1985). Genotype dependent variation in mycorrhizal colonization and response to inoculation of pearl millet. Plant Soil **86**: 113-125.

Kuykendall, L.D. (1987). Isolation and identification of genetically marked strains of nitrogen-fixing microsymbionts of soybeans, p.205-220. In G.H. Elkan (ed.), Symbiotic nitrogen fixation technology. Marcel and Dekker, Inc., New York and Basel.

Lange, R.T. (1961). Nodule bacteria associated with the indigenous leguminosae of South-Western Australia. J. Gen. Microbiol. **61**: 351-359.

Lawrie, A.C. (1983). Relationships among rhizobia from native Australian legumes. Appl. Environ. Microbiol. **45**: 1822-1828.

Lawson, K.A., Barnet, Y.M. and McGilchrist, C.A. (1987). Environmental factors influencing numbers of *Rhizobium leguminosarum* biovar *trifolii* and its bacteriophages in two field soils. Appl. Environ. Microbiol. **53**: 1125-1131.

Leakey, R.R.B. and Last, F.T. (1980). Biology and potential of *Prosopis* species in arid environments, with particular reference to *P. cineraria*. J. Arid Environ. **3**: 9-24.

Lie, T.A., Goktän, Engin, M., Pijnenborg, J. and Anlarsal, E. (1987). Co-evolution of the legume-*Rhizobium* association. Plant Soil **100**: 171-181.

Lim, G. and Ng, H.L. (1977). Root nodules of some tropical legumes in Singapore. Plant Soil **46**: 317-327.

Lock, J.M. (1989). Legumes of Africa. A check-list. Royal Botanic Gardens, Kew.

Lowendorf, H.S. (1980). Factors affecting survival of *Rhizobium* in soils, p.87-123. In M. Alexander (ed.), Advances in microbial ecology, vol.**4**. Plenum Press, New York.

Lucena Costa, N. de and Paulino, V.T. (1990). Response of *Leucaena*

leucocephala to single and combined inoculation with *Rhizobium* and mycorrhiza. *Leucaena* Research Reports (LRR) **11**: 45-46.

Madsen, E.L. and Alexander, M. (1982). Transport of *Rhizobium* and *Pseudomonas* through soil. Soil Sci. Soc. Am. J. **46**: 557-560.

Manjunath, A. and Bagyaraj, D.J. (1984). Response of pigeon pea and cowpea to phosphate and dual inoculation with vesicular arbuscular mycorrhiza and *Rhizobium*. Trop. Agric. (Trinidad) **61**: 49-52.

Marks, G.C. (1991). Causal morphology and evolution of mycorrhizas. Agric. Ecosystems Environ. **35**: 89-104.

Martinez-Romero, E., Segovia, L., Mercante, F.M., Franco, A.A., Graham, P. and Pardo, M.A. (1991). *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena* sp. trees. Int. J. Syst. Bacteriol. **41**: 417-426.

May, S.N. and Bohlool, B.B. (1983). Competition among *Rhizobium leguminosarum* strains for nodulation in lentils (*Lens esculenta*). Appl. Environ. Microbiol. **45**: 960-965.

McDonald, J. (1935). The inoculation of leguminous crops. East Afr. Agric. For. J. **1**: 8-13.

McInroy, S.G., Andrews, M. and Sprent, J.I. (1988). Growth, nodulation and nitrate reductase activity in the aquatic legume *Neptunia plena* (L.) Benth. concentrations. Aquatic Bot. **30**: 379-387.

Michelsen, A. (1992). Mycorrhiza and root nodulation in tree seedlings from five nurseries in Ethiopia and Somalia. For. Ecol. Manage. **48**: 335-344.

Miettinnen, P., Luukkanen, O., Johansson, S., Eklund, E. and Mulatya, J. (1988). *Rhizobium* nodulation in *Prosopis juliflora* seedlings at different

irrigation levels in eastern Kenya. Plant Soil **112**: 233-238.

Miller, M.S. and Pepper, I.L. (1988). Survival of a fast-growing strain of lupin rhizobia in Sonoran desert soils. Soil Biol. Biochem. **20**: 323-327.

Minchin, F.R., Sheehy, J.E. and Witty, J.F. (1986). Further errors in the acetylene reduction assay: Effects of plant disturbance. J. Exp. Bot. **37**: 1581-1591.

Moawad, M. and Schmidt, E.L. (1987). Occurrence and nature of mixed infections of field-grown soybeans (*Glycine max*). Biol. Fertil. Soils **5**: 112-114.

Morrison, J. (1966). Productivity of grass and grass/legume swards in Kenya highlands. East Afr. Agric. For. J. **32**: 19-24.

Naisbitt, T., James, E.K. and Sprent, J.I. (1992). The evolutionary significance of the legume genus *Chamaecrista*, as determined by nodule structure. New Phytol. **122**: 487-492.

Nilsen, E.T., Sharifi, M.R., Rundel, P.W., Jarrell, W.M. and Virginia, R.A. (1983). Diurnal and seasonal water relations of the desert phreatophyte *Prosopis glandulosa* (honey mesquite) in the Sonoran Desert of California. Ecology **64**: 1381-1393.

Nishijima, F., Evans, W.R. and Vesper, S.J. (1988). Enhanced nodulation of soybean by *Bradyrhizobium* in the presence of *Pseudomonas fluorescens*. Plant Soil **111**: 149-150.

Norris, D.O. (1965). Acid production by *Rhizobium*, a unifying concept. Plant Soil **22**: 143-166.

Odee, D.W. (1989). A cross-inoculation study on indigenous rhizobia and selected tree legumes. Research Note No.3, Kenya Forestry Research Institute

(KEFRI), Nairobi.

Odee, D.W. (1990). Some characteristics of the *Rhizobium* symbiosis of *Sesbania sesban* and its potential for biological nitrogen fixation in Kenya, p.203-210. In B. Macklin and D.O. Evans (ed.), Perennial *Sesbania* species in Agroforestry systems. Nitrogen fixing tree association, Hawaii.

Odee, D.W. and Sprent, J.I. (1992). *Acacia brevispica*: A non-nodulating mimosoid legume? Soil Biol. Biochem. **24**: 717-719.

Osonubi, O., Mulongoy, K., Awotoye, O.O., Atayese, M.O. and Okali, D.U.U. (1991). Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings. Plant Soil **136**: 131-143.

Pacovsky, R.S., Silva, P. da, Carvalho, M.T. and Tsai, S.M. (1991). Growth and nutrient allocation in *Phaseolus vulgaris* L. colonized with endomycorrhizae or *Rhizobium*. Plant Soil **132**: 127-137.

Peoples, M.B. and Herridge, D.F. (1990). Nitrogen fixation by legumes in tropical and subtropical agriculture. Adv. Agron. **44**: 155-223.

Pielou, E.C. (1975). Ecological diversity. John Wiley and Sons, New York.

Rolfe, B.G., Gresshoff, P.M., Shine, J. and Vincent, J.M. (1980). Interaction between a non-nodulating and an ineffective mutant of *Rhizobium trifolii* resulting in effective (nitrogen fixing) nodulation. Appl. Environ. Microbiol. **39**: 449-452.

Ross, J.H. (1981). An analysis of the African *Acacia* species: their distribution, possible origins and relationships. Bothalia **13**: 389-413.

Roughley, R.J. (1987). Acacias and their root nodule bacteria, p.45-49. In J.W. Turnbull (ed.), Australian acacias in developing countries: proceedings of an

international workshop held at forestry training centre, Gympie, Qld., Australia, 4-7 August, 1986. ACIAR Proceedings No.16. ACIAR, Canberra.

Roughley, R.J., Wahab, F.A. and J. Sundram. (1992). Intrinsic resistance to streptomycin and spectinomycin among root-nodule bacteria from Malaysian soils. Soil Biol. Biochem. **24**: 715-716.

Sanginga, N., Manrique, K. and Hardarson, G. (1991). Variation in nodulation and N_2-fixation by *Gliricidia sepium/Rhizobium* spp. symbiosis in a calcareous soil. Biol. Fertil. Soils **11**: 273-278.

Schreven, D.A. van. (1964). The effect of some actinomycetes on rhizobia and *Agrobacterium radiobacter*. Plant Soil **21**: 283-302.

Scott, J.M. and Porter, F.E. (1986). An analysis of the accuracy of a plant infection technique for counting rhizobia. Soil Biol. Biochem. **18**: 355-362.

Selker, J.M.L. and Newcomb, E.H. (1985). Spatial relationships between uninfected and infected cells in root nodules of soybean. Planta **165**: 446-454.

Sieverding, E. (1991). Vesicular-arbuscular mycorrhizal management in tropical agrosystems. Deutsch Gessellschaft für Technische Zusammenarbeit (GTZ), Eschborn.

Sneath, P.H. (1984). Numerical taxonomy, p.5-7. In N.R. Krieg and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol.1. The Williams and Wilkins, Co., Baltimore.

Sniezko, R.A. (1987). Range-wide provenance variation in nodulation of *Acacia albida* seedlings. Nitrogen Fixing Tree Research Reports (NFTRR) **5**: 20-21.

Somasegaran, P. and Hoben, H.J. (1985). Methods in Legume - *Rhizobium* technology. NifTAL project and MIRCEN, University of Hawaii.

Sombroek, W.G., Braun, H.M.H. and van der Pouw, B.J.A. (1982). Exploratory soil map and agro-climatic zone map of Kenya, 1980. Exploratory soil survey report no. E1, Kenya Soil Survey, Nairobi.

Souza, D.I.A. de (1969). Legume nodulation and nitrogen fixation studies in Kenya. East Afr. Agric. For. J. **34**: 299-305.

Sprent, J.I. (1986). Potentials for nitrogen fixing legume trees in the tropics. Int. Tree Crops J. **4**: 49-55.

Sprent, J.I. (1989). Which steps are essential for the formation of functional legume nodules? New Phytol. **111**:129-153.

Sprent, J.I. (1993). Harnessing symbiotic association: The potentials of nitrogen fixing trees. In R. Leakey and A. Newton (ed.), Tropical trees: The potential for domestication. Proceedings of ECTF Conference, held in August 1992, Edinburgh, Scotland. In press.

Sprent, J.I., Becana, M. and Sutherland, J.M. (1988). Optimizing nitrogen fixation in legume crops and trees, p.725-734. In H. Bothe, F.J. de Bruijn and W.E. Newton (ed.), Nitrogen fixation: Hundred years after. Gustav Fischer, Stuttgart.

Sprent, J.I. and Faria, S.M. de (1988). Mechanisms of infection of plants by nitrogen fixing organisms. Plant Soil **110**: 157-165.

Sprent, J.I. and Sprent, P. (1990). Nitrogen fixing organisms. Pure and applied aspects. Chapman and Hall, London.

Streeter, J.G. and Salminen, S.O. (1993). Effect of polysaccharide deposition by *Bradyrhizobium japonicum* bacteroids in soybean nodules on nodule function.

Plant Physiol. Biochem. **31**: 73-79.

Sutherland, J.M. and Sprent, J.I. (1993). Nitrogen fixation by legume trees, p.33-63. In N.S. Subba Rao and C. Rodriguez-Barrueco (ed.), Symbioses in nitrogen-fixing trees. Oxford and IBH Publishing Co. PVT Ltd., New Delhi.

Sylvester-Bradley, R., Thornton, P. and Jones, P. (1988). Colony dimorphism in *Bradyrhizobium* strains. Appl. Environ. Microbiol. **54**: 1033-1038.

Toomsan, B., Rupela, O.P., Mittal, S., Dart, P.J. and Clark, K.W. (1984). Counting *Cicer-Rhizobium* using a plant infection technique. Soil Biol. Biochem. **16**: 503-507.

Trinick, M.J. (1980). Relationships amongst the fast-growing rhizobia of *Lablab purpureus*, *Leucaena leucocephala*, *Mimosa* spp., *Acacia farnesiana* and *Sesbania grandiflora*, and their affinities with other rhizobial groups. J. Appl. Bacteriol. **49**: 39-53.

Turk, D. and Keyser, H.H. (1992). Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness. Can. J. Microbiol. **38**: 451-460.

Venkateswarlu, B. and Singh, R.P. (1988). Nodulation and nitrogenase activity patterns of *Leucaena leucocephala* and *Gliricidia sepium* during early stages of growth. *Leucaena* Research Reports (LRR) **9**: 55-58.

Vincent, J.M. (1970). A manual for the practical study of root-nodule bacteria. International Biological Programme Handbook no.15. Blackwell Scientific Publications Ltd., Oxford.

Vincent, J.M. (1981). The genus *Rhizobium*, p.818-841. In M.P. Starr, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (ed.), The prokaryotes. A

handbook on habits, isolation and identification of bacteria, Vol.**1**. Springer-Verlag, Berlin.

Virginia, R.A., Jenkins, M.B. and Jarrell, W.M. (1986). Depth of root symbiont occurrence in soil. Biol. Fertil. Soils **2**: 127-130.

Wadisirisuk, P., Danso, S.K.A., Hardarson, G. and Bowen, G.D. (1989). Influence of *Bradyrhizobium japonicum* location and movement on nodulation and nitrogen fixation in soybeans. Appl. Environ. Microbiol. **55**: 1711-1716.

Whaley, J.W. and Boyle, A.M. (1967). Antibiotic production by *Streptomyces* species from the rhizosphere of desert plants. Phytopathology **57**: 347-351.

Wild, A. (1988). Russell's soil conditions and plant growth. Longman Group UK. Ltd. and John Wiley and Sons, Inc., New York.

Woomer, P., Asano, W. and Pradhan, D. (1990). Environmental factors related to rhizobial abundance in kikuyugrass (*Pennisetum clandestinum*) pasture. Trop. Agric. (Trinidad) **67**: 217-220.

Woomer, P., Bennett, J. and Yost, R. (1990). Overcoming the inflexibility of most-probable-number procedures. Agron. J. **82**: 349-353.

Woomer, P., Singleton, P.W. and Bohlool, B.B. (1988a). Ecological indicators of native rhizobia in tropical soils. Appl. Environ. Microbiol. **54**: 1112-1116.

Woomer, P.L., Singleton, P.W. and Bohlool, B.B. (1988b). Reliability of the most-probable-number technique for enumerating rhizobia in tropical soils. Appl. Environ. Microbiol. **54**: 1492-1497.

Worrall, V. and Roughley, R.J. (1991). Vertical movement of *Rhizobium leguminosarum* bv. *trifolii* in soil as influenced by soil water potential and

water flow. Soil Biol. Biochem. **23**: 485-486.

Young, J.P.W., Downer, H.L. and Eardly, B.D. (1991). Phylogeny of the phototrophic *Rhizobium* strain BTAi1 by polymerase chain reaction-based sequencing of a 16S rRNA gene segment. J. Bacteriol. **173**: 2271-2277.

Yousef, A.N., Al-Nassiri, A.A., Al-Azani, S.K. and Abdul-Hussain, N. (1987). Abundance of peanut *Rhizobium* as affected by environmental conditions in Iraq. Soil Biol. Biochem. **19**: 391-396.

Zang, X., Harper, R., Karsisto, M. and Lindström, K. (1991). Diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees. Int. J. Syst. Bacteriol. **38**: 89-98.

Zar, J.H. (1974). Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs.

Appendix 1. List of seeds and sources used in this study

Species	Provenance/Cultivar*	Source
Acacia brevispica Harms	Kibwezi	KEFRI Seed Centre
Acacia brevispica Harms	Wamba	KEFRI Seed Centre
Acacia elatior Brenan	Turkana	KEFRI Seed Centre
Acacia elatior Brenan	Bura	Collected from experimental site
Acacia mearnsii De Wild.	Kakamega	KEFRI Seed Centre
Acacia mearnsii De Wild.	Kitale	KEFRI Seed Centre
Acacia mellifera (Vahl) Benth.	Loruk	KEFRI Seed Centre
Acacia mellifera (Vahl) Benth.	Lodwar	KEFRI Seed Centre
Acacia mellifera (Vahl) Benth.	Isiolo	KEFRI Seed Centre
Acacia nilotica (L.) Del.	Wamba	KEFRI Seed Centre
Acacia nilotica (L.) Del.	Marigat	KEFRI Seed Centre
Acacia nubica Benth.	Marigat	KEFRI Seed Centre
Acacia nubica Benth.	Lodwar	KEFRI Seed Centre
Acacia nubica Benth.	Wamba	KEFRI Seed Centre
Acacia polyacantha Willd.	Nyeri	KEFRI Seed Centre
Acacia polyacantha Willd.	Kitui	KEFRI Seed Centre
Acacia polyacantha Willd.	Nyamonye	Collected from experimental site
Acacia reficiens Wawra	Katilu	KEFRI Seed Centre

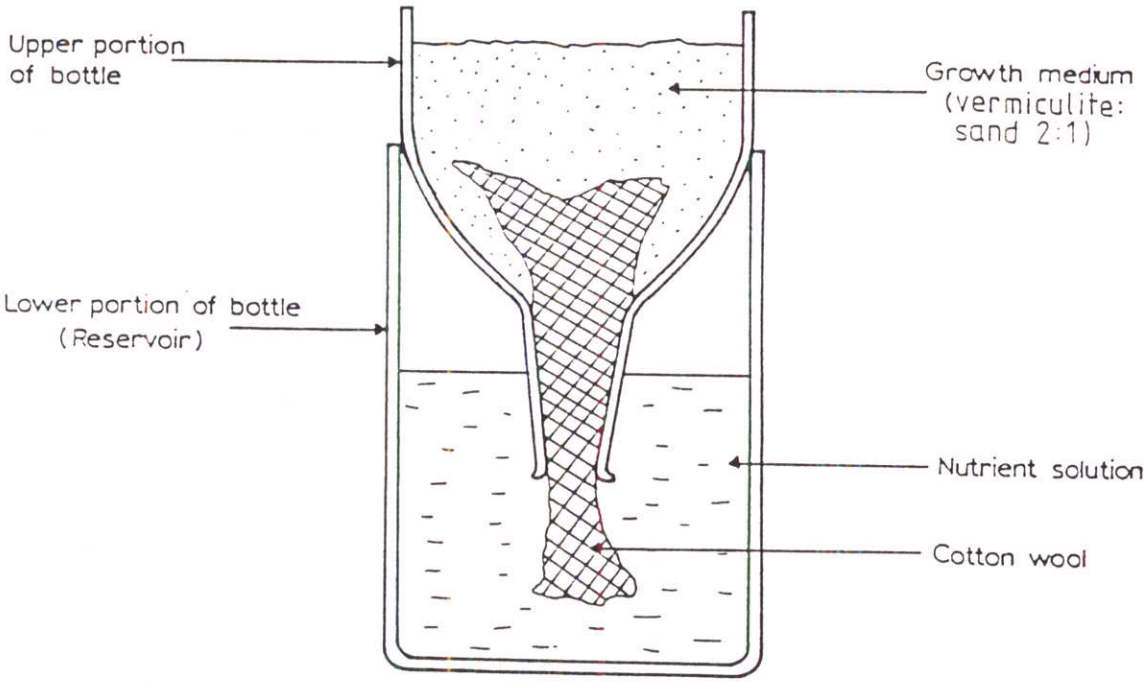
Appendix 1. Continued

Species	Provenance/Cultivar*	Source
Acacia senegal (L.) Willd.	Hunter's Lodge	KEFRI Seed Centre
Acacia senegal (L.) Willd.	Machakos	KEFRI Seed Centre
Acacia tortilis (Forsskal) Hayne	Marigat	KEFRI Seed Centre
Acacia tortilis (Forsskal) Hayne	Mwatate	KEFRI Seed Centre
Acacia tortilis (Forsskal) Hayne	Wamba	KEFRI Seed Centre
Acacia tortilis (Forsskal) Hayne	Kibwezi	KEFRI Seed Centre
Acacia tortilis (Forsskal) Hayne	Lodwar	KEFRI Seed Centre
Acacia xanthophloea Benth.	Athi river	KEFRI Seed Centre
Acacia xanthophloea Benth.	Rongai	KEFRI Seed Centre
Acacia xanthophloea Benth.	Mai Mahiu	KEFRI Seed Centre
Acacia xanthophloea Benth.	Kibwezi	KEFRI Seed Centre
Acacia zanzibarica (S. Moore) Taubert	Bura	KEFRI Seed Centre
Acacia zanzibarica (S. Moore) Taubert	Garissa	KEFRI Seed Centre
Faidherbia albida (Del.) A. Chev.	Kainuk	KEFRI Seed Centre
Faidherbia albida (Del.) A. Chev.	Elgeyo/Marakwet	KEFRI Seed Centre
Faidherbia albida (Del.) A. Chev.	Turgen	KEFRI Seed Centre
Macroptilium atropurpureum (DC.) Urban	Siratro*	CSIRO
Phaseolus vulgaris L.	Canadian Wonder*	Local agricultural shop
Prosopis chilensis L.	Etirae	KEFRI Seed Centre

Appendix 1. Continued

Species	Provenance/Cultivar*	Source
Prosopis chilensis L.	Peru	KEFRI Seed Centre
Prosopis julifora L.	Bura	KEFRI Seed Centre
Sesbania grandiflora (L.) Poiret	Kitui	KEFRI Seed Centre
Sesbania sesban (L.) Merr.	Yala swamp	Collected from experimental site
Sesbania sesban (L.) Merr.	Kakamega	KEFRI Seed Centre
Vigna unguiculata (L.) Walp.	Unknown	Local agricultural shop

Appendix 2.



Modified Leonard jar

Appendix 3.

(a) Yeast extract mannitol agar/broth for growing rhizobia

Mannitol

K_2HPO_4

$MgSO_4.H_2O$

NaCl

Yeast extract

*Agar

All made up in 1 l distilled water.

* for yeast mannitol broth, agar is excluded.

(b) Peptone glucose agar

Glucose (Dextrose)

Peptone

Agar

All made up in 1 l distilled water

Appendix 4. N and P concentrations in plant parts, VAM fungi infection and root:shoot ratios in *A. tortilis* Marigat, *A. tortilis* Mwatate and *A. brevispica* Wamba provenances (± SE, n = 16 unless indicated otherwise)

Parameter	Species and Provenance			F-test[1] level
	A. tortilis		*A. brevispica*	
	Marigat	Mwatate	Wamba	
Leaf N (%)	2.44 ± 0.12	2.64 ± 0.13	2.67 ± 0.12	NS
Stem N (%)	1.81 ± 0.15	1.84 ± 0.10	1.50 ± 0.10	NS
Shoot N (%)	2.13 ± 0.13	2.24 ± 0.11	2.09 ± 0.09	NS
Root N (%)	1.59 ± 0.09	1.73 ± 0.07	1.73 ± 0.11	NS
Total N (%)	1.86 ± 0.11	2.06 ± 0.09	1.43 ± 0.01	NS
Seed N (%)	3.50 ± 0.10	3.25 ± 0.15	2.65 ± 0.15	NT
Shoot P (%)	0.032 ± 0.003*	0.022 ± 0.001	0.023 ± 0.002	S
Root P (%)	0.027 ± 0.003	0.019 ± 0.003	0.025 ± 0.002	NS
Total P (%)	0.030 ± 0.002*	0.022 ± 0.002	0.024 ± 0.001	S
Seed P (%)	0.065 ± 0.002	0.044 ± 0.001	0.037 ± 0.08	NT
VAM infection (%)	32.6 ± 3.6	31.9 ± 4.3	28.1 ± 3.9	NT
Root:shoot ratio	0.68 ± 0.11	0.74 ± 0.07	0.83 ± 0.06	NS

[1] NS = not significant at P = 0.05, S = significant at P = 0.01

* indicates the provenance that was significantly higher than the other two at a given F-test level.

NT = not tested by ANOVA, but compared by SE, n = 4 for seed and n > 10 for VAM fungi infection.