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Soil Biology & Biochemistry 32 (2000) 323–333

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Single and multi-strain rhizobial inoculation of African acacias in nursery conditions

J.M. Sutherland[a,*], D.W. Odee[b], G.M. Muluvi[b,1], S.G. McInroy[a], A. Patel[c,2]

[a]*Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, Scotland, UK*
[b]*Kenya Forestry Research Institute, P.O. Box 20412, Nairobi, Kenya*
[c]*Department of Physiology, University of Dundee, Dundee DD1 4HN, Scotland, UK*

Abstract

Inoculation experiments were conducted in Kenya on seven African *Acacia* species/subspecies (*Faidherbia albida*, *Acacia karroo*, *A. arenaria*, *A. nilotica* ssp. *kraussiana*, *A. tortilis* ssp. *spirocarpa*, *A. tortilis* ssp. *heteracantha*, *A. senegal*) in sterilised and untreated soil. The untreated soil contained 10^3 rhizobia g^{-1}. In six of seven species in untreated soil the multi-strain inoculated plants contained significantly more total nitrogen than control plants. The exception was *A. arenaria* in which significant increase in total nitrogen was achieved only with the single strain inoculum in sterile soil. In *A. tortilis* ssp. *spirocarpa* the single strain was better than the multi-strain inoculum. Significant increases over controls in dry weight ranged from 19 to 75% and in total nitrogen from 11 to 89%. Nitrogen derived from fixation (Ndff) was determined for three species/subspecies using the natural abundance ^{15}N method. Values for fixation for the best treatments in these species were *A. nilotica* 53%, *A. tortilis* ssp. *heteracantha* 45% and *A. tortilis* ssp. *spirocarpa* 44%. These are conservative values because of the relatively small $\delta^{15}N$ values (−2.85‰ for *A. nilotica* and −2.52‰ for both species of *A. tortilis*) determined as the 100% fixation values. We conclude that: inoculation can result in substantial gains in nitrogen fixation in African acacias; multi-strain inoculation is preferable to single strain inoculation in some circumstances; *A. nilotica* and *A. tortilis* have at least moderate nitrogen fixation potential and the wide genetic variation found suggests that substantial improvement may be obtained by selection for this character.

Keywords: African *Acacia* species; Multi-strain inoculation; Tropical rhizobia; Nitrogen fixation; Natural abundance ^{15}N; Nursery conditions

1. Introduction

The importance of acacias in the rural economy of Africa, particularly in semi-arid areas, is widely recognised and efforts in germplasm collection and testing are expanding (Fagg and Stewart, 1994). Their uses include fodder, fuelwood, timber gums and many others such as honey production and medicines. The seed used in this study were from single provenances of the most potentially useful species or subspecies from a large collection of African acacias made by an Oxford Forestry Institute/ODA research project. When planting legume trees in new areas or when performance testing in areas in which some species/provenances may be native and others not, an understanding of their rhizobial requirements is necessary to ensure establishment or fair assessment. While Australian acacias appear to be promiscuous for nodulation (Roughley, 1987) both African and Australian species vary in their strain specificity for high nitrogen fixation as determined using single strain inocula

* Corresponding author. Fax: +44-1382-344-275.
E-mail address: j.m.sutherland@dundee.ac.uk (J.M. Sutherland).
[1] Present address: Department of Biochemistry, Kenyatta University, P.O. Box 43844, Nairobi, Kenya.
[2] Present address: Department of Chemistry, University of St. Andrews, St. Andrews KY16 9ST, Scotland, UK.

0038-0717/00/$ - see front matter
PII: S0038-0717(99)00157-1

(Dreyfus and Dommergues, 1981; Barnet and Catt, 1991; Sun et al., 1992; Turk and Keyser, 1992). Variation in nodulation and nitrogen fixation in individual acacia species has been shown between sites where these species grow naturally (Basak and Goyal, 1980; Odee et al., 1995). Strain competitiveness has been shown to vary with a wide range of environmental factors (Dowling and Broughton, 1986). However, the practice of inoculation with single, highly competitive strains is a risky strategy since strains may not be effective in all environmental conditions, may lose effectiveness, or be superseded by more effective strains while the population of the introduced strain may remain high through seasonal renewal of nodules on the trees (Lal and Khanna, 1993). We therefore, hypothesised that multi-strain inocula may increase the chance of good nodulation in differing environmental conditions. The strategy would also simplify the application of inocula in large performance trials. We anticipated that this may not be as effective as single specific strains but would reduce the risk of complete failure of nodulation in adverse conditions. A nursery experiment was set up at contrasting sites in Kenya to test the efficacy of a multi-strain inoculum in soil with a substantial indigenous rhizobial population. The strains used had been selected for individual species in standard sterile conditions. After the experiment had been set up we found that all strains had lost infectivity during storage. The results of this experiment were nevertheless useful and a second experiment was set up to assess a new multi-strain inoculum against single specific strains in both sterilised and untreated soil. The results of these experiments are presented.

2. Materials and methods

2.1. Experimental sites

The Muguga site was at Kenya Forestry Research Institute (KEFRI) headquarters which is in the subhumid zone at a height of 2000 m. The Kitui site was located in Kwa-Vonza about 21 km west of Kitui town in the semi-arid zone at a height of 1100 m. *Acacia* species dominate the natural vegetation of the latter site but do not occur at the former.

2.2. Seed

Seed of the following species was obtained from Oxford Forestry Institute, Oxford, UK (abbreviations for species names used in the tables are given in brackets): *Faidherbia albida* (Del.) A. Chev., (Fa); *Acacia karroo* Hayne, (Ak); *A. arenaria* Schinz, (Aa); *A. nilotica* (L.) Willd. ssp. *kraussiana* (Benth.) Brenan, (An); *A. tortilis* (Forssk.) Hayne ssp. *spirocarpa* (Hochst. ex A. Rich.) Brennan, (Ats); *A. tortilis* (Forssk.) Hayne ssp. *heteracantha* (Burch.) Brenan, (Ath); and *A. senegal* L. Willd (As). Provenance Machakos (As) was obtained from Kenya Forestry Seed Centre (KFRSC).

Table 1
Host origin and growth rate of strains used in this study. DUS and KFR = Dundee University Sprent and Kenya Forestry Research Institute bacterial culture collections, respectively

Strain	Growth rate	Host	Origin
Experiment 1			
DUS 8	fast	*A. tortilis*	Kenya
DUS 13	slow	*F. albida*	Kenya
DUS 24	fast	*A. senegal*	Kenya
DUS 36	fast	*A. tortilis*	Kenya
DUS 48	fast	*A. nilotica*	Zimbabwe
DUS203	fast	*A. seyal*	Kenya
DUS292	fast	*A. hebeclada*	Zimbabwe
DUS300	fast	*A. nilotica*	Zimbabwe
DUS351	slow	*F. albida*	Zimbabwe
Experiment 2			
DUS 48(1)	fast	*A. nilotica*	Zimbabwe
DUS 453c	slow	*F. albida*	Expt 1
DUS 454a	fast	*A. arenaria*	Expt 1
DUS 455d	fast	*A. tortilis*	Expt 1
DUS 492b	fast	*A. karroo*	Expt 1
KFR 357	fast	*A. senegal*	Kenya

2.3. Growth of plants

Seed of *A. senegal* had poor germination. Surface sterilization was carried out with 1% mercuric chloride. Seed was pretreated with hot water and with one exception, was scarified with a hot wire prior to sowing: the thick seed coat of *A. nilotica* ssp. *kraussiana* required nicking with nail clippers. In experiment 1 seed was sown in sterile sand trays. Trays were sprayed with distilled water 3 times daily and then transferred to the glasshouse from the laboratory after 4 d. After 14 d seedlings were transferred to black plastic tubes (15 × 23 cm) containing c. 1.5 kg Muguga soil. In experiment 2 seed were sown directly into black tubes, 3 seeds per tube, containing the same soil. When more than one seed germinated, only one was retained. In both experiments plants were watered 1 d after inoculation and twice per day thereafter until harvest at 5 months.

2.4. Inoculants

Inoculant strains are listed in Table 1. The methods of Somasegaran and Hoben (1985) were used in preparation of the peat carrier in experiment 1. The single strain inocula were prepared by adding 45 ml of undiluted late log phase broth cultures to 50 g bags of peat and incubating for 1 week. The multi-strain inoculum was prepared by adding 5 ml of fully grown

broth culture of each of the single strains (total 45 ml) to 50 g bags of peat and incubating for 1 week. Inoculation was carried out 14 d after transplanting by making a slurry of peat inoculum in water. Inoculum concentration after application was c. 10^5 rhizobia g^{-1} of soil. When the original single cultures used for preparation of the multi-strain peat inoculum for this experiment were retested on their original hosts under sterile laboratory conditions after the experiment had been set up, all were found to be ineffective. Strains had been stored on YMA slopes at 4°C for 6–12 months with subculturing at 4–6 months. However, a subculture of one strain, DUS 48, was found to have retained effectiveness and was included in experiment 2. The indigenous rhizobial soil population was at least 10^3 rhizobia g^{-1} determined using *F. albida* and *A. polyacantha* as trap hosts and the most probable number enumeration system (MPNES) of Bennett et al. (1990).

In experiment 2 the freshly tested isolates from experiment 1 plus DUS 48 were first inoculated into 50 g bags of sterile filter mud obtained from a local sugar factory and prepared as described in Marufu et al. (1995). To prepare the single strain inocula 42 ml of undiluted late log phase broth cultures were injected into the filter mud bag, and for the multi-strain inoculum, 7 ml of each strain (42 ml in total) were injected into the bags and incubated for 1 week at c. 28°C. Viable counts after incubation were 3.4×10^9 fast growing cells g^{-1} and 7×10^8 slow growing cells g^{-1} of moist carrier. Plants were inoculated 2 weeks after sowing with 10 ml of filter mud slurry diluted with 1/4 strength N-free nutrient solution (Somasegaran and Hoben, 1985). Each plant received a total of c. 10^8 rhizobia.

2.5. Experimental design

Treatments in experiment 1 were: control, which received autoclaved inoculum; and inoculated, which received the first multi-strain peat inoculum, each with 10 replicates for all seven species/subspecies at two sites, Kitui and Muguga. In experiment 2 treatments were: control (C), in which plants received sterilised inoculum; single (S), in which each species/subspecies received a specific strain; and multi-strain inoculum (M), in which all species received the same mixture of strains. Soil was either steam sterilised (S) or untreated (U). There were two blocks of eight replicates each at only one site, Kitui.

In both experiments plants with the same inoculation treatment had to be kept together within blocks to reduce the possibility of contamination by inoculant strains, but species were randomised within these blocks. Between block effects would likely be due only to slight differences in wind exposure and aspect since the same soil was used in all cases. Since both sites were relatively level and evenly exposed these differences would be minimal compared with treatment effects. Seedlings were watered twice daily. The Kitui nursery site was fenced to prevent grazing by gazelle and was guarded at night.

2.6. Analyses

Seedlings in experiment 1 were divided into shoots and roots then dried, weighed and ground. Nodules were removed, weighed while fresh and placed in vials containing silica gel. Ten nodules (where present), five large and five small selected at random from each of 5 plants of all species were used for strain isolation. Some of these isolates were tested in sterile conditions on their primary hosts and the most effective isolate was used in experiment 2.

Nitrogen and carbon analyses were carried out using a Carlo Erba elemental analyser, model 1106, and an atropine calibration standard. Of the 10 plant replicates per treatment in experiment 1, only four were selected at random for C and N analyses. In experiment 2, C and N analyses were carried out on nodules, and the whole plant minus the nodules of all replicates in all blocks and treatments.

In experiment 1 natural abundance ^{15}N was analysed by continuous flow dry combustion followed by mass spectrometry with a Tracermass (Europa Scientific Instruments, Crewe) mass spectrometer. Leaf samples of all plants were analysed separately but nodules remaining (after 50 were used for strain isolation) from all replicate plants per treatment were pooled before analysis. In experiment 2, natural abundance ^{15}N analyses were carried out on all whole plants (minus nodules) and nodule samples from all replicate plants of 3 species (*A. tortilis* ssp. *heteracantha, A. tortilis* ssp. *spirocarpa* and *A. nilotica* ssp. *kraussiana*) grown in untreated soil. The natural abundance of ^{15}N was expressed as $\delta^{15}N\ (‰) = 1000(R_{sample} - R_{standard})/R_{standard}\ (‰)$, where $R =$ mass 29/mass 28 and the standard was calibrated against atmospheric N_2. In estimating nitrogen derived from fixation (Ndff) nonnodulated plants which were using only seed or soil N were used as controls ($\delta^{15}N_o$), and it was assumed that the plants with the smallest $\delta^{15}N$ values were fixing 100% nitrogen ($\delta^{15}N_z$). Values for each plant ($\delta^{15}N_f$) were interpolated between these two values to give the fraction of plant nitrogen derived from fixation as follows: $\%Ndff = 100\ (\delta^{15}N_o - \delta^{15}N_f)/(\delta^{15}N_o - \delta^{15}N_z)$. For the 100% fixation value the average of the two most depleted plants was used. These gave values for *A. nilotica* of $-2.85‰ \pm 0.01$, and for *A. tortilis* of $-2.52‰ \pm 0.03$. Data for the *A. tortilis* subspecies were pooled to obtain this value. Corresponding nodule enrichments were $5.24‰ \pm 1.59$

and 4.55‰ ± 1.56 respectively. Potential nitrogen fixation was estimated as follows: correlations were determined between both plant $\delta^{15}N$ ($\delta^{15}N_f$) and the difference between nodule and plant $\delta^{15}N$ ($\delta^{15}N_{nod\text{-}plant}$); the parameter having the more significant of these correlations was then used to identify both the four plants fixing the most nitrogen (those with either the most negative $\delta^{15}N_f$ or the most positive $\delta^{15}N_{nod\text{-}plant}$) and the four plants fixing the least nitrogen; these were averaged and the difference described as the potential nitrogen fixation.

2.7. Statistics

Data were analysed by multivariate analysis of variance using Statgraphics, Version 6, Manguistics, Inc., Rockville, MD, USA.

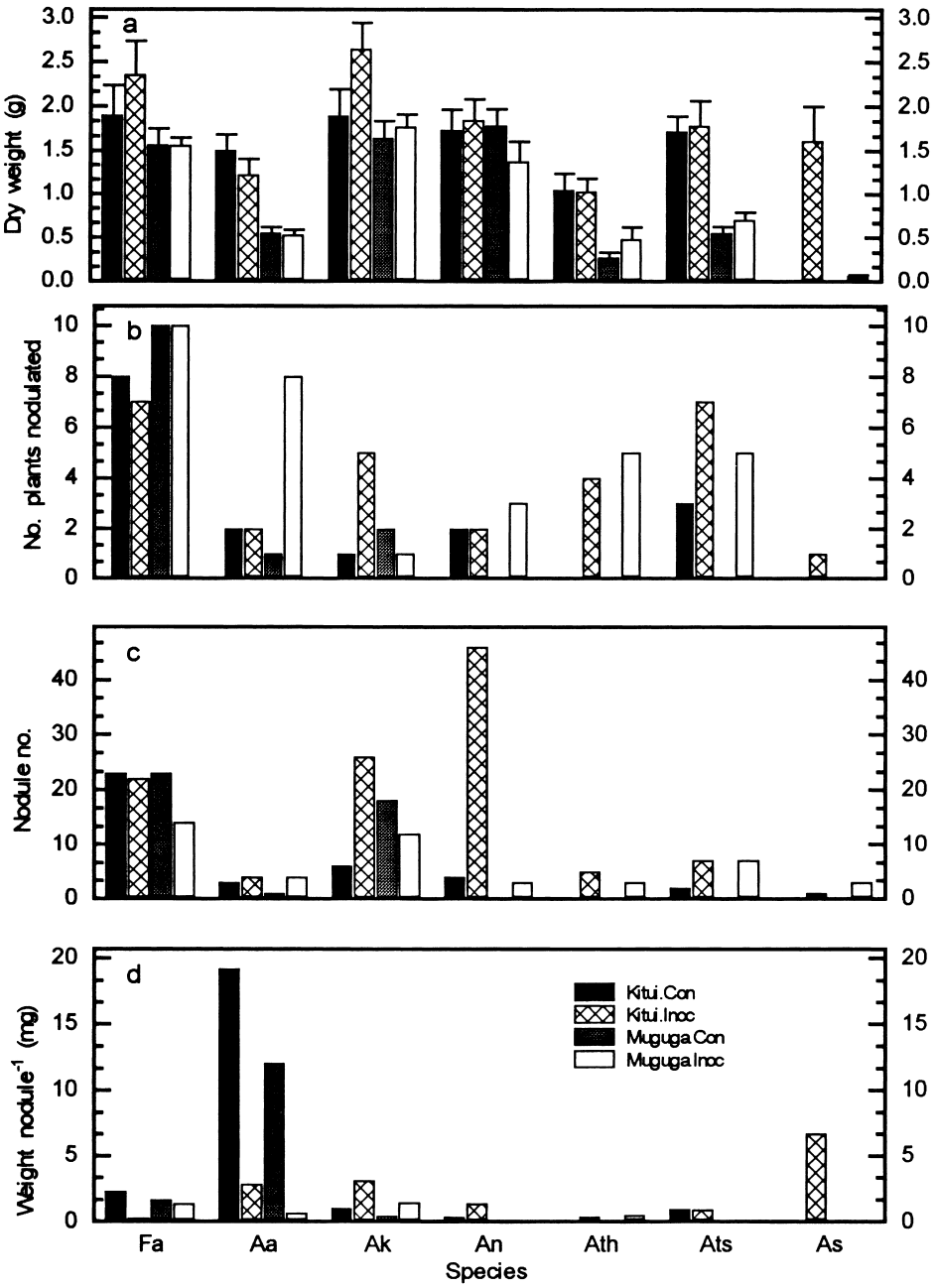

Fig. 1. Data from experiment 1 showing dry weight (a), number of plants nodulated (b), nodule number per plant (c) and weight per nodule (d) in seven species/subspecies of African acacia: *Faidherbia albida* (Fa), *A. arenaria* (Aa), *A. karroo* (Ak), *A. nilotica* ssp. *kraussiana* (An), *A. tortilis* ssp. *heteracantha* (Ath), *A. tortilis* ssp. *spirocarpa* (Ats), *A. senegal* (As). Data for control and inoculated plants at two sites Kitui and Muguga are shown. Data are means, bars indicate standard errors, $n = 10$.

Table 2
$\delta^{15}N$ values in shoots and nodules in experiment 1. Data for shoots are means ± standard error, $n = 10$. For nodules $n = 1$. nd = not determined

Species	Site	Control		Inoculated	
		shoot	nodule	shoot	nodule
F. albida	Kitui	6.54 ± 0.32	6.37	5.16 ± 0.4	5.73
	Muguga	nd	4.55	5.43 ± 0.37	5.36
A. arenaria	Kitui	6.44 ± 0.29	6.37	4.68 ± 0.47	nd
	Muguga	nd	4.55	4.34 ± 0.32	9.45
A. karroo	Kitui	6.24 ± 0.43	nd	5.59 ± 0.67	9.49
	Muguga	nd	nd	5.74 ± 0.22	8.74
A. nilotica	Kitui	5.59 ± 0.29	9.48	4.48 ± 0.18	7.46
	Muguga	nd	nd	3.77 ± 0.35	7.41
A. tortilis ssp. *heteracantha*	Kitui	6.49 ± 0.42	nd	6.16 ± 0.35	nd
	Muguga	nd	nd	3.59 ± 0.27	nd
A. tortilis ssp. *spirocarpa*	Kitui	nd	nd	5.78 ± 0.32	nd
	Muguga	nd	nd	nd	nd
A. senegal	Kitui	nd	nd	6.96 ± 0.30	11.99
	Muguga	nd	nd	nd	nd

3. Results

3.1. Experiment 1

3.1.1. Dry weight

Despite having been inoculated with rhizobia in which subsequent testing showed loss of infectiveness, some positive effects on growth and nodulation were observed. Highly significant site differences ($P =< 0.001$) and site/species interactions ($P =< 0.01$) were found in both growth and nodulation measurements. Total plant dry weights for the best treatment for each species was greater at Kitui than Muguga with those of *A. tortilis* (both subspecies) and *A. arenaria* being 2- to 3-fold greater (Fig. 1a). In three cases, *A. nilotica* ssp. *kraussiana*, *A. karroo* and *F. albida,* the dry weights of control plants did not differ between the two sites. Significant increases in dry weight were achieved with inoculation in two species: *A. karroo* at Kitui and *A. tortilis* ssp. *spirocarpa* at Muguga. Inoculation significantly reduced dry weight of *A. nilotica* ssp. *kraussiana* at Muguga.

3.1.2. Nodulation

The patterns of nodulation differed markedly at the two sites (Fig. 1b,c). In control treatments only *Faidherbia albida* were well nodulated at both sites with eight of 10 plants nodulated at Kitui and 10 of 10 at Muguga. In general, nodulation (both number of plants which nodulated and number of nodules per plant) was increased by inoculation in four of six species at each site but the species differed at the two sites, *A. karroo* being better at Kitui than Muguga and the reverse was found for *A. arenaria* (Fig. 1b,c). There were large differences in weight per nodule between species and treatments: in *A. arenaria* and *F. albida* to a lesser extent, nodules were larger on control than inoculated plants (Fig. 1d).

3.1.3. Nitrogen fixation

While inoculation increased nodulation in *A. arenaria* (Fig. 1b,c) neither dry weight (Fig. 1a) nor total nitrogen (data not shown) were increased, suggesting that nodules were ineffective. Nitrogen analyses were carried out on four plants only per treatment. $\delta^{15}N$ analysis was carried out on nodules pooled from all replicate plants in each treatment and showed large differences (up to six delta units) between shoots (not pooled) and nodules, with nodules enriched with respect to shoots (Table 2). Species showing large differences in one or more treatments were *A. arenaria*, *A. karroo, A. nilotica* ssp. *kraussiana* and *A. senegal*. Neither of the *A. tortilis* subspecies had sufficient nodules for analyses. However, the shoot value for *A. tortilis* ssp. *heteracantha* was significantly lower in the inoculated Muguga plants than in inoculated Kitui plants indicating better fixation in the Muguga plants. The only species in which there was no difference between shoot and nodule $\delta^{15}N$ values was *F. albida*.

3.2. Experiment 2

3.2.1. Dry weight

In five of the seven species/subspecies in untreated soil average dry weight per plant was significantly greater in inoculated treatments than in uninoculated controls (Fig. 2a). Three species (*F. albida, A. tortilis* ssp. *heteracantha*, and *A. senegal*) showed significant ($P =< 0.05$) increases in growth with the multi-strain inoculum and two (*A. nilotica* and *A. tortilis* ssp. *spirocarpa*) with single strain inocula. In a sixth species (*A. arenaria*) the single inoculum increased dry weight in

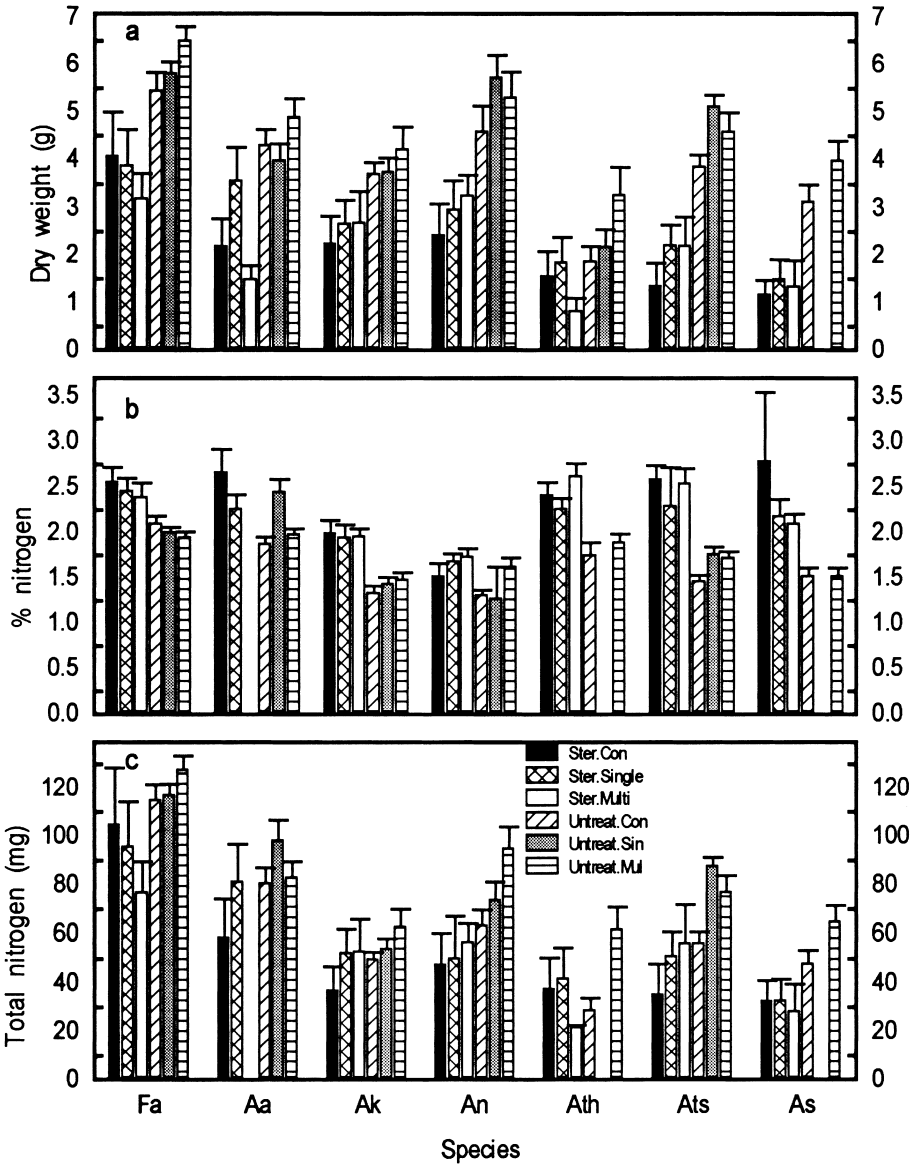

Fig. 2. Data from experiment 2 showing dry weight (a), % nitrogen (b), total nitrogen (c), number of plants nodulated (d), weight per nodule (e) and nodule number per plant (f), for seven species/subspecies and three treatments: control (con), single strain inoculum (single) and multi-strain inoculum (multi) in sterilised (ster) and untreated soil (untreat). Data are means, bars indicate standard errors, $n = 16$.

sterilised but not in untreated soil. There was a 2- to 3-fold increase in dry weight between the worst and best treatments for each species. Overall plant growth was significantly greater ($P =< 0.001$) in untreated (mean 4.27 g) than sterilised (mean 2.35 g) soil with one exception, *A. tortilis* ssp. *heteracantha,* which had significantly lower dry weight than all other species. There was no difference between the sterilised and untreated control treatments. Species ranked as follows according to their average dry weight for all treatments *F. albida* > *A. nilotica* > *A.tortilis* ssp. *spirocarpa* > *A. arenaria* > *A. karroo* > *A. senegal* > *A. tortilis* ssp. *heteracantha*. *F. albida* had significantly greater ($P =< 0.05$) dry weight than all other species.

3.2.2. Nitrogen content

In untreated soil there were significant increases in nitrogen content (%N) with the multi-strain inoculum in *A. karroo, A. nilotica* and *A. tortilis* ssp. *spirocarpa* (Fig. 2b). The last species also increased %N with the single strain inoculum. %N of *A. nilotica* also significantly increased with the multi-strain inoculum in sterilised soil but other species showed significantly lower %N with inoculation in sterilised soil. In six of seven species the multi-strain inoculum in untreated soil resulted in significant ($P =< 0.001$) increases in total nitrogen (Fig. 2c) over controls, although in *A. tortilis* ssp. *spirocarpa* the single strain inoculum resulted in significantly greater increased total nitrogen than the

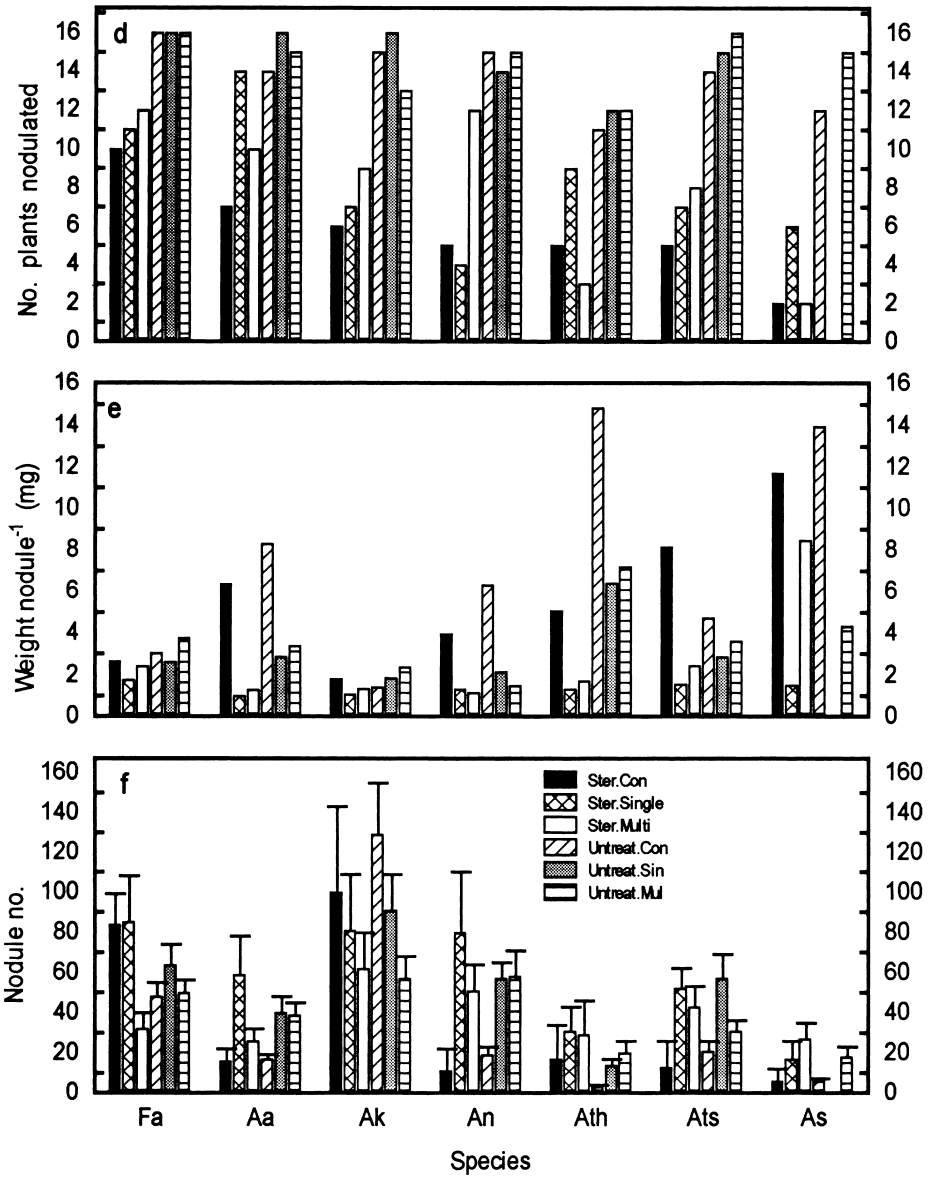

Fig. 2 (*continued*)

multi-strain inoculum. In the remaining species (*A. arenaria*) a significant increase was achieved only with the single strain inoculum.

3.2.3. Nodulation

More plants were nodulated in untreated (range 11–16) than sterilised (range 2–14) soil (Fig. 2d). In sterilised soil, all treatments had both nodulated and non-nodulated plants. There were between 2- and 20-fold (avg. 5.5-fold) differences in average dry weight between these in all species and treatments (Table 3). In sterilised soil, two species (*A. tortilis* ssp. *heteracantha* and *A. senegal)* showed fewer plants nodulated with the multi-strain than with single inocula suggesting that strains for these species do not compete well. Five of the seven species had fewer, larger nodules on control than inoculated plants (Fig. 2e,f). The exceptions were *F. albida*, in which nodule number decreased with the multi-strain inoculum in sterilised soil, and *A. karroo* in which nodule number decreased with inoculation in both soils (Fig. 2e,f). There were large differences in nodule dry weight and nodule number per plant between the two experiments despite similar growth periods (Figs. 1 and 2).

3.2.4. Nitrogen fixation

Significant increases over controls in dry weight ranged from 19 to 75%, and in total nitrogen from 11 to 114% (Fig. 2a,c). $\delta^{15}N$ analysis was carried out on three species to determine (a) the percentage of nitro-

Table 3
Dry weight (g) of nodulated and nonnodulated replicates of each inoculation treatment in sterilised soil in experiment 2. Data are means ± standard errors

Species	Control		Single		Mixed	
	+nod	−nod	+nod	−nod	+nod	−nod
F. albida	6.00 ± 1.05	0.90 ± 0.27	4.98 ± 0.9	1.49 ± 0.29	3.79 ± 0.58	1.44 ± 0.57
A. arenaria	3.98 ± 0.7	0.44 ± 0.12	3.80 ± 1.4	1.15 ± 0.66	1.82 ± 0.31	1.00 ± 0.45
A. karroo	3.98 ± 0.67	1.23 ± 0.39	4.38 ± 1.4	1.36 ± 0.41	4.71 ± 0.81	0.70 ± 0.15
A. nilotica	5.74 ± 1.09	1.78 ± 0.45	5.82 ± 0.69	2.04 ± 0.36	3.90 ± 0.34	1.30 ± 0.32
A. tort. het.	3.81 ± 0.65	0.32 ± 0.08	3.18 ± 0.65	0.15 ± 0.03	2.15 ± 0.4	0.54 ± 0.16
A. tort. spir.	3.75 ± 1.02	0.50 ± 0.14	3.18 ± 0.65	1.49 ± 0.44	4.38 ± 0.8	0.54 ± 0.16
A. senegal	3.03 ± 0.77	0.87 ± 0.21	2.62 ± 0.67	0.81 ± 0.33	5.55 ± 0.59	0.66 ± 0.27

gen due to fixation (%Ndff), and (b) the potential nitrogen fixation (defined in Materials and Methods). $\delta^{15}N$ values were determined in whole plants minus nodules and in nodules of all replicates of *A. nilotica* and the two subspecies of *A. tortilis*. Primary data for each replicate are shown for one treatment only (Table 4). These show wide variation in $\delta^{15}N$ values in both plants and nodules. In general there was less $\delta^{15}N$ enrichment than in experiment 1 (Table 2). This reflects the greater growth and nitrogen fixation in experiment 2. Potential nitrogen increase was the percentage difference between the four plants which were fixing most nitrogen and the four fixing the least in the same treatment as determined by the $\delta^{15}N$ method, and depended on a significant correlation between total nitrogen and either the $\delta^{15}N_f$ or $\delta^{15}N_{(plant\text{-}nod)}$ (Table 5). In two cases a better correlation was found with the former value, in three other cases with the latter value, and in two cases there was no significant correlation (Table 5). Differences in potential fixation with this method ranged from 1.48- to 3.22-fold while actual increases in total nitrogen of inoculated treatments over controls ranged from 1.16- to 2.14-fold. These data are complementary rather than comparable. For example the species which showed the greatest increase in total nitrogen by the nitrogen difference method (*A. tortilis* ssp. *heteracantha*) was that species in which the controls were shown by the $\delta^{15}N$ method to be fixing the least nitrogen. Percent Ndff values ranged from 28 to 53%. Maximum %Ndff for the three species/subspecies were *A. nilotica* 53%, *A. tortilis* ssp. *heteracantha* 45% and *A. tortilis* ssp *spirocarpa* 44%. These are conservative values because of the relatively small values (−2.85 and −2.52‰ for *A. nilotica* and *A. tortilis* respectively) used to indicate 100% fixation.

4. Discussion

These experiments have provided new information on nodulation and nitrogen fixation in African acacias at a range of scales from the specific to the general. They have also given positive answers to the questions: can inoculation in nursery conditions in untreated soil improve nodulation, nitrogen fixation and growth; does multi-strain inoculation have advantages over single-specific strain inoculation; and is there scope for genetic improvement in nitrogen fixation in African acacias?

4.1. Specific effects

In both experiments *A. tortilis* ssp. *heteracantha* showed a greater response to inoculation than did *A. tortilis* ssp. *spirocarpa* while growth was better in the latter, possibly reflecting the more southerly range of the former subspecies and eastern African range of the latter. In experiment 2, $\delta^{15}N$ analysis showed that the nodulated controls in the former subspecies were fixing less nitrogen (28%) than those in the latter (40%) suggesting lack of effectiveness of the indigenous strains for the southern subspecies. However, data for

Table 4
Dry weight, nitrogen and $\delta^{15}N$ data for 15 replicate *A. tortilis* ssp. *spirocarpa* plants supplied with a multi-strain inoculum in untreated soil

Plant	DW(g)	%N	Total N (mg)	$\delta^{15}N_{plant}$	$\delta^{15}N_{nod}$	$\delta^{15}N_{diff}$
1	2.06	1.98	41	3.14	2.80	−0.34
2	2.87	1.70	49	1.97	1.37	−0.60
3	3.70	1.57	58	1.98	2.27	0.29
4	3.51	1.81	64	0.48	1.76	1.28
5	3.94	1.61	64	2.36	0.28	−2.08
6	4.10	1.62	66	3.68	1.71	−1.97
7	3.46	1.98	68	−0.67	3.69	4.36
8	4.72	1.49	71	1.57	1.38	−0.19
9	4.57	1.64	75	1.20	1.00	−0.20
10	4.29	1.82	78	1.78	1.92	0.14
11	6.34	1.35	86	1.03	2.75	1.72
12	6.43	1.39	89	−0.57	1.87	2.44
13	4.97	2.07	103	−0.61	1.71	2.32
14	7.40	1.61	119	−2.42	4.87	7.29
15	6.72	1.95	131	−1.65	2.92	4.57

Table 5
Various assessments of nitrogen content and fixation in control and inoculated treatments in three species in experiment 2. Numbers are means or calculated from means. Standard errors of means are given where appropriate. nd = not determined. Asterisks indicate level of significance $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Species/treatment	Total N (mg)	% Increase[a] total N	Potential[b] increase total. N	%Ndff[c] δ^{15}N	Correlation[d] coefficient
A. nilotica					
Control	63.44	nd	70	43 ± 9	0.574
Single strain	74.14	16	117	42 ± 10	-0.736^{**}
Multi-strain	95.14	49	nd	53 ± 5	-0.414
A. tortilis ssp. *heteracantha*					
Control	29.07	nd	48	28 ± 7	0.858^{*}
Multi-strain	62.15	114	222	45 ± 8	-0.751^{**}
A. tortilis ssp. *spirocarpa*					
Control	56.73	nd	74	40 ± 8	0.845^{***}
Multi-strain	77.47	37	93	44 ± 7	0.809^{***}

[a] Percentage increase in total nitrogen.
[b] Potential percentage increase in total nitrogen.
[c] Percent nitrogen derived from fixation calculated by the δ^{15}N method.
[d] Correlation coefficient of the regression between total nitrogen and plant δ^{15}N (negative numbers) or between total nitrogen and the difference between plant and nodule δ^{15}N (positive numbers).

this species must be treated with caution because Odee et al. (1995) found unpredictable nodulation behaviour in various Kenyan provenance — soil combinations. The δ^{15}N value of −2.52‰ for the 100% fixing plants used here is lower than the values of −2‰ or 0‰ which are commonly used as estimates when measured values are not available as is the case in most field conditions. The estimates of fixation (28–53%) are also correspondingly lower. The potential of 62% for *A. tortilis* ssp. *raddiana* found by Ndoye et al. (1995) would then be similar to that found in this study.

The Ndff of 53% found here for *A. nilotica* with the multi-strain inoculum suggests that this species had moderate nitrogen fixation potential. Woldemeskel and Sinclair (1998) found acetylene reduction rates in this species comparable with those for high potential species such as *Leucaena leucocephala*.

Sanginga et al. (1990) and Ndoye et al. (1995) have found similarly small inoculation response in pot experiments with *F. albida* to that shown here. In all these studies untreated soil was also used allowing the possibility of ineffective nodulation with nonspecific strains. In natural stands of *F. albida* in desert lowlands of Namibia Schulze et al. (1991) also found very small fixation rates. Perhaps *F. albida* can only be effectively nodulated in soils in which bradyrhizobia predominate. In view of its economic importance in agroforestry systems in semi-arid areas the low response to inoculation of *F. albida* requires further investigation.

4.2. General growth effects

Inoculation with the multi-strain inoculum increased dry weight significantly in five of the seven species in experiment 2 and in two species in experiment 1. Better performance of multi-strain than single strain inoculants was also found by Somasegaran and Bohlool (1990) on three species of field crops and by Paau (1989) on soyabean. A longer growth period in experiment 1 may have resulted in growth differences between treatments in other species. The Kitui-grown plants in experiment 1 were half the size of those in experiment 2 in spite of similar growth periods and soil and watering regimens. This may have been due either to strain deterioration during storage, or to the fact that in experiment 1 plants remained in the cooler conditions of Muguga for 1 month before being transferred and confirms one of the findings of that experiment that Kitui is the more representative site for conducting acacia trials.

4.3. Soil–microbial interactions

That complex microbial interactions occurred in these experiments is suggested by: the increased nodulation in experiment 1 in spite of deterioration of inoculant strains; the better overall performance of the multi-strain inoculum compared to single strain inocula in experiment 2; and the large difference in growth between sterilised and untreated soil in experiment 2. The nature of these interactions, whether between inoculant strains, inoculant strains and indigenous rhizobial strains or other soil microflora is at present largely a matter for speculation.

4.4. Nitrogen fixation

The greater increase in total nitrogen (29% overall) than dry weight (21% overall) for the multi-strain

inoculum in untreated soil in experiment 2 showed the importance of nitrogen determination in assessing inoculation effects. The great variation in growth and nitrogen fixation shown by $\delta^{15}N$ analysis in experiment 2 confirms the inadequacy of only carrying out nitrogen analyses on four of the 10 replicates selected at random for each treatment in experiment 1. Relative nodule enrichment in $\delta^{15}N$ has often been correlated with nodule activity (Shearer et al., 1982). The large relative enrichments in $\delta^{15}N$ found in the nodules relative to shoots in four of the five species for which data were available in experiment 1 suggested that these species were fixing nitrogen. The lack of enrichment in nodules of *F. albida* suggested that the most competitive strains for this species were ineffective. That significant differences in growth were not found for the species with actively fixing nodules may have been due to slow growth resulting from the cool conditions at Muguga discussed above. Values for percent nitrogen derived from fixation obtained using the $\delta^{15}N$ method can vary widely depending on the reference plants selected (see for example Högberg, 1990; Shearer and Kohl, 1993; Sprent et al., 1996). We were able to avoid this problem by using as reference plants any nonnodulated plants of the species under test. The wide variation in values and the good correlations found between total plant nitrogen and both $\delta^{15}N_{plant}$ and $\delta^{15}N_{nodule}$ within treatments enabled us to use one or other of these correlations to estimate the potential for improvement of nitrogen fixation by selection. Values for this potential ranged from 1.48- to 3.22-fold. This is likely to be a more realistic measure of potential for improvement of nitrogen fixation than that obtained by simple comparison of total nitrogen in nodulating versus nonnodulating plants in the same treatment: there was and average difference of 5.5-fold between these plants in experiment 2 but this value includes both the genetic variation in growth as well as that in nitrogen fixation potential.

4.5. Relative effectiveness of the multi-strain inoculum

The significant increases in dry weight and total nitrogen reported here were achieved with the same multi-strain inoculum on a wider genetic range of plant material than is implied by the single genus status of the *Acacia* species. It is widely accepted that this large genus requires division (Chappill and Maslin, 1995). The species included here represent the two main sections of African *Acacia* (Vassal, 1972) which are likely to become at least two new genera. Each species also represents much greater genetic variability than do field crops in which rhizobial strains are selected for particular varieties. Nevertheless the percentage gains in yield resulting from rhizobial inoculation of these crops (range 30–50%, Sprent and Sprent, 1990) are of the same order of magnitude as those reported here. Much greater gains than those reported here are likely to be achievable for acacias because: (i) relatively few strains for each species were tested and much better strains should not be difficult to find; (ii) strains were selected for good performance in unstressed conditions; (iii) the same multi-strain inoculum was used here on a broad genetic range of species; and (iv) the indigenous soil rhizobial population was high.

We conclude: that substantial gains in nitrogen fixation may be made by the use of inoculation in African acacias growing in nursery conditions; that the use of multi-strain inocula is preferable to single strain inocula in some circumstances; that *A. nilotica* and *A. tortilis* have at least moderate nitrogen fixation potential; and that this potential could be considerably increased by selection.

Acknowledgements

This work was funded by the Department for International Development, UK Government. We also wish to thank the staff of the Biotechnology Laboratory, Agroforestry Working Group, KEFRI for their support and enthusiasm, and Professor J.I. Sprent, University of Dundee for stimulating discussions.

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