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Soil acidity in relation to groundnut-*Bradyrhizobium* symbiotic performance

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Abstract

Effects of soil acidity on groundnut-*Bradyrhizobium* symbiotic performance were studied in a potted, sandy soil in a glasshouse in Zimbabwe. The soil was limed to soil-pH levels of 5.0 and 6.5. Soil acidity negatively affected plant development, measured as leaf area and plant dry weight, while nodulation was enhanced. This acidity-enhanced nodulation was most evident when nodulation was caused by the indigenous *Bradyrhizobium* population. Effects of soil acidity differed between groundnut cultivars and *Bradyrhizobium* spp. strains, the former having greater importance. Two *Arachis hypogaea* L. Spanish-type cultivars, Falcon and Plover, performed equally well at neutral soil pH, but Falcon was more acid tolerant. Comparison of the symbiotic performance in neutral versus acid soil of two *Bradyrhizobium* spp. strains, MAR 411 (3G4b20) and MAR 1510 (CB 756), showed that MAR 411 performed superiorly in neutral soil, but MAR 1510 in acid soil. The indigenous *Bradyrhizobium* population was more effective than was inoculation with strains MAR 411 or MAR 1510. Comparison of twelve *Bradyrhizobium* spp. strains for their symbiotic performance in acid soil showed that some strains were totally ineffective under acidity stress (MAR 253, MAR 967 and MAR 1506), while others performed well. *Bradyrhizobium* spp. strain MAR 1576 (32 H1) ranked highest for nitrogen accumulation, plant dry weight and leaf area, with strains MAR 1555 (TAL 11) and MAR 1510 following closely. Nitrate fertilisation of groundnut plants led to soil alkalinisation, while nitrogen fixation resulted in soil acidification. Soil acidity in combination with soil sterilisation gave rise to symptoms associated with Al and Mn toxicity.

Introduction

Groundnut is a leguminous crop predominantly grown in the semi-arid (sub)tropics. In Zimbabwe short-season, Spanish-type groundnuts are cultivated within the subsistence farming sector, either for protein-rich food or as a cash crop. Most of the tropical soils under groundnut cultivation are highly weathered, sandy and acid.

Soil acidity in general constitutes a major constraint for legume cultivation by reducing plant growth, nodulation and yield (Munns, 1978, 1986; Russell, 1978). Besides proton toxicity, low soil pH is associated with many other soil acidity factors such as toxicities of Al and Mn and deficiencies of Ca, Mg, Mo and P (Foy, 1984). Liming may ameliorate most of these infertility factors, but is unaffordable in low-input agriculture, warranting the need to select for acid tolerance.

The influence of several acidity factors has been studied for a number of legumes, with an emphasis on forage and pasture legumes. Several grain legumes that are nodulated by *Bradyrhizobium* spp. have also been studied, with an emphasis on soybean (e.g. Cline and Kaul, 1990; Taylor et al., 1991), and a few studies on cowpeas (e.g. Keyser et al., 1979), lespedeza (Cline and Ngewoh-Senwo, 1993) and groundnuts (e.g. Adams and Pearson, 1970; Blamey and Chapman, 1982; Chong et al., 1984; Munns and Fox, 1977;

Shamsuddin et al., 1992; Tang et al., 1991). Tropical legumes, which are predominantly nodulated by *Bradyrhizobium* strains, are generally more acid tolerant than temperate legumes (Munns, 1986).

Groundnut nodulation, growth and nitrogen fixation was optimal in the pH range from 5.7–6.3 (Chong et al., 1984). Groundnut yield and nodulation in an acid soil could be improved by lime ($CaC0_3$), but not by gypsum ($CaS0_4$) application. It was deduced that an acid-induced Al toxicity rather than Ca deficiency caused poor groundnut performance in this acid soil (Blamey and Chapman, 1982). In general, nodulation is impaired under acidity stress (Munns, 1978). However, groundnut nodulation in solution culture was depressed if the pH was raised through bicarbonate addition (Tang et al., 1991).

Screening *Bradyrhizobium* spp. strains in laboratory media showed that a high Al concentration constitutes the most severe acidity stress factor (Keyser and Munns, 1979 a, b). The incidence of acid-tolerant *Bradyrhizobium japonicum* strains was higher in acid soil than in neutral soil (Asanuma and Ayanaba, 1990). It appears appropriate to include strains that are isolated from acid soils in acid tolerance studies.

The mode of nitrogen nutrition of a legume, either dinitrogen, nitrate or ammonium uptake, influences the soil pH (reviewed by Nye, 1986), by modifying the (overall) uptake pattern of cationic and anionic nutrients (Aguilar and Van Diest, 1981). Nitrate uptake implies uptake of additional anions thus leading to negative charge accumulation inside the root which is balanced by bicarbonate extrusion, resulting in soil alkalinisation. Ammonium (a cation) assimilation will result in soil acidification (Arnold, 1993; Riley and Barber, 1971). Symbiotic nitrogen fixation means uptake of uncharged dinitrogen, which results in an overall positive charge accumulation inside the root being balanced by proton extrusion, and so leading to soil acidification (Nyatsanga and Pierre, 1973).

We report here on the effectiveness of *Bradyrhizobium* spp. strains, either indigenous or inoculated, in symbiosis with two groundnut cultivars in a potted, sandy soil at neutral pH (6.5) compared to acid pH (5.0). Twelve strains were compared for their symbiotic performance in acid soil, devoid of an indigenous rhizobial population. Soil pH was measured to assess whether the mode of nitrogen nutrition in groundnuts affected soil acidity.

Materials and methods

Two *Arachis hypogaea* cultivars were grown with different *Bradyrhizobium* spp. strains, the latter either inoculated or indigenous. The effectiveness of their symbiosis was assessed in an acid and in a neutral soil. The experiment was performed in Marondera, Zimbabwe in a glasshouse between March 23 and June 15, 1992, which spans the end of summer to the beginning of winter.

Soil

A sandy soil was used to fill pots as described previously (Van Rossum et al., 1993). Dolomitic limestone, containing approximately 78% $CaC0_3$, 16% $MgC0_3$ and 6% impurities, was used to raise the soil pH from its original level of 3.8. Applications of 250 and 1500 mg kg^{-1} soil resulted in a soil pH of 5.0 and 6.5, respectively. Soil pH was measured in a 20% (w/v) soil in 10 m*M* $CaCl_2$ suspension. Soil was either steam autoclaved (sterilised) for 3 h at 120°C or left natural i.e. not autoclaved (unsterilised). Soil pH did not change with sterilisation.

Bradyrhizobium *spp. strains*

The eleven strains used in this study have been listed previously (Van Rossum et al., 1993). One strain was added: MAR 1576, with synonyms 32 H1, TAL 1380 and IC 7080, which was originally isolated from *Crotolaria paulina*. Strains were stationary cultured, at 28°C with daily shaking, in YM medium (Van Rossum et al., 1993) for 11 days to yield optical densities, measured spectrophotometrically (660 nm), between 0.4 and 0.8.

Experimental design and statistics

The experimental design involved five factors each with several levels: (1) soil sterilisation with two levels: sterilised and unsterilised soil, (2) soil pH with two levels: initial soil pH of 5.0 and 6.5, (3) groundnut cultivars with two levels: Falcon and Plover, (4) inoculation with fifteen levels: twelve *Bradyrhizobium* spp. strains, non-inoculated (control), nitrate-fertilised/uninoculated (control) and indigenous microbial population, and (5) blocking (replication) with three levels: three blocks. The experiment was not fully factorial.

Within the experiment three sections were distinguished:

1. unsterilised soil × two soil-pH levels × two cultivars × 6 replicates (i.e. 3 blocks comprising 2 replicates each).
2. sterilised soil × two soil pH-levels × two cultivars × two strains plus two controls × 3 blocks.
3. sterilised soil × acid soil × two cultivars × 12 strains plus two controls × 3 blocks.

For statistical analyses a completely randomised design was used for the first two sections and a completely randomised block design for the third experimental section, since this section showed significant block differences. The error mean square was calculated and from that the F-statistic (if the factor had two levels) or the least significant difference based on Student's t-distribution (if the factor had more than two levels).

Plant culturing

Two *Arachis hypogaea* L. subspecies *fastigiata* variety *vulgaris* (i.e. Spanish type) cultivars Falcon and Plover were used. Both cultivars were bred in Zimbabwe. Seeds were selected for uniform size, rinsed thoroughly with tap-water and six times with sterile distilled water before planting. Six seeds were planted per pot. Each seed was inoculated, if required, with 1.0 mL *Bradyrhizobium* culture. The uninoculated control received only YM medium. The treatments with unsterilised soil received neither inoculum nor YM medium. The nitrate-fertilised control received 1.0 mL 0.5 *M* $NaNO_3$ per seed and 4.0 mL in the base of the pot at planting and 10.0 mL in the base during plant growth, with intervals of 14 days. After planting and inoculating, the soil was covered with a layer of heat-sterilised paraffined coarse sand to reduce contamination. Plant nutrients were added 4 days after planting to the bases of the pots. The plant nutrient solution was as described previously (Van Rossum et al., 1993) with a 3.0-times higher $MgSO_4$ and a 2.4-times lower $NaNO_3$ (starter N) concentration. Pots received a further dose of 1.0 mL 0.5 *M* $MgSO_4$ in their bases at 30 days after planting. The sterilised, inoculated treatments reived a total of 0.57 mMole (starter) nitrate kg^{-1} soil, while the nitrogen controls received an additional 6 doses (each 14 days) of 1.79 mMole nitrate kg^{-1} soil. At 11 days after planting, plants were thinned to two per pot and these were re-inoculated with 2.0 mL culture per plant at 16 days after planting. Plants were sprayed with a fungicidal mix (Mancozeb and Benomyl), 17 days after planting, to minimise *Cercospora* infection. Pots were watered through the bases with sterile distilled water whenever the bases of the pots became dry. Temperatures were maintained between 18–31°C by heating during night-time and cooling/ventilation during day-time.

Harvesting

Plants were harvested between 77–84 days after planting. Plants were uprooted from the pots. Nodules were detached from the roots, counted and their dry weights (drying at 70°C to constant weight) determined. Leaf area, plant dry weight (all biomass, except kernels for the few cases that pods were already formed) and percentage nitrogen in the plant dry weight were determined as described previously (Van Rossum et al., 1993). Total nitrogen accumulation (mg) includes an estimate of the amount of nitrogen in the kernels, if they were formed at all. Kernel %N was taken to be 4.2%, a value based on previous research (Van Rossum et al., 1993). All values are per two plants.

Soil pH was determined of a soil sample that was drawn from uniformly mixed pot soil after harvesting.

Results and discussion

Symbiotic performance in acid compared to neutral soil

Table 1 shows data on the symbiotic performance of groundnut plants as affected by acidity in a sandy soil, either unsterilised soil or sterilised, inoculated soil.

Nodulation in the unsterilised soil with indigenous *Bradyrhizobium* spp. strains was increased at low soil pH compared to neutral soil pH. Nodule dry weight increased by 57% ($p < 0.001$) and nodule numbers increased with 21% ($p = 0.15$). Plant development, measured as leaf area and plant dry weight, was significantly reduced by 26% and 21%, respectively, due to soil acidity.

The effects caused by acidity in the sandy soil that had been sterilised and subsequently inoculated with *Bradyrhizobium* spp. strains MAR 411 or MAR 1510 were analogous to those found for unsterilised soil with an indigenous *Bradyrhizobium* population. The main difference between unsterilised and sterilised soil concerned nodule dry weight, being increased in the former and decreased in the latter as a result of soil acidity. This seemingly contradictory observation may be

Table 1. Effect of soil acidity on groundnut symbiotic performance with indigenous *Bradyrhizobium* strains in an unsterilised soil and with inoculated *Bradyrhizobium* strains (MAR 411 and MAR 1510) in a sterilised soil. Values are per two plants and are means of 12 pots[a]

Variable	Nodulation		Plant development		Total N
	Number	DW[b] (mg)	Leaf area (cm^2)	DW (g)	(mg)
Unsterilised soil with an indigenous Bradyrhizobium *population*					
Acid soil	150	371	914	14.05	338.4
Neutral soil	124	237	1237	17.74	407.6
Difference (neutral-acid)	-26	-134*** [c]	323***	3.69***	69.2**
Sterilised soil with inoculation					
Acid soil	222	169	747	10.60	263.1
Neutral soil	176	215	1090	15.48	361.0
Difference (neutral-acid)	-46	46**	343***	4.88***	97.9***

[a] Values are averaged for two cultivars and c.q. for the two inoculant strains.
[b] Dry Weight.
[c] Asterixes denote probability levels; *: $p = 0.05$–0.10; **: $p = 0.01$–0.05; ***: $p = 0.01$.

explained by the adverse growth conditions, due to Al and Mn toxicities (see below), observed with the sterilised, acid soil treatment but not with the unsterilised soil treatment. In the sterilised soil, too, a larger number of nodules were formed under acid conditions, but they were limited in their development and could therefore not lead to an increase of nodule dry weight.

A reduction in plant growth due to soil acidity is commonly found for most legumes, e.g. alfalfa, bean, soybean and also groundnut, being accompanied by reduced nodulation (e.g. Wolff et al., 1993; Pijnenborg, 1990; Cline and Kaul, 1990; Chong et al., 1984). Optimal soil pH levels are usually between 5.5 and 6.5. The increased nodulation of groundnut in an acid compared to a neutral soil, observed in this experiment, is rather exceptional. Chong et al. (1984) reported on one exceptional groundnut-*Bradyrhizobium* combination that nodulated preferentially at a pH of 5.1. In that case both symbiotic partners originated from tropical soils, which are generally acid. Tang et al. (1991) reported a depressed nodulation with groundnut at a high bicarbonate concentration i.e. high pH. They found a similar effect with lupins, which showed reduced nodulation at pH above 6.0 (Tang and Robson, 1993). Experiments with beans (*Phaseolus vulgaris*) showed that nodulation and growth was much improved when one or both of the symbionts were acid tolerant (Vargas and Graham, 1988).

The increased nodulation at a lower pH, which was observed in this experiment, may be explained in several ways. Firstly, it may indicate ecochemical adaptation to adverse soil conditions, such as acid tolerance. Secondly, it may be caused by the positive effect of an acid-induced soil factor that assists in nodule initiation. Such a putative factor may render the plant root more susceptible to nodulation or may increase the infectivity of the *Bradyrhizobium* strain. Thirdly, it might be a response to nitrogen deficiency.

To state that nodulation is reduced at high pH (compare Tang and Robson, 1993), rather than increased at low pH, is less likely, because the plants were growing optimally at the higher pH (6.5), which would lead to the expectation that nodulation, too, is optimal at that (higher) pH.

Plants grew better in the unsterilised soil than in the sterilised, inoculated soil at both soil acidity levels. This could have the dual cause of a greater symbiotic effectiveness of the indigenous *Bradyrhizobium* spp. population compared to the inoculated strains and/or a negative effect on soil fertility caused by sterilisation. As the groundnut plants developed healthily at neutral soil-pH in both unsterilised and sterilised soil, it was concluded that the indigenous strains (possibly in concert with other beneficial micro-organisms) were more effective than inoculated strains (MAR 411 and MAR 1510).

The groundnut plants developed healthily in all treatments except in the sterilised, acid soil. Plants within this treatment were stunted, with narrow leaves which showed black spots and upward curled edges. These symptoms were reversed when the acidity of the soil was reduced during plant growth. This became evident for the nitrate-fertilised plants, which alkalinise the soil, which were less affected than the other treatments (see paragraph on strain performance and Table 3). Plant nutrient solution was added after soil sterilisation, which makes it unlikely that the symptoms were caused by a mineral deficiency, but rather by a toxicity. The symptoms of nutrient toxicities described for Al and Mn fit the observed symptoms well (Foy, 1984; Helyar, 1978). It is assumed that the Al and Mn toxicity symptoms were caused by a combination of acidity and sterilisation of this sandy soil. Soil acidity is generally associated with high levels of Al and Mn, besides low availabilities of Ca and Mo (Pijnenborg, 1990). Steam sterilisation of soil is known to increase solubility of minerals (Warcup, 1957). Rovira and Bowen (1966) found that steam sterilisation led to an increase of soluble Mn levels in a pH-neutral soil, while Boyd (1971) observed Mn toxicity in groundnuts grown in autoclaved soil. The occurrence of heat- or steam-induced toxicities may also depend on the amounts of (fixed) minerals present in the soil that is being autoclaved. We found no toxicity symptoms in groundnuts grown in an autoclaved sandy soil with very low levels of minerals and organic matter (so-called 'Zimbabwean-Kalahari sand'). High Mn levels in plants may cause secondary effects like Ca deficiency, by blocking Ca translocation from the root to the shoot (Marschner, 1986). Moreover, toxicity may be exerted in sterilised soil due to the elimination of soil micro-organisms that could detoxify the soil (Rovira and Bowen, 1966).

Cultivar performance in acid compared to neutral soil

Data pertaining to cultivar performance as influenced by soil pH in both unsterilised soil and sterilised, inoculated soil are presented in Table 2.

In neutral soil the differences between Falcon and Plover are marginal. In the unsterilised soil the differences do not reach significance beyond the $p = 0.10$ level. Falcon performed better than Plover in the sterilised, inoculated soil regarding plant dry weight and nitrogen accumulation, but not for leaf area.

In acid soil, both unsterilised soil and sterilised, inoculated soil, all parameters were higher for Falcon than for Plover, the difference being significant for most parameters.

In acid soil Falcon is thus superior to Plover. The superior performance of Falcon over Plover in acid, sterilised soil is shown for two commercially available groundnut inoculant strains and for a total of 12 inoculation treatments. Data obtained as the mean of the 12 inoculation treatments show that Falcon performed significantly better ($p < 0.01$) than Plover concerning nodule dry weight, leaf area, plant dry weight and total nitrogen accumulation; the number of nodules formed tended also to be greater on Falcon than Plover ($p = 0.14$).

The results obtained with unsterilised soil would best predict the responses to be expected under field conditions, since inoculation of groundnut is not common in Zimbabwean subsistence farming.

Bradyrhizobium *spp. strain performance in acid compared to neutral soil*

Table 3 shows data on the performance of two *Bradyrhizobium* spp. strains (MAR 411 and MAR 1510), a non-inoculated treatment and a nitrate-fertilised treatment in sterilised soil with acid or neutral soil-pH.

When the performance of the two inoculated treatments is compared it is clearly seen that the (relative) performance of a strain is dependent on soil acidity. At neutral soil-pH, strain MAR 411 performed better than MAR 1510. This was reversed at acid soil pH, strain MAR 1510 now being the best of the two. These results indicate that the effectiveness of an inoculant strain should be tested at the soil pH which prevails in the (agricultural) area for which the strain will be recommended.

The uninoculated treatment showed that the soil did not keep free of *Bradyrhizobium* throughout the course of the experiment, which is practically unfeasible in a long term (80 days) pot experiment conducted in a glasshouse in the subtropics with forceful cooling/ventilation facilities. Nevertheless it was clear from plant observations that the uninoculated plants were initially not provided with symbiotically fixed nitrogen, because they yielded plant dry weights which were significantly lower (36–70%) than those of the other treatments. It is therefore assumed that the influence of contaminating strains on the results of the inoculated and nitrate-fertilised treatments was negligible. The uninoculated control at low pH had a significantly greater number of nodules than at neutral soil-pH in

Table 2. Effect of soil acidity on symbiotic performance of two groundnut cultivars with indigenous *Bradyrhizobium* strains in an unsterilised soil and with inoculated *Bradyrhizobium* strains (MAR 411 and MAR 1510) in a sterilised soil. Cultivar performance is also shown for acid, sterilised soil as a mean over twelve inoculant strains. Values are per two plants

Variable	Nodulation		Plant development		Total N
	Number	DW[a] (mg)	Leaf area (cm^2)	DW (g)	(mg)
Unsterilised soil with indigenous strains (n = 6)					
Falcon on neutral soil	139	230	1287	18.48	403.2
Plover on neutral soil	109	245	1189	17.00	412.0
Difference (Falcon-Plover)	30	-15	98	1.48	-8.8
Falcon on acid soil	171	406	1059	16.46	419.5
Plover on acid soil	129	336	769	11.65	257.3
Difference (Falcon-Plover)	42	70	290***	4.81***	162.2***
Sterilised soil with inoculation (n = 6)[c]					
Falcon on neutral soil	164	209	1044	16.86	390.4
Plover on neutral soil	187	221	1136	14.09	331.6
Difference (Falcon-Plover)	-23	-12	-92*	2.77**	58.8**
Falcon on acid soil	235	182	818	11.97	289.8
Plover on acid soil	209	156	676	9.23	236.4
Difference (Falcon-Plover)	26	26	142**	2.74**	53.4**
Sterilised soil with inoculation (12 strains: n = 36)					
Falcon on acid soil	225	197	690	11.17	277.0
Plover on acid soil	194	164	558	8.41	207.5
Difference (Falcon-Plover)	31	33***	132***	2.76***	69.5***

[a]Dry Weight.
[b]Asterixes denote probability levels; *: $p = 0.05$–0.10; **: $p = 0.01$–0.05; ***: $p < 0.01$.
[c]Values are averaged for the two inoculant strains.

agreement with our above-mentioned conclusion that acidity enhances nodulation.

The nitrate-fertilised treatment was less affected by an (initially) acid soil-pH than the inoculated treatments. The major reason for this is thought to be the increase of soil pH (Table 3) as an effect of nitrate uptake by groundnut plants. This increase in soil pH (1.3 units) caused that the plants grown at an initial soil-pH of 5.0 did not show the above described toxicity symptoms, which occurred with all other groundnut plants grown in acid, sterilised soil.

In other studies assessing the effects of soil acidity, the effects are often divided into those exerted on the legume, on the rhizobial symbiont and on their symbiosis. The nitrogen-fertilised treatment is then used to determine the response of the legume itself to acidic conditions. If effects observed with a legume grown under nitrogen-fixing conditions supercede those found under nitrogen-fertilised conditions, the additional effects are allocated to the symbiosis (e.g. Pijnenborg, 1990; Cline and Kaul, 1990). It is shown here that such an approach is probably flawed if soil pH changes are not taken into consideration. Therefore soil pH must be monitored during (or at the end of) the growth period (see also last paragraph).

It is certainly not denied that effects of soil acidity on the legume itself overrule those on its symbiosis, since nodule development and functioning are dependent on photosynthesis. This may be seen from comparing nodulation by an acid-tolerant *Bradyrhizobium* spp. strain (MAR 1587) on an acid-tolerant cultivar (Falcon) with nodulation on an acid-sensitive cultivar

Table 3. Effect of soil acidity on *Bradyrhizobium* strain performance compared to nitrate-fertilised and uninoculated treatments in sterilised soil. Values are per two plants and are means of 6 pots, i.e. average for two groundnut cultivars

Variable	Nodulation		Plant development		Total N	Final soil pH
	Number	DW[a] (mg)	Leaf area (cm^2)	DW (g)	(mg)	
Performance in neutral soil (initial soil-pH = 6.5)						
MAR 1510	180	225	1024	13.71	327.3	6.8
MAR 411	172	206	1156	17.24	394.6	6.3
Nitrate-fertilised	8	14	897	14.65	328.4	7.7
Uninoculated	35	31	310	6.20	99.3	7.1
Strain-difference (1510–411)	8	19	-132**[b]	-3.53* * *	-67.3**	
Performance in acid soil (initial soil-pH = 5.0)						
MAR 1510	196	197	828	11.25	280.2	4.3
MAR 411	248	141	665	9.95	246.0	4.7
Nitrate-fertilised	100	99	797	11.88	286.8	6.3
Uninoculated	151	126	496	6.94	170.1	4.8
Strain-difference (1510–411)	-52	56**	163* * *	1.30	34.2	
Statistics						
LSD at $p = 0.05$	87	47	102	2.60	60.9	
LSD at $p = 0.01$	117	64	137	3.50	81.9	

[a]Dry Weight.
[b]Asterixes denote probability levels; *: $p = 0.05$–0.10; **: $p = 0.01$–0.05; * * *: $p < 0.01$.

(Plover). Falcon showed increased nodulation at acid compared to neutral pH, of 160 and 97 mg nodule dry weight per plant, respectively. However, Plover showed a reduced nodulation at acid compared to neutral pH, of 121 to 189 mg nodule dry weight per plant, respectively (data at neutral pH were derived from Van Rossum et al., 1993). Clearly, the effects of acidity on the legume host itself overruled those on its symbiosis. A similar tendency was observed when the less acid-tolerant strains MAR 411 and MAR 1510 were assessed (see Table 2).

Bradyrhizobium *spp. strain performance in acid soil*

The performance in sterilised, acid soil of twelve *Bradyrhizobium* spp. strains as groundnut microsymbiotic partners and a non-inoculated treatment, are presented in Figure 1.

The twelve strains were ranked based on nitrogen accumulation (Fig. 1A), which correlates well with both plant dry weight and leaf area, since all three parameters gave comparable ranking orders (Fig. 1A, B and C). However, a ranking based on nodule dry weight would be largely different, reaffirming our previously reported finding that nodulation in groundnut is poorly correlated to any of the yield parameters (Van Rossum et al., 1993).

Strain MAR 1576 ranked highest for nitrogen accumulation, plant dry weight and leaf area, performing significantly (at $p = 0.10$) better than the third ranking strain, which is the commercial inoculant strain MAR 1510. All the other strains, except the three lowest ranking strains, ranked in one group with MAR 1510 at $p = 0.05$ probability, based on nitrogen accumulation and plant dry weight.

MAR 1510 gave a high leaf area over plant dry weight ratio, indicating that this strain specially favours leaf development. This feature of strain MAR 1510 was also observed under neutral soil pH conditions by Van Rossum et al. (1993).

The three lowest ranking strains, MAR 253, MAR 967 and MAR 1506, performed similarly to the uninoculated control, indicating that those strains were ineffective under acid soil conditions.

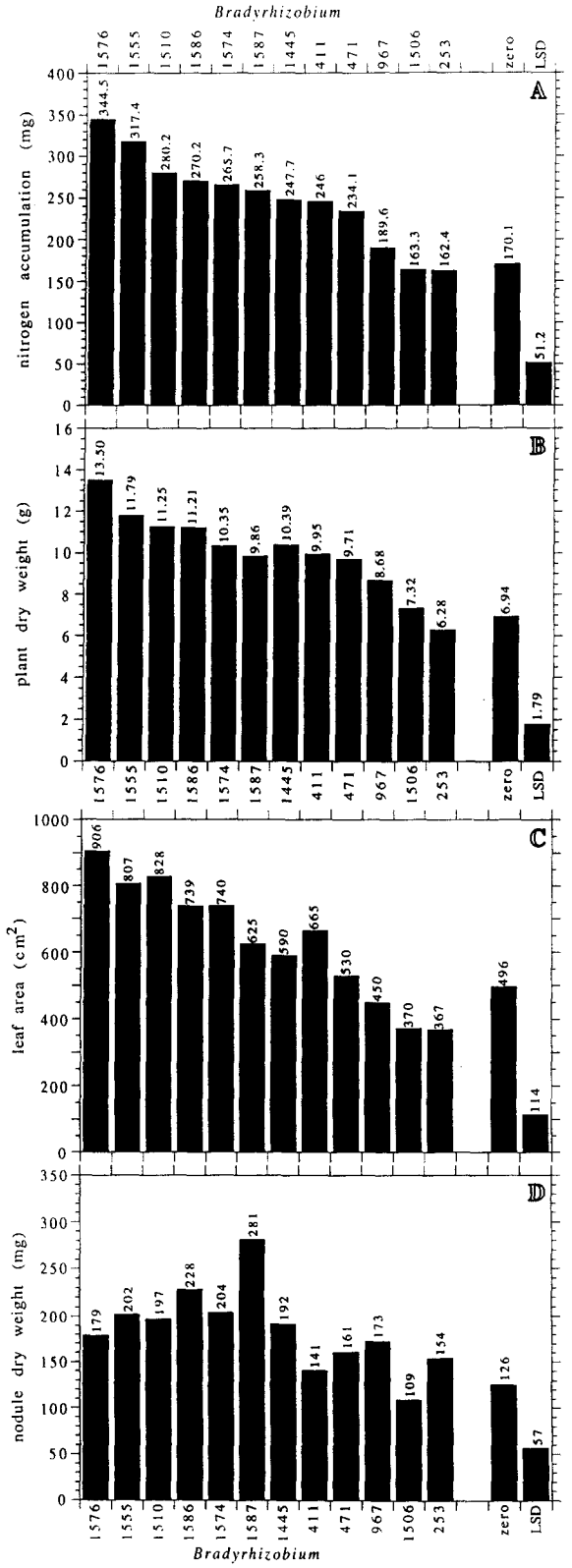

Fig. 1. Symbiotic performance of 12 *Bradyrhizobium* spp. strains and the uninoculated control (0) in acid, sterilised soil for nitrogen accumulation (**A**), plant dry weight (**B**), leaf area (**C**) and nodule dry weight (**D**). Values are per two groundnut plants and are means of 6 pots. The LSD value ($p = 0.05$) is presented as a bar.

In conclusion, the most effective strain under acid soil conditions was MAR 1576, followed by MAR 1555 and MAR 1510.

Strains MAR 1587 and MAR 1586 ranked highest for nodule dry weight (Fig. 1D) and also for nodule number (data not shown). Those strains were, among the twelve employed strains, the most successful in establishing nodules with groundnut in acid soil. These strains had been recently isolated from groundnut nodules harvested from plants grown in an acid, sandy soil of a similar type to that used in this experiment. This indicates that selection of strains for nodulation competence under adverse soil conditions is best performed on strains that were naturally exposed to those stresses. Nodulation in groundnut is, however, poorly correlated to yield: better nodulation may therefore not translate into better symbiotic performance.

Strains MAR 1510 and MAR 1555 were previously tested under neutral soil conditions and were found to rank in the highest group for nitrogen accumulation, leaf area and seed dry weight (Van Rossum et al., 1993). Strain MAR 1555 showed a remarkable increase in nodulation due to soil acidity since this strain ranked lowest for nodule dry weight at neutral pH (66 mg plant^{-1}), while it gave a large nodule dry weight at acid pH (101 mg plant^{-1}, see Fig. 1D).

Soil acidification caused by nitrogen fixation

Soil acidification occurred as a result of nitrogen fixation in the sandy soil used in this experiment (Fig. 2). The opposing effects of nitrogen fixation and nitrate assimilation on soil pH were most clear in the soil with an initial pH of 5.0 (Fig. 2A): nitrogen fixation led to soil acidification and nitrate assimilation led to soil alkalinisation. Soil acidification due to nitrogen fixation showed that groundnuts had a similar nutrient uptake pattern as other legumes (Nyatsanga and Pierre, 1973).

Soil acidification by nitrogen fixing groundnuts in an initially acid soil was better correlated to nitrogen accumulation than to plant dry weight, leaf area or nodule dry weight, in this order. This provided additional evidence that proton extrusion was indeed a result of nitrogen fixation. Inoculated treatments giving soil alkalinisation were, presumably, not fixing nitrogen, but utilising combined nitrogen (present as residual soil nitrogen or starter nitrogen) for their growth. These treatments, which accumulated less than 200 mg N, corresponded to the treatments that were concluded to be ineffective in symbiotic nitrogen fixation in acid

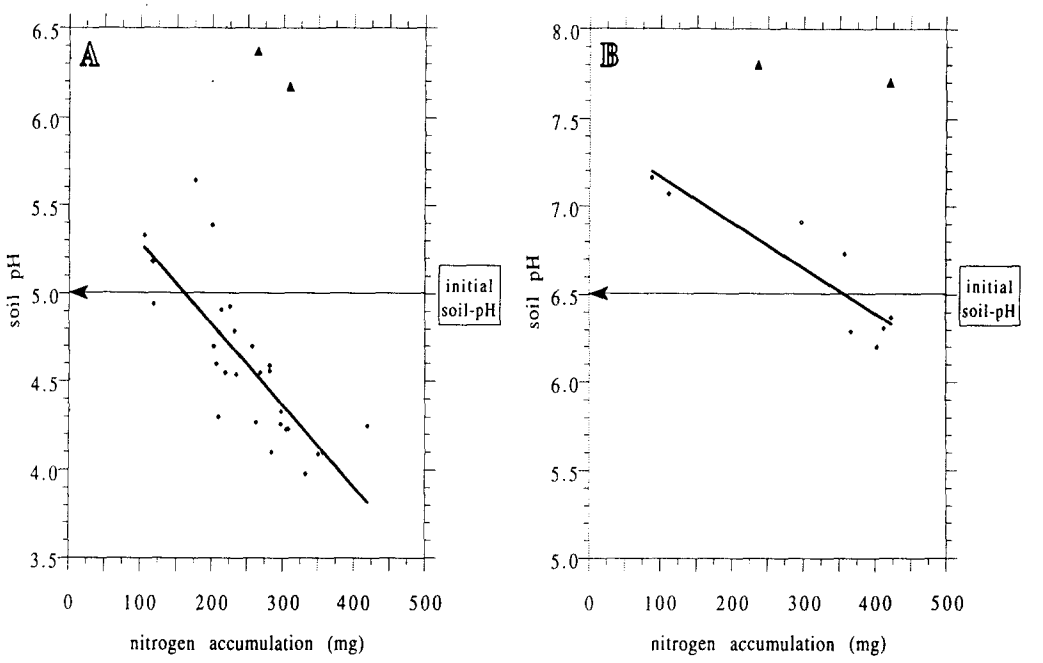

Fig. 2. Effect of nitrogen fixation and nitrate fertilisation on soil pH in an initially acid soil (**A**) and in an initially neutral soil (**B**). Values for nitrogen accumulation are given per two plants and are means of 3 pots. Triangles show data for nitrate-fertilised plants. The line shows the linear relationship between soil pH and nitrogen accumulation in nitrogen fixing groundnuts.

soil, since they were not different from the uninoculated control (Fig. 1A).

The effect of nitrate fertilisation on soil pH for the treatments with an initially neutral pH, was similar to that for soil with an initially acid pH. The uninoculated treatments, with nitrogen accumulation values below 125 mg, showed soil alkalinisation, indicative for the absence of (or very low) nitrogen fixing activity. The other treatments with nitrogen accumulation values of 275–425 mg showed only small effects on final soil-pH.

It is of interest that both acidification and alkalinisation due to nitrogen fixation or nitrate assimilation, respectively, was approximately 100-times higher in the initially acid soil compared to the initially neutral soil. Recalculation of data given by Riley and Barber (1971) for soybeans, grown at initial pH levels of 5.2 and 6.7, lead to a similar conclusion. This may be due to the different buffering capacity of the soil at different pH levels or in a changed nutrient uptake pattern of the plant.

Conclusions

Two closely related groundnut varieties that performed equally well at neutral soil-pH were found to respond very differently to soil acidity. The symbiotic performance of two commercially available (in Zimbabwe) groundnut inoculant strains was also differentially influenced by soil acidity, one strain performing superiorly in neutral soil, the other in acid soil. This showed that groundnut host and *Bradyrhizobium* spp. strain selection should be performed under conditions that closely resemble actual (agricultural) soil conditions, such as acidity, if a host and/or strain recommendation has to be made.

We found that the strains originating from an acid, sandy soil were more nodulation competent than most of the other strains when tested in an acid soil, but had similar nodulation competence in a neutral soil. Moreover, an indigenous *Bradyrhizobium* population performed superiorly when compared to inoculated strains. This showed that ecochemical adaptation of *Bradyrhizobium* spp. strains to a stress factor should be utilised in selection programmes, by including strains that were isolated from soils where those stresses prevail.

In general it was found that soil acidity reduced groundnut plant performance, but enhanced nodulation.

If a strain effect on symbiotic performance is anticipated, nodulation competence of that strain under certain stress factors should be established. However, in groundnut, the improvement of nitrogen fixation by *Bradyrhizobium* strain selection should not be overemphasised, since nodulation is poorly correlated to yield, while being the parameter that is mainly affected by the choice of the strain.

It was also shown that the mode of nitrogen nutrition of groundnuts influenced soil pH. Allocating acid tolerance to the plant or its symbiosis may be based on faulty comparisons if nitrogen-nutrition dependent effects on soil pH are not taken into consideration.

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