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A comparison of the cation/anion balance of ten cultivars of *Trifolium subterraneum* L., and their effects on soil acidity

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Summary Ten cultivars of subterranean clover were grown in pots on a poorly buffered, sandy soil of pH 4.9; the plants were dependent throughout upon symbiotic fixation for their supply of N. There were some marked increases in soil acidity which resulted in changes in pH of 0.76 to 1.08 (mean 0.94) units. Increasing soil acidity was associated with an increasing total content of excess cations, *i.e.* $(Ca^{2+}+Mg^{2+}+K^{+}+Na^{+})-(H_2PO_4^{-}+SO_4^{2-}+Cl^{-})$, which ranged from 15.8 to 21.9 meq pot^{-1}. The differences between the cultivars in their effects on acidity were largely related to differences in growth rather than to marked differences in the concentration of total cations or inorganic anions. There was no obvious grouping amongst the ten cultivars in terms of either their effects on soil pH, or on their uptake of manganese.

Introduction

Poor legume growth on acid soils often results from a high solubility and availability of aluminium (Al) and/or manganese (Mn) ions[4]. The solubility of Al in the rhizosphere and the content of Mn in plants can be considerably altered by the form of nitrogen (N) taken up by plants and the resultant changes in the balance of uptake of cations and anions[6,7]. When differences in this balance are caused by the form of N supplied, increased acidity is associated with a lowered content of excess cations[6,7], *i.e.* the difference between the contents of total cations and total inorganic anions (and which is numerically equal to the organic acid content). For plants dependent upon the same source of N, however, it is to be expected that those with a greater content of excess cations will be associated with a greater increase in acidity in the rhizosphere[12,13]. Indeed, it has been proposed that at least part of the variation in genotypic tolerance to Al toxicity may be associated with variation in excess cation uptake and changes in rhizosphere acidity[2].

Legumes which are dependent on symbiotically fixed N remove an excess of cations over anions from the soil solution, and thus increase acidity at the root

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surface[1,5,6,7,9]. It has been estimated[9], for example, that the removal of 1 tonne (dry weight) of lucerne (*Medicago sativa* L.) requires the addition of 60 kg $CaCO_3$ to neutralize the acidity produced in the rhizosphere. At very short distances from the roots there may be major changes in pH[10] and these will have important implications for the nutrition of the plant during a growing season. There are major differences between cultivars of subterranean clover (*Trifolium subterraneum* L.) in their tolerance to excess Al and Mn[11]. Little is known, however, about differences between cultivars in their excess cation uptake and the effect that such differences may have in the development of rhizosphere acidity.

In the present studies we have examined the excess cation uptake of ten cultivars of subterranean clover solely dependent upon symbiotic fixation for their supply of N. The clover was grown, in pots, on a poorly buffered, sandy soil from Western Australia; changes in soil pH and in the uptake of Mn were examined.

Materials and methods

The soil was a cultivated, sandy soil (pH 4.91, 1:5 soil:0.01 *M* $CaCl_2$) from Bodallin in the eastern wheat belt of Western Australia. Both the soil and the general experimental procedures have been fully described elsewhere[6,7]. Briefly, three kilogram soil (air dried) were weighed into polythene-lined plastic pots, and the following rates (mg pot^{-1}) of basal nutrients were applied and mixed with the soil; KH_2PO_4, 1000; K_2SO_4, 435; $CaCl_2.2H_20$, 214; $MgSO_4.4H_20$, 60; $CuSO_4.5H_20$, 15; $ZnSO_4.7H_20$, 30; $MnSO_4.4H_20$, 45; $CoSO_4.7H_20$, 1.25; H_3BO_3, 2.5 and $Na_2MoO_4.2H_20$, 2.0. No combined N was added to the soil. The soil was brought to field capacity (*i.e.* 12% of soil weight) and maintained at this water content by regular weighing and addition of de-ionised water throughout the experiment. Ten cultivars of subterranean clover were sown during July, and immediately after emergence the seedlings were thinned so that 16 plants per pot remained. There were six cultivars of the sub-species *subterraneum,* two of *yanninicum* and two of *brachycalycinum* (Table 1). A suspension of *Rhizobium trifolii* Dang (strain TA1) was applied to each seed at sowing.

There were four replicate pots of each cultivar, as well as four pots containing no plants, and these were arranged in four randomized blocks in root cooling tanks (with temperatures held at 18–20°C) housed in the glasshouse. At day 62 after sowing the plants were harvested by cutting the shoots level with the surface of the soil, and the roots from duplicate pots were separated from the soil by careful sieving and washing. Sub-samples of the sieved soil were air dried, and pH determined in a 1:5 suspension of soil in 0.01 *M* $CaCl_2$.

Nitric acid/perchloric acid digests of sub-samples of roots and shoots from duplicate pots of each cultivar were analysed for Ca, Mg, Na, K and Mn (atomic absorption spectrophotometry) and P (colorimetrically). Nitrogen (Kjeldahl), S (X-ray fluorescence) and Cl (chloride titrator) contents were also measured. The ash alkalinity of shoots from duplicate pots of each cultivar was also determined by heating 0.5g samples slowly in a muffle furnace to 400°C followed by 1 hour at 500°C; the resultant ash was treated with 0.5 ml *M* HCl, and the residual acid titrated against 0.25 *M* NaOH.

Results

(i) *Plant growth and N contents*

The ten cultivars differed considerably in the dry weights of their shoots after 62 days *i.e.* from 14.4 (Dwalganup) to 19.9 (Yarloop) g pot^{-1} (Table 1).

Table 1. Dry weight (g pot^{-1}) and nitrogen concentration (% in dry matter) of ten cultivars of subterranean clover

		Dry weight		N concentration	
Cultivar	Sub-species	Shoots	Roots	Shoots	Roots
Dinninup	*subterraneum*	15.7	2.3	3.63	3.39
Mt. Barker	,,	17.3	2.9	3.79	3.30
Seaton Park	,,	16.4	2.4	3.66	3.53
Dwalganup	,,	14.4	1.9	3.21	3.34
Daliak	,,	13.8	1.6	3.69	3.40
Nungarin	,,	14.4	2.0	3.80	3.51
Yarloop	*yanninicum*	19.9	3.6	3.32	3.42
Trikkala	,,	15.6	2.1	3.70	3.57
Clare	*brachycalycinum*	18.9	3.3	3.30	3.37
65180 B	,,	15.9	2.3	3.83	3.59
s.e. (df)		0.48 (30)	0.20 (10)	0.046 (10)	0.091 (10)

There were also considerable differences in the ratios of shoot weight:root weight which ranged from 5.45 (Clare) to 8.63 (Daliak). Nitrogen concentrations in both shoots and roots did not vary to any large extent between the cultivars.

(ii) *Cation and inorganic anion content*

The concentrations of individual cations (Table 2) varied considerably between cultivars, but the same pattern was not displayed by each element. Variation between cultivars in the concentration of total cations ($Ca^{2+}+Mg^{2+}+K^{+}+Na^{+}$) in their shoots to some extent reflected differences in growth. Thus the total content of cations ranged from 24.6 (Nungarin) to 30.2 (Clare) meq pot^{-1}. Some of the variation in total anion ($H_2PO_4^- + SO_4^{2-} + Cl^-$) concentrations also arose from the differences in growth; thus, the concentrations ranged from 43 (Yarloop) to 65 (Dwalganup) meq 100 g^{-1}, whilst total contents ranged from 7.7 (Daliak) to 10.0 (Mt Barker) meq pot^{-1}. The pattern of variation in anion contents did not coincide with that in cation contents. There was thus a range in excess cations, *i.e.* ($Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}$) − ($H_2PO_4^- + SO_4^{2-} + Cl^-$), from 104 (Yarloop) to 135 (65180 B and Daliak) meq 100 g^{-1} and from 15.8 (Nungarin) to 21.9 (Clare) meq pot^{-1}. As very little N would have been supplied from the soil, excess cation content can be assumed to be equivalent to the difference between the sum of cations ($Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}$) absorbed and the sum of anions ($H_2PO_4^- + SO_4^{2-} + Cl^-$) absorbed. No attempt was made to differentiate between organic and inorganic sulphur; however, sulphur would have been absorbed as SO_4^{2-}, and would be present in this form in the digested and ashed samples.

Table 2. Cation and inorganic anion contents of shoots and roots of ten cultivars of subterranean clover. Values (meq 100 g^{-1}) are means for plants from two replicate pots

	Shoots							Roots						
Cultivar	Ca^{2+}	Mg^{2+}	K^+	Na^+	$H_2PO_4^-$	SO_4^{2-}	Cl^-	Ca^{2+}	Mg^{2+}	K^+	Na^+	$H_2PO_4^-$	SO_4^{2-}	Cl^-
Dinninup	80	27	66	3	13	18	19	20	52	58	17	22	18	7
Mt. Barker	73	33	68	4	14	21	23	17	50	49	13	19	19	4
Seaton Park	72	32	67	4	14	22	20	19	54	45	15	19	19	5
Dwalganup	75	29	77	5	14	29	21	17	58	59	20	32	23	5
Daliak	74	29	78	5	15	18	23	19	51	78	16	29	26	7
Nungarin	65	26	76	5	13	27	21	18	51	60	19	24	25	5
Yarloop	59	29	56	4	10	17	16	19	51	37	13	19	15	4
Trikkala	64	32	77	4	14	21	17	20	43	49	19	21	18	7
Clare	60	33	62	4	10	18	16	19	44	43	14	18	16	7
65180B	73	39	68	5	14	18	18	18	42	50	12	22	15	11

Excess cation concentrations in the roots were always lower than in the shoots, and cultivars with a high concentration in the shoots did not always have a correspondingly high concentration in their roots. Thus the difference in excess cation concentration between shoots and roots ranged from 15 (Nungarin) to 55 (65180B) meq 100 g^{-1}.

In previous studies[6,7] with subterranean clover, cv. Seaton Park, as well as with other species, ash alkalinity has provided a good measure of excess cations. Amongst eight of the present ten cultivars there was reasonably good agreement (*i.e.* differences of 8 per cent or less) between ash alkalinity and excess cation contents (Table 3). With the two exceptions *i.e.* Nungarin and Trikkala, the values of ash alkalinity were, respectively, 15 per cent lower and 15 per cent higher than the values for excess cations.

Table 3. Ash alkalinity and excess cation content (meq 100 g^{-1}) of shoots of ten cultivars of subterranean clover, and final pH of soil (1:5, soil:0.01 *M* $CaCl_2$)

Cultivar	Ash alkalinity	Excess cations*	Soil pH
Uncropped soil	—	—	4.92**
Dinninup	124	126	3.91
Mt. Barker	126	121	3.90
Seaton Park	127	121	3.97
Dwalganup	130	122	4.02
Daliak	146	135	4.04
Nungarin	127	110	4.16
Yarloop	100	104	3.84
Trikkala	105	124	4.12
Clare	117	116	3.95
65180B	146	135	3.90
s.e.	3.9 (10 df)	—	0.032 (22 df)

* Excess cations = $(Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}) - (H_2PO_4^{-} + SO_4^{2-} + Cl^{-})$

** Initial pH = 4.91.

(iii) *Soil pH*

Even although the rooting density in the pots was low (on average 0.81 g dry roots kg^{-1} air dry soil), there were some marked increases in acidity, resulting in changes of from 0.76 to 1.08 (mean 0.94) pH units (Table 3). The pH of the uncropped soil did not change significantly. In contrast to our previous studies[6,7], increasing acidity was associated with increasing ash alkalinity (excess cation) contents. However, the concentrations of excess cations and ash alkalinity were not well correlated with final soil pH (Table 4) (the data for excess cations provided better correlations than those with ash alkalinity, and only these have been included). However, if total excess cation contents are considered, the relationship with the final pH is much improved (Table 4), and is further improved if the excess cation contents of the roots are also included (Table 4).

Table 4. Relationship between excess cation content (Ca^{2+} + Mg^{2+} + K^+ + Na^+) − ($H_2PO_4^-$ + SO_4^{2-} + Cl^-) and manganese content of ten cultivars of subterranean clover, and final soil pH

Variable x	Variable y	Regression equation	Correlation coefficient
Excess cations			
Shoots (meq 100 g^{-1})	Final pH	y = 3.97+0.001	0.110
Shoots (meq pot^{-1})	Final pH	y = 4.76−0.040 x	−0.752
Shoots+roots (meq pot^{-1})	Final pH	y = 4.80−0.038 x	−0.804
Shoots (meq 100 g^{-1})	Mn in shoots (μg g^{-1})	y = 20+2.99 x	0.590
Shoots+roots (meq pot^{-1})	Mn in shoots (μg g^{-1})	y = 26.9+2.38 x	0.532
Final soil pH	Total Mn, shoots+roots (mg pot^{-1})	y = 10.0−2.09 x	−0.664

(iv) *Mn uptake and distribution*

Both the concentration and the total contents of Mn in shoots and roots varied considerably between the cultivars (Table 5). As in previous studies[6,7], the Mn contents were correlated with excess cation contents but only moderately well (Table 4); there was, however, a better relationship between total Mn uptake and the final pH, *i.e.* increasing uptake with increasing acidity.

Discussion

There was no obvious grouping amongst the present ten cultivars either in terms of their excess cation contents, or in the final pH of the soil around their roots. The three sub-species of subterranean clover differ in their natural distribution and soil preference. Thus sub-species *subterraneum* is found on acid to neutral soils, *yanninicum* on acid, waterlogged soils and *brachycalycinum,* on slightly acid to alkaline (pH 7.5) soils[8]. It might therefore have been expected that if different excess cation contents had evolved on different soil types as a means of tolerating acidic conditions and excesses of Al and/or Mn, there would have been some similarity between the members of a particular

Table 5. Manganese content of shoots and roots of ten cultivars of subterranean clover grown with N supplied through fixation (values are means for analyses of plants from two replicate pots)

	Concentration ($\mu g\ g^{-1}$)		Total content ($mg\ pot^{-1}$)		
Cultivar	Shoots	Roots	Shoots	Roots	Proportion in roots (%)
Dinninup	77	140	1.19	0.36	23
Mt. Barker	74	101	1.26	0.29	19
Seaton Park	71	163	1.13	0.38	25
Dwalganup	79	155	1.20	0.30	20
Daliak	80	216	1.11	0.38	25
Nungarin	59	210	0.84	0.42	33
Yarloop	85	129	1.70	0.48	22
Trikkala	79	135	1.23	0.27	18
Clare	82	193	1.49	0.64	30
65180B	97	199	1.59	0.43	21
s.e. (10 df)	2.9	10.8	0.051	0.045	

sub-species. On the present evidence, it seems unlikely that this is the case for subterranean clover.

Differences between subterranean clover cultivars in their tolerances of excess levels of Al and Mn in solution culture have been demonstrated recently[11]. The present plants contained concentrations of Mn that may be considered to be normal, and each cultivar may respond differently when subjected to excessive levels. The cultivars common to the present studies and to those of Osborne *et al.*[11], may be ranked in the following order of decreasing tolerance to excess Mn: Dinninup, Seaton Park, Yarloop, Trikkala, Daliak and Mt. Barker. None of the characteristics determined for these cultivars in the present studies, *i.e.* Mn concentration, Mn total contents, retention of Mn by roots, excess cation contents or the magnitude in the changes in the final soil pH can be ranked in a similar order. It should be noted, however, that the present plants were dependent upon symbiotically fixed N, whereas those in the study of Osborne *et al.*, were almost entirely dependent on nitrate-N, the uptake of which would have resulted in increasing alkalinity in the culture solution. It would be of interest to know whether the same ranking of tolerance to Mn would be found in clovers that were dependent upon symbiotically fixed N, and further to compare the behaviour of Al in plants dependent on symbiotically fixed N with those dependent on combined N.

The relationship between excess cations in the plants and final soil pH was in the expected direction, *i.e.* increasing acidity with increasing content of excess cations in the whole plant. Studies of interspecific differences of Al tolerance in wheat have been related to differences in excess cation uptake and therefore to pH changes at the root surface[2]. Any such relationship for subterranean clover would appear to be complex. In this species, differences amongst cultivars in changes in pH in soil near their roots result, in large part, from differences in growth.

It has been suggested that the development of acidity in soils under legume-based pastures is due to an accumulation, over the long-term, of organic matter and an increased cation exchange capacity[14]. However, when produce that has utilised N obtained through symbiotic fixation is removed from the site of production, the imbalance in the uptake of cations and anions may be an additional and/or alternative cause of acidification. Some of the increased acidity that occurs during a growing season will be neutralized by the return of plant material to the soil, either directly or through the grazing animal[3]. Unless all is returned, however, there will be some residual acidity which will be buffered to varying degrees depending upon the initial pH, cation exchange capacity, clay content and organic matter content of the soil. The effects will be greatest in poorly buffered soils, such as that in the present study.

The immediate changes in pH (*i.e.* during the growing season), along with the associated changes in other chemical properties will, as indicated by the model of Nye[10], be much greater in the soil in immediate contact with the root than those measured in the bulk soil. However, over the longer term, there may be a build-up of acidity in the bulk soil which will result in significant changes in the chemical status of clover-dominated pasture soils and the subsequent mineral nutrition of the plants growing upon them.

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