A comparison of soil tests to predict the growth and nodulation of subterranean clover in aluminium-toxic topsoils

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Abstract

Total Al concentration or pH in 1:5 10 mM CaCl_2 extracts and exchangeable Al in 100 mM BaCl_2 extracts cannot always distinguish between Al-toxic and Al-nontoxic topsoils. Our objectives were to compare the abilities of different measures of Al and pH in various extracts to predict the effects of acidity on growth and nodulation of subterranean clover. In a glasshouse experiment, *Trifolium subterraneum* L. cv. Mt Barker was grown in acidic soils from 3 sites in the Western Australian wheatbelt with different histories of phosphate fertilizer application. The pH was adjusted to give a range of 3.8–7 in the centrifuged soil solution (SS). Total (Al-tot), reactive Al (8-hydroxyquinoline-extractable, Al-HQ) and pH were measured in SS and 1:5 extracts of KCl, CaCl_2 and LaCl_3 . Another method of estimating reactive Al (Al which reacts with Chelex-100) was also measured in SS only. Other measurements included exchangeable Al and H, Ca in SS, and P in SS and the CaCl_2 extracts. Both plant growth and early nodulation decreased with increasing acidity. Plant growth in the acidified and unlimed treatments of all soils was best described by Al-HQ in SS, KCl or CaCl_2 ($r^2 = 0.68\text{--}0.70$). Multiple regression of relative yield against Al or pH with the concentration of P in SS increased the percentage variation explained by 10% and 30%, respectively. Early nodulation was well correlated ($r^2 = 0.67\text{--}0.91$) with pH or exch. H, Al-tot or exch. Al and Al-HQ. No improvement in the correlation was gained by including P using multiple regression. At constant ionic strength, increasing the valence of the extracting cation decreased the ability of soil tests to distinguish phytotoxic Al.

Introduction

Al toxicity can affect productivity in a farming system both directly by decreasing yield of sensitive crop and pasture species and indirectly by decreasing the amount of N supplied to crops grown in rotation with Al-sensitive legumes. The importance to agriculture of identifying Al problems in soils with low pH-buffering capacities lies in the high costs of applying lime relative to returns over the short term. So far, no soil test has proven capable of predicting Al toxicity.

In acidic conditions, decreased growth of subterranean clover can be due to Al toxicity directly affecting plant growth (Munns, 1965) and/or to poor nodulation limiting nitrogen fixation (Coventry and Evans, 1989). Decreased nodulation and damage to root hairs are observed at concentrations of monomeric Al as low as $6.4 \mu\text{M}$ (Kim et al., 1985a) as determined by the 30-min aluminon method (Blamey et al., 1983).

In simplified acid solutions with careful control of pH, ionic strength and concentration of ligands forming complexes with Al, it has been

possible to demonstrate that decreased plant growth is well correlated with the activity of the Al^{3+} ion (henceforth denoted (Al^{3+}) , Cameron et al., 1986) or the sum of the activities of inorganic monomeric Al species ($\Sigma(\text{Al}_{\text{mono}})$, Blamey et al., 1983). In complete nutrient solutions and soils, calculation of speciation of Al is not possible because there are no thermodynamic data available for Al complexes with phosphate or fulvic and humic acids.

Wright et al. (1987) attempted to simplify the speciation calculations by using the concentration of reactive Al in the soil solution of subsoils as determined with three colorimetric methods to estimate $\Sigma(\text{Al}_{\text{mono}})$ from which (Al^{3+}) was calculated. Prediction of growth reduction by (Al^{3+}) was only marginally better than by the measured concentrations of reactive Al and was improved in one case by including (Ca^{2+}) in multiple regression equations with pH and (Al^{3+}) .

In topsoils, where organic ligands can be present in sufficient amounts to form complexes with a large proportion of the dissolved Al, there still appears to be no satisfactory method for measuring the toxic fraction. Al which reacts within 15 seconds with 8-hydroxyquinoline (8-HQ) (James et al., 1983) is a good estimate of toxic Al in the presence of fluoride (Noble et al., 1988). However, this method appears to extract a variable proportion of Al which is complexed with organic ligands (James et al., 1983; Whitten and Ritchie, 1991). Given that organic acids have an ameliorating effect on Al toxicity (Hue et al., 1986; Tan and Binger, 1986), the inability of 8-HQ to distinguish toxic from non-toxic Al concentrations in soils (Adams and Hathcock, 1984) may be due to differences in the amount of organically complexed Al extracted or by differ-

ences in the supply of ameliorating factors such as Ca. Wright and Wright (1987) also used Al which reacts with 8-HQ to estimate $\Sigma(\text{Al}_{\text{mono}})$ and (Al^{3+}) in topsoils and again found in one case that including a Ca term improved their model such that $(\text{Ca}^{2+})/\Sigma(\text{Al}_{\text{mono}})$ was a better predictor of growth reduction of subterranean clover than either $\Sigma(\text{Al}_{\text{mono}})$ or (Al^{3+}) . Prediction of Al toxicity across a range of soils may therefore require inclusion of factors such as Ca or P which have been shown to alleviate Al toxicity (Blamey et al., 1983; Cameron et al., 1986) as well as the ability to discriminate between toxic and non-toxic forms of Al.

The objectives of our work were: i) to compare the abilities of different types of soil extract and methods of estimating toxic Al to predict the growth and nodulation of subterranean clover in the presence of various amounts of Al in three topsoils from the Western Australian wheatbelt; ii) to identify which, if any, ameliorating factors could improve the predictive value of the soil test; and iii) to establish the relationship between pH or Al in the soil solution and the corresponding measurement in salt extracts.

Materials and methods

Soil types

Three sites in the Western Australian wheatbelt (Table 1) were sampled to a depth of approximately 10 cm after clearing the surface of plant debris. The soils were air-dried and sieved (≤ 2 mm) immediately after collection. All sites had been cleared of native vegetation of at least 10 years. Soil 1 came from near Meckering (latitude $31^{\circ}36'$ S, longitude $116^{\circ}65'$ E) and was under

Table 1. Summary of soil properties

Soil	Rainfall mm yr^{-1}	Northcote classification	pH ^a	Al ^b	Org. C ^c (%)	Particle size (%)		
						Sand	Silt	Clay
1	450	Dy5.51	4.4	0.18	1.0	90	4	6
2	450	Dy5.51	4.4	0.20	1.0	89	4	7
3	300	Gn2.21	4.8	0.09	1.3	80	3	17

^a 1:5 10 mM CaCl_2 .

^b Exchangeable Al, 1:10 100 mM BaCl_2 , $\text{cmol}_c \text{ kg}^{-1}$.

^c Yeomans and Bremner, 1988.

pasture. Soil 2 came from near Goomalling (31°18' S, 116°50' E) and had been continuously cropped for the past 10 years. Soil 3 came from near Merredin (31°29' S, 118°17' E) and was in the crop phase of a short crop: pasture rotation.

Experimental design

In a glasshouse pot trial *Trifolium subterraneum* L. cv. Mt Barker with and without rhizobial inoculation was grown in 3 soils with 8 pH treatments and three replicates in randomized blocks. Uninoculated treatments received fertilizer N. Soil analyses were carried out on separate subsamples of the soils which had the same treatments as the plant growth subsamples except for N and trace elements.

Nutrient addition and pH amendment

The soil pH was adjusted with dilute HCl (range: 0.06–0.8 cmole H⁺ kg⁻¹) or precipitated CaCO₃ (equivalent to 1 and 2 × 10³ kg ha⁻¹ assuming a bulk density of 1.6 and depth of incorporation 15 cm). The unamended treatment received only de-ionised water. The target pHs in the soil solution were 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.5 and 6.5. All treatments were incubated at field capacity at 40°C for 8 days which is equivalent to 32 days at 20°C according to the Arrhenius equation (Castellan, 1970). Nutrient solutions supplying (mg kg⁻¹ soil) Na₂MoO₄ 2H₂O, 0.67; H₃BO₃, 0.83; CuSO₄ 5H₂O, 5; ZnSO₄ 7H₂O, 10; MnSO₄ H₂O, 15; CoSO₄ 7H₂O, 0.4; K₂SO₄, 145; and KH₂PO₄, 110 (soils 1 and 2) and 176 (soil 3) were then applied as solutions to the surface of the soil which was then dried and mixed. These P rates were selected from a preliminary P response experiment to produce 90% maximum growth in each soil in order to minimise the ameliorating effects that higher rates of P could have on any Al toxicity in the acidified treatments. N was added to the +N treatments at the rate of 24 mg N kg⁻¹ as NH₄NO₃ immediately before sowing and every ten days thereafter. All reagents were analytical grade.

Glasshouse experiment

Germinated seed with radicles of approximately

2 mm were sown into 25 evenly spaced 10-mm depressions in soil at field capacity in lined 3-kg pots. The plants were thinned to 20 plants per pot on day 7. Inoculated treatments received 1 mL of a suspension containing approximately 10⁷ colony-forming units of *Rhizobium leguminosarum* bv *trifolii* TA1 which was pipetted into the depressions before seeding. The replicates were arranged in randomised blocks in separate root-cooling tanks in which the temperature was maintained at approximately 20°C. Air temperature varied between approximately 15–30°C. Pots were watered daily with de-ionised water, initially to 80% field capacity and later to field capacity as demand increased. Damping-off was controlled with 2 treatments on days 13 and 20 with a systemic fungicide (Fongarid®, Ciba-Geigy, Basle, Switzerland). Harvest commenced on day 35 with 1 replicate harvested on each of 3 successive days.

Soil extracts and analyses

Three types of soil extract were obtained. Soil solutions were extracted by centrifugation (Gillman, 1976) after incubating the soils at field capacity for 16 hr at 20°C. Salt extracts were obtained by shaking the soil end-over-end for 16 hours at 20°C at a soil:solution ratio of 1:5 (w/v) with 30 mM KCl, 10 mM CaCl₂, and 5 mM LaCl₃ (ionic strength 0.03) or at a soil:solution ratio of 1:10 (w/v) in 100 mM BaCl₂ to determine exchangeable ions. All extracts were filtered through 0.45-μm membrane filters (Millipore type HA).

Reactive Al was estimated by the 8-HQ method (James et al, 1983, hence referred to as Al-HQ) in the soil solutions and 1:5 extracts and by Chelex-100®, a cation exchange resin (Al-res; Whitten and Ritchie, 1991) in the soil solutions only. Total Al (Al-tot) was measured in the LaCl₃ extracts with 8-HQ using the same reagents as James et al. (1983) for total Al, and in all other extracts with pyrocatechol violet based on the method of Dougan and Wilson (1974). Pye-Unicam SP8-150 or SP6-550 UV-Vis spectrophotometers were used for the colorimetric determinations. Al standards were made up at pH 3 from a stock solution of AR-grade Al wire dissolved in HCl using mercury as a catalyst.

Ca, Mg, K and Na in the soil solutions and BaCl_2 extracts were determined by flame atomic absorption spectrophotometry (FAAS) on a Perkin Elmer model 403. Standards and sample dilutions were made with a solution of 1000 mg L^{-1} Cs as CsCl in 100 mM HCl . A nitrous oxide flame was used for Ca and Mg determinations to remove potential interference from P, Al and Si. Phosphorus was measured by the molybdenum-blue method (John, 1970) in the soil solutions and CaCl_2 extracts. Measurements of pH in the extracts and soil solutions were made on the day of extraction with a combination electrode and an Orion Research EA 940 meter. Electrical conductivity in the soil solution was measured with an Activon PT-18 digital conductivity meter with automatic temperature correction and calibrated with standard solutions of KCl. The relative concentration of dissolved organic matter in the soil solution was estimated by measuring the spectrophotometric absorbance at 240 nm with a 1-mm quartz cuvette.

The ionic strength of the soil solution was estimated from the electrical conductivity of the soil solution using the formula of Gillman and Bell (1978).

The phosphate retention index (PRI) of each soil was determined in triplicate by shaking 1 g air-dry soil for 16 h at 20°C in 10 mL 10 mM KCl containing 10 mg L^{-1} P as KH_2PO_4 . The solution was then centrifuged, filtered through a $0.45\text{-}\mu\text{m}$ filter (Millipore type HV), and the P concentration determined by the molybdenum-blue method (John, 1970). The PRI was then calculated as the amount of P adsorbed by the soil divided by the final concentration of P.

Plant analyses and growth measurements

Shoots were dried and ground in a hammer mill to pass through a 1-mm screen. After further drying at 70°C overnight, subsamples from each replicate were digested in a 4:1 nitric:perchloric acid mix (Johnson and Ulrich, 1959) and analysed for Ca, Mg by FAAS and P by the molybdovanadate method (Hanson, 1950). Nodules were counted on the uppermost 20 mm of the taproot and on the first 10 mm of laterals originating within this zone.

Data analysis

Growth data were related to soil parameters by fitting exponential models (Genstat 5; Rothamsted Statistics Department, 1987) and by step-wise multiple linear regression using forward selection (Statview 1.03; Abacus Concepts, Inc., 1988).

Results

Effects of acidity

In all three soils, the concentrations of Al-tot and reactive Al in the soil solution and 1:5 extracts (Fig. 1) increased as pH decreased. Plant growth and early nodulation decreased with increasing Al (Fig. 2; data for Al-HQ only presented).

Concentrations and forms of Al in soils

At a constant pH, the concentration of total Al in the different extracts tended to be highest in the soil solution and decreased in the order $\text{LaCl}_3 > \text{CaCl}_2 > \text{KCl}$. In any one extract, the concentration of Al tended to be lower in soil 3 than soils 1 and 2. The total amount of Al extracted per unit weight of soil by each type of extract decreased in the order $\text{BaCl}_2 > \text{LaCl}_3 > \text{CaCl}_2 > \text{KCl} > \text{soil solution}$.

The two measures of reactive Al extracted different proportions of Al-tot from the soil solution. The cation exchange resin extracted most of Al-tot from the soil solutions of all soils. This contrasts with previous work in which the proportion of Al which exchanged with the resin was controlled by the ratio of Al to organic complexing groups (Whitten and Ritchie, 1991). The concentration of Al-HQ in the soil solution and the KCl and CaCl_2 extracts was $< 50\%$ of Al-tot, whereas it was nearly 90% of Al-tot in LaCl_3 extracts. The concentration of Al-HQ in the soil solution and 1:5 extracts was linearly related to Al-tot in the same extract, but the proportion of Al-HQ was greater ($p < 0.05$) in soil 3 than in soils 1 and 2, except in LaCl_3 in which it was approximately the same in all 3 soils (Fig. 3).

Fig. 1. The concentration of Al-tot (μM) in the soil solution plotted against the pH. Error bars are standard errors of means.

Correlation between the pH in the soil solution and 1:5 extracts

The pH of the 1:5 extracts from all soils was linearly correlated with the pH of the soil solution ($r^2 \geq 0.98$ in all cases). The pH of the KCl extract was not significantly different from that measured in CaCl_2 . The pH in both KCl and CaCl_2 was higher than in the soil solution, except at the higher rate of lime. In contrast, the pH in LaCl_3 was similar to that in the soil solution in the most acidic treatment, but was much lower in the limed treatments and was significantly less ($p < 0.05$) than in KCl and CaCl_2 .

Relationship between reactive Al in the soil solution and 1:5 extracts

The relationships between the concentration of reactive Al in the soil solution and the corresponding measurement in 1:5 extracts were non-linear and showed increasing curvature as the valence of the extracting cation increased from +1 for KCl to +3 for LaCl_3 . The exponential regression equations ($y = A + B e^{-cx}$, where A, B and c represent the intercept, asymptote and curvature, respectively) for Al-HQ in the 1:5 extracts (y) vs Al-HQ in the soil solution (x)

were $y = 31.36 - 29.97 e^{-0.0025x}$, $y = 35.42 - 34.69 e^{-0.0112x}$ and $y = 144.60 - 146.29 e^{-0.0501x}$, for KCl, CaCl_2 and LaCl_3 , respectively. The differences in curvature were significant ($p < 0.05$) in all cases, and the intercept and asymptote for LaCl_3 were also significantly different from the corresponding values for KCl and CaCl_2 .

P, Ca, Mg, K and Na in soil solution and 1:5 extracts

The concentration of P in CaCl_2 increased slightly with increasing acidity in soils 1 and 2, but there was no clear effect of pH on the concentration of P in the soil solution in any of the soils. The concentration of P in the soil solution was highest in soil 2 (average $42.1 \mu\text{M}$), followed by soil 1 ($15.4 \mu\text{M}$) and lowest in soil 3 ($6.1 \mu\text{M}$). The relationship between the concentration of P in the CaCl_2 extract and that in the soil solution was linear over all soils ($y = 0.06 + 0.27x$, $r^2 = 0.96$). The phosphate retention index (PRI) of each soil increased as the pH decreased and was higher in soil 3 (3.8–5.6) than in soil 1 (1.2–2.5) and soil 2 (1.1–2.3). The concentrations of P in the soil solution and CaCl_2 extract were not linearly related to the PRI.

Fig. 2. Relative shoot dry weight expressed as a percentage of the maximum within each soil and N source (a) and number of early nodules (b) plotted against the concentration of Al-HQ (μM) in the soil solution.

Fig. 3. The concentration of Al-HQ (μM) plotted against the concentration of Al-tot (μM) in 1:5 30 mM KCl extracts (a), 1:5 10 mM CaCl_2 extracts (b), 1:5 5 mM LaCl_3 extracts (c) and soil solution (d). Error bars are standard errors of means.

Fig. 3. (continued).

Adding acid increased the concentrations of Ca (2–20 mM), Mg (2–11 mM), K (5–12 mM) and Na (4–10 mM) in the soil solution in all soils, whereas adding lime only increased Ca (2–5 mM). The ionic strengths in the soil solutions increased from approximately 0.03 to 0.09.

UV absorbance of soil solution

The UV absorbance at 240 nm decreased in all soils as the pH decreased and the concentration of Al increased. The values were slightly lower in soil 3 than in soils 1 and 2, except in the limed treatments.

Growth and nutrient uptake by subterranean clover

Plant growth was at a maximum within each soil type at the higher rate of lime addition in the N-fixing treatments in all soils, and at the lower rate of lime when fertilised with NH_4NO_3 . The order of decreasing response to acidity for plant growth was soil 1 > soil 3 > soil 2 and for nodulation soil 2 > soil 1 > soil 3.

As acidity increased, the concentration of Ca in the whole tops in soils 1 and 3 and of Mg in all

soils increased. The P concentration in plants grown in soils 1 and 3 decreased as Al-HQ increased (Fig. 4).

Root dry weights decreased with increasing acidity in all treatments, whereas root length decreased only in the N-fixing treatments in soils 1 and 2 (data not shown). Over all treatments, there was a linear relationship between root weight and root length ($y = 0.0987 + 0.0034x$, $r^2 = 0.61$, slope significant at $p < 0.05$).

Correlation of yield and nodulation with different measures of pH and Al

The number of early nodules decreased as acidity increased, and increased as plant growth increased. The relationship between nodulation or relative yield and measures of pH and Al over the entire range of pH treatments could be described by an exponential equation of the form $Y = A + Be^{-cx}$. Using this model for all soils, relative yield was correlated better with Al-HQ ($r^2 = 0.76$ – 0.78) than with Al-tot and Al-res ($r^2 = 0.68$ – 0.74) or pH ($r^2 = 0.70$ – 0.77) in the soil solution and the KCl and CaCl_2 extracts. Both exchangeable H and Al were correlated with relative yield ($r^2 = 0.71$ and 0.80 , respec-

Fig. 4. The concentration of P (% w/w) in the whole tops of subterranean clover plotted against Al-HQ (μM) in the soil solution. Error bars are standard errors of means.

tively). With the LaCl_3 extracts there was a poorer correlation between relative yield and pH ($r^2 = 0.61$) or both measures of Al ($r^2 = 0.58$ – 0.59). Early nodulation was well correlated with pH ($r^2 = 0.87$ – 0.92) and all measures of Al ($r^2 = 0.81$ – 0.94). In most cases, correlation coefficients were higher for individual soils and N treatments than when the data from all soils were combined (data not presented).

The response curves for plant growth plotted against pH in all extracts, exchangeable H and Al and Al in LaCl_3 extracts were characterised by a plateau with a wide separation between the data points for the limed treatments and those of the unamended and acidified treatments. With the limed data excluded, Al-HQ explained more of the variation in relative yield (Table 2; $r^2 = 0.68$ – 0.70), than Al-tot and exchangeable Al ($r^2 = 0.54$ – 0.64) or exchangeable H and pH ($r^2 = 0.49$ – 0.57) in the soil solution and the CaCl_2 and

KCl extracts. Early nodulation (Table 3) was correlated slightly better with pH ($r^2 = 0.81$ – 0.91) than with Al-tot ($r^2 = 0.74$ – 0.90) and Al-HQ ($r^2 = 0.67$ – 0.87).

Stepwise multiple linear regression (using forward selection) between relative yield over all soils from the acidified and unlimed treatments and each measure of pH or Al combined with the concentration of P in the soil solution explained more variation than the corresponding soil test parameter alone (Table 2). The coefficients for P were all highly significant ($p < 0.01$). An additional small increase in percentage variation explained was gained in some cases by including the concentration of Ca in the soil solution as a third explanatory variable. However, the improvement gained by including a term for P or P and Ca in most cases fell short of the r^2 values for relative yield vs the corresponding single soil test parameter for individual soils.

Table 2. Variation (significant at $p < 0.05$) in relative yield of subterranean clover in acidified and unlimed treatments as explained by pH, Al-tot, Al-HQ, Al-res, exchangeable Al and H with the concentration of P and Ca in the soil solution and quadratic term for the Al and pH measurements, using stepwise multiple regression

Model	Extract				
	Soil solution	1:5			
		KCl 0.03 M	CaCl_2 0.01 M	LaCl_3 0.005 M	BaCl ₂ 0.1 M
pH	0.55	0.57	0.49	0.37	0.58
+P SS	0.84	0.87	0.80	0.84	0.79
+Ca SS	0.88	NS	0.82	0.86	NS
+quadratic	NS	0.88	NS	NS	NS
Al-tot	0.64	0.63	0.54	0.35	0.64
+P SS	0.78	0.84	0.88	0.84	0.85
+Ca SS	NS	0.87	0.90	0.86	NS
+quadratic	0.85	0.89	NS	NS	NS
Al-HQ	0.68	0.70	0.70	0.37	
+P SS	0.77	0.80	0.88	0.79	
+Ca SS	NS	NS	NS	0.85	
+quadratic	0.83	0.83	NS	NS	
Al-res	0.64				
+P SS	0.78				
+Ca SS	NS				
+quadratic	0.84				

NS = improvement in r^2 not significant.

Table 3. Variation (significant at $p < 0.05$) in number of early nodules in acidified and unlimed treatments as explained by pH, Al-tot, Al-HQ, Al-res, exchangeable Al and H with the concentration of P and Ca in the soil solution and quadratic term for the Al and pH measurements, using stepwise multiple regression

Model	Extract				
	Soil solution	1:5			
		KCl 0.03 M	CaCl_2 0.01 M	LaCl_3 0.005 M	BaCl ₂ 0.1 M
pH	0.86	0.91	0.81	0.79	0.78
+P SS	0.94	NS	NS	0.84	NS
+Ca SS	0.89	NS	NS	0.89	NS
+quadratic	NS	NS	NS	NS	NS
Al-tot	0.74	0.86	0.90	0.76	0.86
+P SS	NS	NS	NS	0.81	NS
+Ca SS	NS	NS	NS	0.85	NS
+quadratic	0.89	0.92	NS	NS	NS
Al-HQ	0.67	0.73	0.87	0.73	
+P SS	NS	NS	NS	NS	
+Ca SS	NS	NS	NS	NS	
+quadratic	0.81	0.81	0.91	NS	
Al-res	0.73				
+P SS	NS				
+Ca SS	NS				
+quadratic	0.88				

NS = improvement in r^2 not significant.

The inclusion of a quadratic term for the major explanatory variable in the above multiple regressions was significant only for the Al measurements in the soil solution and all measurements in the KCl extracts, and accounted for between 2–7% of the variation (Table 2).

With similar multiple regression analysis for early nodulation, Ca and P were significant ($p < 0.05$) explanatory variables only with pH in the soil solution and with pH and Al-tot in the LaCl_3 extracts, but only increased the percentage variation explained by 5–8%. The quadratic term of the major explanatory variable was significant ($p < 0.05$) for all Al measurements in the soil solution and KCl extracts and for Al-HQ in the CaCl_2 extracts (Table 3).

The UV absorbance estimate of dissolved organic matter was not significant in any of the multiple regression analyses.

Discussion

Al measured by the 15 sec. 8-HQ method explained more of the variation in relative yield in the acidified and unlimed treatments than either the total concentration of Al or pH in the corresponding extract. Including the concentration of P in the soil solution as a secondary explanatory variable increased the percentage of variation in relative yield explained by each soil test parameter. Little or no improvement was gained by including Ca in the multiple regression analysis. The concentration of Al-HQ in 1:5 extracts in KCl or CaCl_2 at the same ionic strength as the soil solution are equally good predictors of Al toxicity as that in the soil solution. At a constant ionic strength, increasing the charge on the extracting cation in 1:5 extracts lowered the pH of the extract and increased both the amount of Al extracted from the soil and the proportion of uncomplexed Al.

Plant growth

In the soils studied, not all soluble Al was phytotoxic and phosphorus was a more important ameliorating factor than calcium. The main chemical differences between the three soils were the higher proportion of Al-HQ in soil

3 compared with soils 1 and 2, and the range of concentrations of P in the soil solution. The latter reflects the differences between their P histories and P-fixing capacities, and appears to have resulted in a range of P:toxic Al ratios which span a threshold value below which there is no ameliorative effect. Not only was the absolute yield of dry matter higher in soil 2 than in soils 1 and 3, but the percentage decrease in yield at any concentration of Al or pH tended to be less in soil 2 than in soils 1 and 3. The argument for soil 2 having sufficient P to partly counteract Al toxicity is also supported by the decrease in P content of the whole tops with increasing acidity in soils 1 and 3, but not in soil 2. This decrease was proportionately greater than the corresponding decrease in P concentration in the soil solutions. In contrast, Kim et al. (1985b) observed an increase in P content of the tops of subterranean clover when pH decreased from 4.5 to 4.0 in the absence of Al. A possible explanation for this difference is that uptake of P is inhibited by Al at low concentrations of P, but once the P concentration is above a certain threshold, Al toxicity is mitigated without a decrease in P uptake.

Munns (1965) found that in solution culture Al decreased the concentration of P in shoots of subterranean clover as a result of decreased root length and that increasing the concentration of Ca prevented this. The relatively small percentage of variation explained by the inclusion of Ca in multiple regression of relative yield against Al or pH with P and Ca in the soil solution may be due to the concentration of Ca being more than adequate in each soil.

Nodulation

Neither P nor Ca improved the ability of the Al soil tests to predict nodulation in the presence of Al. This may reflect a lower requirement by the rhizobia for Ca and/or P compared with growth of the host plants. Nodulation was affected less by Al and/or H toxicity in this experiment than was the case in the solution-culture studies of Kim et al. (1985a). They found that nodulation of subterranean clover was significantly decreased at $6.4 \mu\text{M}$ monomeric Al and pH 4.5 compared with the zero Al treatments. In this

work, nodulation was at least 75% of maximum at pH of 4.2–4.4 and Al-HQ of 11–22 μM . The concentration of P (7–40 μM) and Ca (2–10 μM) in our soil solutions were higher than in the solution cultures of Kim et al. (1985a) and may have been sufficiently high to decrease the toxicity of Al to the rhizobia or nodulation process. Alternatively, the more concentrated inoculant in this work (10^7 mL^{-1}) compared with that in the solution studies (10^4 mL^{-1}) may account for the apparent greater tolerance of Al and low pH in this experiment. Robson (1970) found that the critical pH for nodulation of medic grown in acidic soil decreased as the inoculant density increased.

Improving soil tests for Al toxicity

Combining estimates of phytotoxic Al and ameliorating nutrients in soil tests raises the problem of sampling strategy. In the pot experiment described here, the soil had been mixed thoroughly, whereas in the field some degree of vertical stratification would be expected. Soil pH profiles under subterranean clover for at least 5 years can develop a sharp decline in pH below the top 2 cm (Williams, 1980; Conyers and Scott, 1989; McLaughlin et al., 1990). This may also occur in shorter rotations where cultivation disturbs but does not mix or invert the top 10 cm. McLaughlin et al. (1990) found that in soils which had been under subterranean clover pasture for up to 8 years, the concentration of P extracted in $\text{HF}_4\text{F}/\text{HCl}$ was highest in the top 2 cm and decreased with depth, and concentration of Al extracted in 1:5 0.01 M CaCl_2 was low in the top 2 cm and high from 2 to 10 cm. They suggest that prediction of lime and fertiliser requirements on such soils may be improved by taking separate soil samples from 0–2 and 2–10 cm depths. The Al:P molar ratio in the soil solution could increase with depth within the 2–10 cm layer, and a given concentration of Al in the soil solution may be more toxic at 10 cm than at 2 cm. McLaughlin and James (1989) have shown that P in the surface layers does not have an ameliorating effect on Al toxicity at depth. Soil tests for Al toxicity may therefore improve, if measurements of Al and ameliorating nutrients are made on samples taken from 2–5 cm and 5–10 cm.

Although pH appeared to be an adequate predictor for the effects of acidity on nodulation in our experiment, this may not be the case in the field. Preliminary results from analyses of soils from lime trials in the field over a range of soils indicate that the relationship between the concentration of Al and pH in the soil solution varies more than was the case in the experiment described here. The 15-sec 8-HQ method still appears to be the best colorimetric method available for estimating phytotoxic Al in soils with relatively low organic content. Recent studies by Kerven et al. (1989a) confirm that in the presence of fulvate, 8-HQ reacts with less organically complexed Al than other commonly used short-term colorimetric methods. At pH 4.5 and 50 μM Al present as 100% Al-fulvate, 46% of the complexed Al reacted with 8-HQ in 15 sec, whereas 82% reacted with aluminon in 30 min. In similar Al-fulvate solutions (Al 100 μM , pH 4.5) approximately 58% and 66% of the organically complexed Al reacted with pyrocatechol violet in 30 and 60 sec, respectively (Kerven et al., 1989b).

Comparison of extracts

At a constant ionic strength, increasing the charge on the extracting cation decreased the correlation between plant growth parameters and different measures of Al by decreasing the pH, increasing Al-tot and increasing the proportion of Al-HQ. This suggests that it is more important to measure the soluble toxic forms of Al than to know the soil's ability to replenish the soluble toxic forms. The more linear relationship between Al-HQ in the KCl extracts and that in the soil solution compared with the CaCl_2 and LaCl_3 suggests KCl at the ionic strength of the soil solution is more representative of the concentration of toxic Al in the soil solution. This may be important when comparing soils which differ markedly in exchangeable Al.

The proportion of Al-tot that reacted with 8-HQ in 15 sec. increased with the charge of the extracting cation, possibly because the ability of the cation to displace Al from organic complexes also increased with charge. For example, in an hypothetical solution containing 50 μM of both Al and citrate (which has a similar affinity for Al as humic acid, Ritchie et al., 1988), the concen-

tration of Al^{3+} at pH 4 may be estimated to be 3, 7 and 37 μM in 30 mM KCl, 10 mM CaCl_2 and 5 mM LaCl_3 , respectively. Evidence that Al-HQ may be able to distinguish between organically complexed Al and inorganic monomeric Al is provided by the differences in the Al-HQ concentrations of the soils and how that difference decreased according to the type of extracting cation in the order $\text{KCl} > \text{CaCl}_2 > \text{LaCl}_3$. The KCl extracts of soil 3 contained a greater proportion of Al-HQ than the KCl extracts of soils 1 and 2, whereas there was no difference between the soils in the proportion of Al-HQ in the LaCl_3 extracts. The implication that there was less soluble organically complexed Al in soil 3 is consistent with the lower UV absorbance of its soil solution in comparison to soils 1 and 2.

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References

- Abacus Concepts Inc. 1988 Statview v 1.03. Abacus Concepts Inc., 1984 Bonita Ave., Berkeley, CA.
- Adams F and Hathcock P J 1984 Aluminium toxicity and calcium deficiency in acid subsoil horizons of two coastal plains soil series. *Soil Sci. Soc. Am. J.* 48, 1305–1309.
- Blamey F P C, Edwards D G and Asher C J 1983 Effects of aluminium, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. *Soil Sci.* 136, 197–207.
- Cameron R S, Ritchie G S P and Robson A D 1986 Relative toxicities of inorganic aluminium complexes to barley. *Soil Sci. Soc. Am. J.* 50, 1231–1236.
- Castellan G W 1970 *Physical Chemistry*. Addison-Wesley, Reading, MA.
- Conyers M K and Scott B J 1989 The influence of surface incorporated lime on subsurface soil acidity. *Aust. J. Exp. Agric.* 29, 201–207.
- Coventry D R and Evans J 1989 Symbiotic nitrogen fixation and soil acidity. In *Soil Acidity and Plant Growth*. Ed. A D Robson. pp 103–137. Academic Press, Australia.
- Dougan W K and Wilson A L 1974 The absorptiometric determination of aluminium in water. A comparison of some chromogenic reagents and the development of an improved method. *Analyst* 99, 413–430.
- Gillman G P 1976 A centrifuge method for obtaining soil solution. CSIRO Aust. Div. Soils, Div. Rep. No. 16.
- Gillman G P and Bell L C 1978 Soil solution studies on weathered soils from tropical North Queensland. *Aust. J. Soil Res.* 16, 67–77.
- Hanson W C 1950 The photometric determination of phosphorus in fertilizers using the phosphovanadomolybdate complex. *J. Sci. Food Agric.* 1, 172–173.
- Hue N V, Craddock G R and Adams F 1986 Effect of organic acids on aluminium toxicity in subsoils. *Soil Sci. Soc. Am. J.* 50, 28–34.
- James B R, Clarke C J and Riha S J 1983 An 8-hydroxyquinoline method for labile and total aluminium in soil extracts. *Soil Sci. Soc. Am. J.* 47, 893–897.
- John M K 1970 Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Science* 109, 214–220.
- Johnson C M and Ulrich A 1959 Analytical methods for use in plant analysis. *Calif. Agric. Exp. Stn. Bull.* No. 766.
- Kerven G L, Edwards D G, Asher C J, Hallman P S and Kokot S 1989a Aluminium determination in soil solution. I. Evaluation of existing colorimetric and separation methods for the determination of inorganic monomeric aluminium in the presence of organic acid ligands. *Aust. J. Soil Res.* 27, 79–90.
- Kerven G L, Edwards D G, Asher C J, Hallman P S and Kokot S 1989b Aluminium determination in soil solution. II. Short term colorimetric procedures for measurement of inorganic monomeric aluminium in the presence of organic acid ligands. *Aust. J. Soil Res.* 27, 91–102.
- Kim M K, Asher C J, Edwards D G and Date R A 1985a Aluminium toxicity: Effects on growth and nodulation of subterranean clover. *Proc. of the XV Intern. Grasslands Conference*.
- Kim M K, Edwards D G and Asher C J 1985b Tolerance of *Trifolium subterraneum* cultivars to low pH. *Aust. J. Agric. Res.* 36, 569–78.
- McLaughlin M J and James T R 1989 The effect of subsurface aluminium on the growth and uptake of surface-applied phosphorus by wheat seedlings. *J. Plant Nutr.* 12, 603–620.
- McLaughlin M J, Baker T G, James T R and Rundle J A 1990 Distribution and forms of phosphorus and aluminium in acidic topsoils under pastures in south-eastern Australia. *Aust. J. Soil Res.* 28, 371–385.
- Munns D N 1965 Soil acidity and growth of a legume. II. Reactions of aluminium and phosphate in solution and effects of aluminium, calcium and pH on *Medicago sativa* L. and *Trifolium subterraneum* L. in solution culture. *Aust. J. Agric. Res.* 16, 743–755.
- Noble A D, Sumner M E and Alva A K 1988 Comparison of aluminon and 8-hydroxyquinoline in the presence of fluoride for assaying phytotoxic aluminium. *Soil Sci. Soc. Am. J.* 52, 1059–1063.
- Ritchie G S P, Nelson M P and Whitten M G 1988 The estimation of free aluminium and the competition between fluoride and humate anions for aluminium. *Comm. Soil Sci. Plant Anal.* 19, 857–871.

- Robson A D and Loneragan J F 1970 Nodulation and growth of *Medicago truncatula* on acid soils. 1. Effect of calcium carbonate and inoculation level on the nodulation of *Medicago truncatula* on a moderately acid soil. Aust. J. Agric. Res. 21, 427–434.
- Rothamsted Statistics Department 1987 Genstat 5 release 1.2. Rothamsted Experimental Station, Harpenden, Hertfordshire, U.K.
- Tan K H and Binger A 1986 Effect of humic acid on aluminium toxicity in corn plants. Soil Science. 141, 20–25.
- Uren N C 1989 Rhizosphere reactions of aluminium and manganese. J. Plant Nutr. 12, 173–185.
- Whitten M G and Ritchie G S P 1991 Soil Tests for Aluminium Toxicity in the Presence of Organic Matter: Laboratory Development and Assessment. Comm. Soil Sci. Plant Anal. 22 (*In press*).
- Williams C H 1980 Soil acidification under clover pasture. Aust. J. Exp. Agric. Anim. Husb. 20, 561–567.
- Wright R J and Wright S F 1987 Effects of aluminium and calcium on the growth of subterranean clover in Appalachian soils. Soil Science 143, 341–348.
- Wright R J, Baligar V C and Wright S F 1987 Estimation of phytotoxic aluminium in soil solution using three spectrophotometric methods. Soil Science 144, 224–232.
- Yeomans J C and Bremner J M 1988 A rapid and precise method for routine determination of organic carbon in soil. Comm. Soil Sci. Plant Anal. 19, 1467–1476.