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PHOSPHATE NUTRITION EFFECTS ON ALUMINIUM AVOIDANCE OF *MUCUNA PRURIENS* VAR. *UTILIS*

K. HAIRIAH,*‡ M. VAN NOORDWIJK,†§ I. STULEN,‡ F. W. MEIJBOOM† and P. J. C. KUIPER‡

*Brawijaya University, Faculty of Agriculture, Malang 65145, Indonesia; †DLO-Institute for Soil Fertility Research, P.O. Box 30003, NL 9750 RA Haren, The Netherlands and ‡University of Groningen, Department of Plant Biology, P.O. Box 14, NL 9750 AA Haren, The Netherlands

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HAIRIAH K., VAN NOORDWIJK M., STULEN I., MEIJBOOM F. W. and KUIPER P. J. C. *Phosphate nutrition effects on aluminium avoidance of* Mucuna pruriens *var.* utilis. ENVIRONMENTAL AND EXPERIMENTAL BOTANY 33, 75–83, 1993.—The response of roots of *Mucuna pruriens* var. *utilis* to aluminium depends on the duration of exposure and the type of experiment. In a short-term study, the usual reduction of root elongation rate by Al was found. In contrast, a study for a longer period of time, 4 weeks, showed a stimulation of root dry weight and root surface area for 110 or 185 μM Al^{3+}, compared to a no-Al control. In a split-root experiment, the response of root growth to the presence of Al^{3+} in its environment depended on the presence or absence of Al^{3+} around other parts of the same root system. It was concluded that Al avoidance, rather than Al toxicity or tolerance, explained the root response of *Mucuna* to acid subsoil conditions in the field. The question was addressed as to whether the Al-avoidance reaction is related to a local response of plants to P stress, leading to increased branch root development close to a P source. Increasing the P supply to the roots indeed resulted in the disappearance of the Al-avoidance reaction in *Mucuna*. An experiment with the more Al-tolerant *Centrosema pubescens* demonstrated that Al avoidance is not restricted to *Mucuna* species. The Al avoidance described here has an important bearing on selection of crop cultivars for a better root development in acid subsoils. To obtain cultivars with a deep root development in acid subsoils, it is not sufficient to screen for Al tolerance of single roots or intact root systems in a homogeneous medium; it is necessary to test for Al avoidance in heterogeneous media.

INTRODUCTION

ROOT ecological studies can be classified[18] following a scale of increasing complexity of the experimental system: (1) short-term studies of a single root in a homogeneous environment (water with solutes); (2) longer-term studies of an intact root system in a homogeneous environment, and (3) studies of crops and plants with a root system growing in a heterogeneous (soil) environment (Fig. 1). Plant physiologists tend to study systems at the first and second level, plant ecologists working in the field encounter the third. The question now arises as to whether the response in the more complex third system can be understood from results obtained with the simpler ones, 1 and 2.

In most studies on Al toxicity and Al tolerance the—often implicit—assumption is made that the

§To whom all correspondence should be addressed.

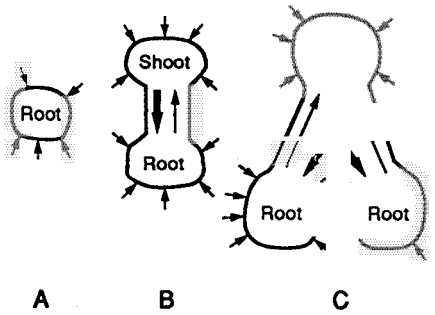

FIG. 1. Three levels of complexity in root studies:[18] (A) single root; (B) shoot/root interaction in homogeneous root environment; (C) split-root study of response to heterogeneity in the root environment.

response observed at the single root level and intact root system in a homogeneous root environment is equivalent to that in a heterogeneous soil. For the leguminous cover crop *Mucuna pruriens* var. *utilis* (velvet bean) the three types of experiment led to markedly different conclusions. Single roots in a short-term study (several hours) showed the usual reduction in elongation rate when exposed to Al^{3+} (unpublished results). Experiments with whole plants with intact root systems for 4 weeks showed[5,9] that solutions with 110 and 185 μM Al^{3+} may lead to a stimulation of root dry weight and root surface area. In split-root experiments (heterogeneous root environment) preferential root development in the control side was found[9] when a choice was offered between nutrient solutions with (+) and without (0) Al. The response of root growth to the presence of Al^{3+} in its environment was positive when no other choice was given and negative in the absence of Al^{3+} around other parts of the same root system. This relative 'Al avoidance', rather than absolute Al tolerance or toxicity, explains root response to acid subsoil conditions in the field; an acid subsoil in which roots can be formed if no alternative is given, after removal of the topsoil, was avoided in situations with choice.[7] Al-avoidance reactions in this sense may help to explain why selection of Al-tolerant genotypes based on experiments with homogeneous media may fail to be successful for field trials.

BENNET and BREEN[1] presented a schematic model of the response of root growth to the presence of Al^{3+} around the root cap, which accounts for both stimulation and inhibition of cell division and elongation depending on Al^{3+} concentration and Al^{3+}/Ca^{2+} ratio. This model, based on a homogeneous root environment, does not include feedback from other parts of the root system. It cannot explain the Al avoidance observed for *Mucuna*.

The Al-avoidance response appears to complement the response to local sources of P in P-stressed plants. Therefore, we formulated[9] the hypothesis that Al avoidance is actually based on internal P shortage in Al-exposed roots, due to precipitation of aluminium phosphates. A consequence of this hypothesis is that Al avoidance should be more pronounced under low P nutrition and absent when plants are well supplied with P, since the response to local sources of P disappears under these conditions.[3,4] The aim of the present paper is to test this hypothesis.

Only part of the Al added to nutrient solutions stays in the physiologically toxic monomeric form. Experiments with inorganic nutrient solutions have shown that phytotoxicity was best correlated[2] with the solution activity of Al^{3+}, which constitutes the main proportion of the monomeric fraction at low pH. In previous experiments with *Mucuna*[9] the pH of the solution was corrected daily, resulting in considerable fluctuations in pH and a decrease in the concentration of monomeric Al^{3+}. For the present experiments a continuous pH-stat system was used to maintain a more stable monomeric Al concentration.

To check whether Al avoidance is specific for *Mucuna*, a comparison was made with *Centrosema pubescens*, a leguminous cover crop which is known to be more Al-tolerant than *Mucuna* and which was deeply rooted on a site where *Mucuna* was shallowly rooted.[6]

MATERIAL AND METHODS

Material

Seeds of *Mucuna pruriens* (L.) DC var. *utilis* (Wall. ex Wright) Baker ex Burck and *Centrosema pubescens* Bth. were obtained from Usaha Dagang (UD) Sri Bharata, Nglegok, Blitar, East Java, Indonesia, and were germinated as previously described.[5] After about 2 weeks (*Mucuna*) or 4

weeks (*Centrosema*), uniform plants were chosen and transferred to a split-root system, in which the two halves of the root system, each in a pot of 5 l, could be exposed to circulating nutrient solutions with (+) or without (0) Al, for 4 weeks. The total content of each solution system (storage reservoir plus pots) was 250 l.

Pilot experiment with Centrosema pubescens

As we had no previous experience of the Al response of *Centrosema pubescens*, a simple experiment with four Al levels (nominal concentrations: 0, 185, 370 and 555 μM; monomeric Al 0, 133, 305, 465 μM) was carried out, with three replicates. A modified 1/4 strength Hoagland solution with a 1 : 1 ratio of NH_4^+ and NO_3^- was used with pH 4.2. The pH of the solution remained more or less constant; the solution was refreshed once a week and plants were harvested after 2 weeks.

Experimental conditions

Three experiments were carried out in a glasshouse with controlled temperature and humidity conditions at the DLO-Institute for Soil Fertility Research, Haren, The Netherlands, under natural light conditions. Day temperature was kept at 27–30°C, night temperature at 20°C and relative air humidity at about 95%, close to that under natural conditions. Experiment 1 (*Mucuna*, high P) was conducted in May–June 1991 and it was directly followed by experiment 2 (*Mucuna*, low P) in June–July 1991. Experiment 3 (*Centrosema*, low P) was carried out in July–August 1991.

Treatments

Plants with two halves of the root system exposed to the same solution, either with (+/+) or without (0/0) Al, were compared with plants where the two halves were in different solutions (0/+), in a randomized block design with six replicates. To test whether Al avoidance was related to preferential root growth at a local P supply, a third pot (1 l) containing an aerated solution of 1 mM KH_2PO_4 was used in experiments 1 and 2. Three roots from each side of the split root system were carefully led, through drinking straws (6 mm diameter), into this container.[9]

Composition of nutrient solution and pH stat

A modified 1/4 strength Hoagland solution[9] was circulated in the system. Al was added at amounts of 0.111 mM_c per day up to the specified concentration to avoid drastic fluctuations in the pH of the solution. Daily water uptake was compensated for by adding the same nutrient solution as used initially. A pH-stat system maintained the pH of the solution in the range 3.8–4.0 by continuous addition of 1 M HNO_3, based on the signal of a pH electrode. Other nutrients were regularly added depending on the observed external alkaline effect of the plants and on uptake ratios measured in a previous experiment[5] where, of a total anion uptake of 3.1 mM_c/g dry wt, 2.1 was accompanied by cations and the remaining 1.0 was accompanied by external alkalization. For the anions, the uptake ratio of N : P : S was 20 : 1 : 2; for the cations, the K : Ca : Mg uptake ratio was 3 : 4.5 : 1. Nutrients were added to the reservoir when the calculated concentration, based on these ratios and the observed external alkaline effect, fell below 80% of the initial value. Regular measurements of the pH and electrical conductivity (*EC*) of the solution were used as a check on the pH stat and the nutrition scheme. Solution samples were taken every other day for determination of the concentration of inorganic monomeric Al.[8–10] $AlCl_3$ was added to the solution to compensate for any decrease in Al^{3+}. The concentration and activity of monomeric Al species were computed by means of a corrected version of GEOCHEM.[15,17] Calculations were based on initial ion concentrations.

In experiments 2 and 3 a lower initial P concentration was used than in experiment 1, and P additions were based on 50% of the estimated plant demand.

Harvest and chemical analysis

Plants were harvested after 4 weeks in solution (6 or 8 weeks after sowing). Excised roots were rinsed three times in deionized water, blotted between two layers of tissue paper, weighed after 30 sec in a household centrifuge, and then dried in an oven at 100°C for 48 hr and weighed again. Root diameter and root length were measured[5] from subsamples stored in a freezer at −20°C. Shoot and root samples (dry) were analysed[5] for total N, P, K, Ca, Mg and Al, in duplicate for

experiments 1 and 2, and in triplicate for experiment 3. Cation contents are expressed on a tissue water basis,[11] N and P on a dry weight basis.

Statistical analysis

Results were analysed with ANOVA (analysis of variance) by using the GENSTAT 5 computer program,[16] and the Duncan Multiple Range Test, when significant ($P < 0.05$) treatment effects were found. Aluminium avoidance was tested by an ANOVA on the difference in root weight between the two sides of the root systems; the difference between the two sides in 0/0 and +/+ plants was used as background variation for that in 0/+ plants.

RESULTS

Al *response of* Centrosema

Shoot growth of *Centrosema* was not affected by nominal Al concentrations up to 555 μM (measured Al_{mono} up to 465 μM), while root weight was slightly, but not significantly, stimulated (Fig. 2). The shoot:root ratio on a fresh weight basis decreased significantly ($P < 0.05$), but on a dry weight basis the decrease was not significant. On the basis of these results a nominal Al concentration of 370 μM was chosen for the split-root experiment with *Centrosema* in the last 2 weeks of the 4-week experimental period.

Al *and* P *concentration of nutrient solution*

Calculations with GEOCHEM showed that 80–90% of the nominal Al concentration should have been in the monomeric form (Table 1); measurement of Al_{mono} showed only about 50–60% of the nominal Al concentration in freshly made solutions; the remainder may have been due to either polymeric Al formation or to Al–P precipitation. Figure 3 shows the Al_{mono} and P concentrations in the course of the three experiments. In experiment 1 (high P; Fig. 3A), the measured concentration of Al_{mono} was initially lower than expected; by addition of $AlCl_3$ in the last week of the experiment values above 100 μM were obtained. The P concentration in solution was maintained at the initial level in the 0-Al solution, except for the last week. In the +Al solution P concentration decreased more rapidly, but stayed above 20 μM, except for the last measurement. It seems likely that aluminium phosphate precipitation was the cause of the reduction of both monomeric Al and phosphate in the solution. In experiment 2 (low P; Fig. 3B), the monomeric Al concentration was kept in the range 130–150 μM, except for the last 2 days. P concentration started at half the level of experiment 1 and decreased to values below 20 μM within 10 days in the +Al solution and after 20 days in the control solution. In experiment 3 (*Centrosema*, low P; Fig. 3C), the monomeric Al concentration was doubled in the last 2 weeks; the P concentration followed the same pattern as in experiment 2.

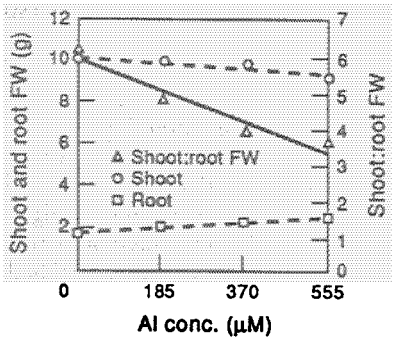

FIG. 2. Response of *Centrosema* shoot and root dry weight to four Al levels in a pilot experiment.

Shoot and root growth

Results on shoot and root dry weight for the three split-root experiments are shown in Fig. 4. In both *Mucuna* experiments a negative effect of Al^{3+} on shoot growth was evident with an intermediate value for the 0/+ treatment; absolute values cannot be compared as the two experiments were not performed at the same time. In *Centrosema* only a slight and non-significant reduction in shoot weight was found while the nominal Al concentration was twice as high. In all three experiments, exposure to Al led to a decrease in root dry weight (Fig. 4B), especially in the low P experiment with *Mucuna*. A significant effect on shoot:root ratio on a dry weight basis was only found in the high P experiment with *Mucuna*.

Table 1. Aluminium supply and speciation of monomeric aluminium calculated from the GEOCHEM program for the nutrient solutions used (pH 4.0); case I is based on the nominal Al concentration, case II on measured monomeric Al concentration (1 min PCV method[10]); all concentrations expressed as μM; ionic strength mM

	Exp. 1		Exp. 2		Exp. 3	
Total Al	185		185		370	
Total P	150		100		100	
Ionic strength	8.7		7.6		7.9	
Al-phosphate	29		20		36	
Case	I	II	I	II		II
Al^{3+}	87	51	89	70	194	28
$Al(OH)^{2+}$	5.3	3.2	5.6	4.4	12.2	8.1
$Al(OH)_2^+$	0.3	0.2	0.3	0.3	0.7	0.5
$Al(SO_4)^+$	63	41	69	57	127	93
$\Sigma Al_{monomeric}$	156	95	164	131	334	230
$\Sigma Act.\ Al_{monomeric}$	97	67	106	86	208	147

Response to split-root treatments

Figure 5 shows the response of the two halves of the split-root system. In the high P experiment with *Mucuna* no significant difference was found in dry weight of the two sides of the split-root systems, in agreement with our hypothesis that Al avoidance is related to P stress. In the low P experiment a clear Al avoidance reaction was seen in the 0/+ plants. The 0 side of 0/+ plants had a significantly higher dry weight than that of 0/0 plants, and the + side had a significantly lower value than that of +/+ plants. For *Centrosema*, a significant Al avoidance was also observed, but 0 roots of 0/+ and 0/0 plants or + roots of 0/+ and +/+ plants were not significantly different. *Centrosema* roots growing in a + Al solution were thicker and had a lower specific root length (L_{rw}) than roots growing in a control solution (Table 2).

Response to a third pot with KH_2PO_4

In an experiment[9] at the same nominal Al and P concentration as experiment 1, but with daily pH control instead of a pH stat, Al-treated plants made significantly more roots in the third pot, with only KH_2PO_4. In experiment 1 little root

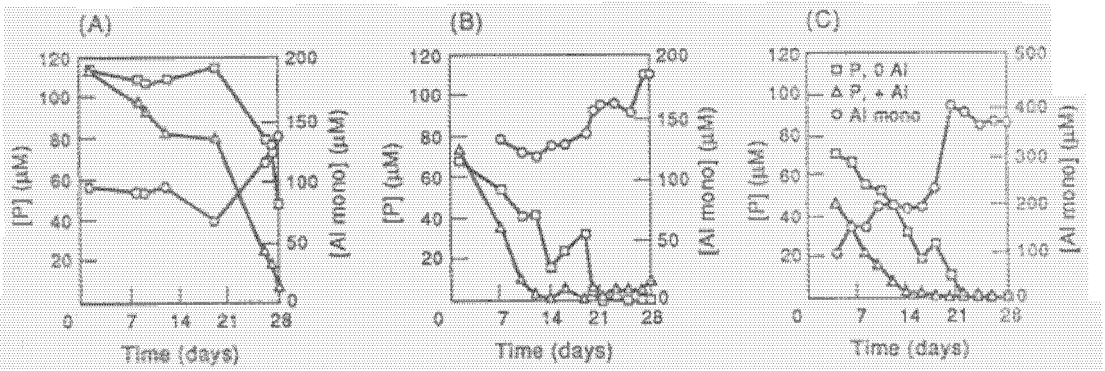

FIG. 3. Composition of nutrient solution during experiments: (A) experiment 1—*Mucuna*, high P; (B) experiment 2—*Mucuna*, low P; (C) experiment 3—*Centrosema*, low P (N.B. a different scale is used for monomeric Al).

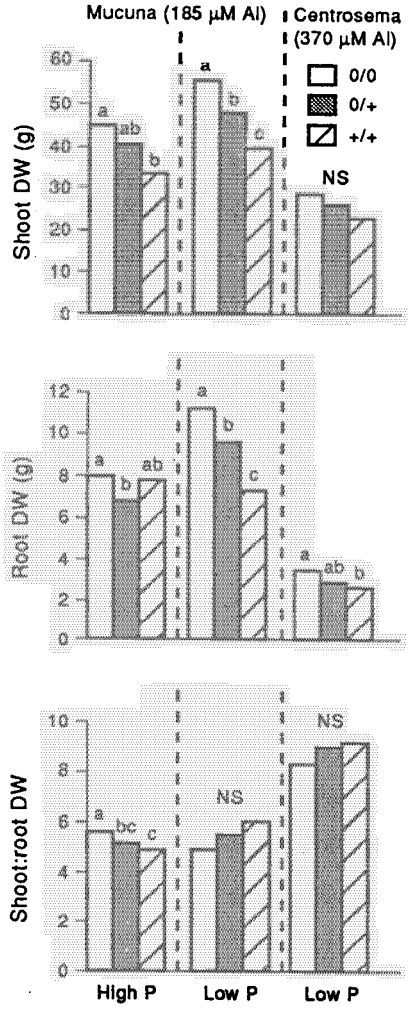

FIG. 4. Total shoot and root dry weights and shoot : root ratio in three split-root experiments (two with *Mucuna*, one with *Centrosema*) with varying P and Al levels. Values with different letters are significantly different ($P < 0.05$).

development was found in the third pot, and treatment effects were not significant (Table 3); root development was similar to that in no-Al plants in the previous experiment.[9] In experiment 2, root development was much stronger and similar to that of Al-treated plants in the previous experiment.[9]

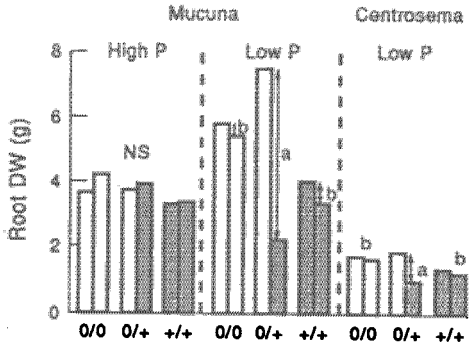

FIG. 5. Root dry weight in the two sides of a split root system in three experiments. Same Al levels and statistical analyses as shown in Fig. 4.

Chemical composition of shoot and root

Table 4 shows the chemical compositions of shoot and root in the two *Mucuna* experiments. Table 5 shows the same for the *Centrosema* experiment. The N:P ratio (Mol/Mol) for the shoot was about 50 in experiments 1 and 3 and about 80 in experiment 2. Al treatment resulted in a significant increase in root and shoot P and K^+ content in experiment 1. Ca^{2+} and Mg^{2+} concentrations in root and shoot were decreased in most cases. Root contents of Ca^{2+}, Mg^{2+}, and the sum of cations were lower in *Centrosema* than in *Mucuna*.

DISCUSSION

Al *avoidance and* P *stress*

Mucuna root development in the third pot, containing only KH_2PO_4, indicated P stress in the low P experiment, and sufficient P in the high P experiment (Table 3). These results coincide with

*Table 2. Root parameters in experiment 3 (*Centrosema*); average root diameter, specific root length (length per unit dry weight), L_{rw}, and specific root surface area (surface area per unit dry weight), A_{rw}; figures followed by different letters are significantly different* ($P < 0.05$)

Plant	0/0	0/+	0/+	+/+
Root	0	0	+	+
Diameter (mm)	0.257^b	0.256^b	0.318^a	0.334^a
L_{rw} (m/g)	366^a	362^a	318^b	204^c
A_{rw} (m^2/g)	0.30^{NS}	0.29	0.32	0.21

Table 3. Root development in a third compartment, containing 1 mM KH_2PO_4, in experiments 1 and 2 (A); DW = root dry weight; L_{rw} = specific root length; differences between Al treatments within each experiment were not statistically significant; for comparison results are given (B) of a high P experiment with daily pH control[9] performed at the same time as experiment 1

	DW (mg)	L_{rw} (m/g)	Length (m)
A. Experiment 1, High P			
0/0	65^{NS}	66^{NS}	2.6^{NS}
0/+	54	92	4.8
+/+	70	65	4.5
Experiment 2, Low P			
0/0	233^{NS}	99^{NS}	29^{NS}
0/+	236	87	27
+/+	188	81	16
B.			
0/0	35^{b}	87^{NS}	3.2^{b}
0/+	231^{a}	122	29.2^{a}
+/+	125^{ab}	105	13.7^{ab}

the Al avoidance observed: a pronounced effect in the low P experiment and no effect in the high P experiment (Fig. 5). Altogether, our present results support the hypothesis that Al avoidance is related to the response to local P sources of plants with an overall insufficient P supply. This conclusion was also supported by our data on P content of roots and shoots. In the high P experiment, P content of roots was increased in the presence of Al in the solution. In the low P experiment, P contents in both shoot and root were about 40% lower than in the high P experiment, and no increase due to Al treatment was found.

A comparison of the present results with earlier ones, with pH adjustment only once a day,[9] shows that the same nominal Al concentrations caused a more pronounced reduction of shoot growth in the pH stat with a constantly high monomeric Al concentration, rather than weekly peaks each time the solution was refreshed.

The effect of Al treatment on Ca and Mg contents of root and shoot was similar to those

Table 4. Chemical composition of Mucuna *shoot and root material; values followed by different letters are significantly different* ($P < 0.05$)

	RWW (g/g)	N_{tot} (%)	P_{tot} (μmol/g)	K^+	Ca^{2+}	Mg^{2+} (mmol$_c$/l)	Al^{3+}	ΣCat
High P, Shoot								
0/0	6.46^{NS}	4.7^{NS}	65^{b}	29^{b}	150^{a}	21^{a}	0.2^{b}	300^{b}
0/+	6.23	4.4	62^{b}	207^{a}	135^{ab}	20^{a}	1.6^{a}	364^{a}
+/+	6.28	4.6	77^{a}	181^{a}	124^{b}	12^{b}	1.8^{b}	318^{b}
High P, Root								
0/0	18.1^{NS}		71^{c}	103^{b}	7.9^{a}	12^{b}	0.9^{b}	124^{b}
0/+ 0	18.6		75^{bc}	101^{b}	7.6^{a}	11^{b}	0.8^{b}	120^{b}
0/+ +	19.3		85^{a}	107^{b}	4.9^{b}	19^{a}	21.9^{a}	153^{a}
+/+	18.1		78^{b}	121^{a}	4.2^{b}	17^{a}	20.7^{a}	163^{a}
Low P, Shoot								
0/0	5.19^{NS}	4.5^{NS}	41^{NS}	146^{b}	155^{a}	13.8^{NS}	0.9^{NS}	316^{NS}
0/+	4.87	4.4	39	158^{b}	149^{b}	13.7	1.1	323
+/+	4.94	4.2	41	205^{a}	129^{a}	15.1	2.1	351
Low P, Root								
0/0	18.3^{NS}		44^{NS}	78^{NS}	6.8^{NS}	37^{a}	6.4^{b}	128^{NS}
0/+ 0	17.6		42	79	7.2	40^{a}	6.1^{b}	133
0/+ +	18.4		43	82	6.0	25^{b}	20.8^{a}	134
+/+	18.2		44	80	6.0	28^{b}	24.3^{a}	138

RWW, Relative water weight; (fresh–dry weight)/dry weight. The presence of a third pot, with KH_2PO_4, had no significant effect on chemical composition of root or shoot.

Table 5. *Chemical composition of* Centrosema *shoot and root material*

	RWW (g/g)	N_{tot} (%)	P_{tot} (μmol/g)	K^+	Ca^{2+}	Mg^{2+} ($mmol_c/l$)	Al^{3+}	ΣCat
Shoot								
0/0	3.90^{NS}	3.3^{NS}	48^{NS}	150^{NS}	146^{a}	19^{a}	0.2^{NS}	316^{NS}
0/+	3.75	3.2	47	191	125^{b}	20^{a}	1.0	337
+/+	3.57	3.4	40	176	86^{c}	12^{b}	0.7	275
Root								
0/0	12.4^{NS}		74^{NS}	72^{NS}	4.0^{a}	12^{NS}	1.6^{b}	90^{NS}
0/+	11.9		74	75	4.0^{a}	14	1.8^{b}	95
0/+	11.2		93	66	2.5^{b}	10	27.6^{a}	106
+/+	11.4		81	59	3.1^{ab}	8.7	25.1^{a}	96

Same terminology as in Table 4.

reported before,[9] but slightly more pronounced (except for roots of experiment 1). Previously, no Al treatment effects on K content of root or shoot were found; with the pH stat K^+ concentrations of root and shoot were increased due to the Al treatment. Shoot Al contents were not increased by using the pH stat.

The use of the pH stat had no effect on phosphate nutrition of the plants at the same nominal P concentration of the solution in the absence of Al, but it improved phosphate nutrition in the presence of Al, as evident from the response to the third pot with KH_2PO_4. Both Al and P concentrations in the root were lower in the pH stat experiment than in the similar experiment with daily pH control, which showed a clear Al avoidance.[9]

In the experiments, Al × P interactions could not be avoided in the nutrient solution, as shown in Fig. 3. Apart from possible aluminium phosphate precipitation on the root surface and/or in the intracellular regions of the root tip,[12,13] the results may have been caused by complexation and/or precipitation in the nutrient solution.

Comparison of Mucuna *and* Centrosema

The expectation that *Centrosema pubescens* would be more tolerant to Al was confirmed by the results. At 370 μM Al, *Mucuna* virtually failed in a previous experiment;[5] *Centrosema* showed a comparatively small negative effect on both shoot and root growth at this concentration. Still, a statistically significant Al-avoidance reaction was recorded, though less pronounced than for *Mucuna* in the low P experiment. At the same P concentration, *Centrosema* had a higher P concentration in the roots than *Mucuna* but a comparable concentration of shoot P. The higher Al tolerance of *Centrosema* might be related to a more efficient P uptake than found in *Mucuna*. In a field experiment in Lampung (S. Sumatera, Indonesia), *Centrosema* was deep rooted,[6] while *Mucuna* can grow in the subsoil when no choice is offered, but is shallowly rooted if a more favourable topsoil is present.[7] The results presented in this paper indicate that the Al content of this subsoil is not yet critical for *Centrosema*. No essential difference in Al avoidance, at higher Al concentrations, exists between the two species. It therefore follows that Al avoidance is not restricted to *Mucuna* and may possibly be encountered in other species as well.

CONCLUDING REMARKS

Practical relevance of the results presented here may be found in improving selection procedures for Al-tolerant genotypes, which are now based on plant performance in homogeneous root environments; Al-avoidance reactions may lead to disappointing performance in soil profiles with a topsoil which is less toxic, as found for *Mucuna* here.

If Al avoidance is indeed related to preferential root growth in P-rich zones by plants short in P, increased P supply in the topsoil might improve

root development in acid subsoils, even when the P fertilizer does not reach the subsoil. Such an effect has been described[14] for two Al-susceptible wheat cultivars in a split-root experiment using an acid subsoil. This effect, at the third level of complexity (compare Fig. 1), gives a new interpretation to the old statement "P stimulates root growth", which is not true at the single root level or in a homogeneous medium.

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