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Response of representative cover crops to aluminum toxicity, phosphorus deprivation, and organic amendment

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Abstract. This study aimed to: (1) determine the effect of P depletion and presence of Al on root and shoot growth of representative cover crops, and on their nutrient uptake; (2) characterise the composition of root exudation under P and Al stress in nutrient solution; (3) evaluate the ability of aqueous extracts of composts in reducing Al phytotoxicity. Plants of cowpea (*Vigna unguiculata* subsp. *unguiculata*), black oat (*Avena strigosa*), and lablab (*Lablab purpureous*) were cultivated in different nutrient solution compositions and concentrations for 3 weeks. It was found that Al at concentration of 20 and 200 μmol/L increased citrate exudation at least 8 and 24 times, respectively, for cowpea and 18 and 36 times, respectively, for lablab, as compared with the blank. However, no release of organic acids occurred due to P deprivation, suggesting that citrate exudation was a specific response to excess Al. No response in organic acid release was observed for black oat under the stress of P deficiency or Al toxicity. Although the presence of Al in solution did not significantly affect chlorophyll content in leaves, it decreased root and shoot weight, as well as root length, surface area, volume, and number of tips. Organic extracts alleviated aluminum toxicity, improving plant growth and ameliorating plant nutrition status. Yard waste extract was more effective in enhancing plant growth than GreenEdge extract in plants under Al stress.

Additional keywords: complexation, nutrient solution, organic acid exudation.

Introduction

Aluminum (Al) toxicity to plants is a major problem in some acidic soils. The presence of organic substances often alleviates Al phytotoxicity, particularly low molecular weight organic acids (LMWOA) such as citric, malic, and oxalic acid, among others (Hue *et al*. 1986; Ma 2000; Jones *et al*. 2003) and humic acids (Gerke 1994; Elkins and Nelson 2002). These organic acids can form stable complexes with Al, thereby reducing its activity in acidic soils. Management techniques that focus on increasing organic matter content in soil, such as adoption of crop systems with high carbon input, use of cover crops, amendment with composts, and use of conservative tillage systems are promising strategies for alleviating Al toxicity without using a large amount of lime (Salet *et al*. 1999; Sumner and Yamada 2002; Muhrizal *et al*. 2003).

Low molecular weight organic acids originate from several sources, including microbial activity, root exudation, and decomposition of plant residues (Jones 1998; Jones *et al*. 2003). The mechanism of Al tolerance in some plants is often associated with LMWOA exudation from the roots under Al stress conditions ((Ma 2000; Silva *et al*. 2001; Barcelò and Poschenrieder 2002; Kochian *et al*. 2002, 2005; Li *et al*. 2002). Similarly, some plant species can enhance LMWOA exudation under P deficiency stresses and, in some cases, Al-induced LMWOA exudation has been shown to be related to P nutritional status in the plant (Gaume *et al*. 2001; Dong *et al*. 2004; Ligaba *et al*. 2004; Liao *et al*. 2006; Jemo *et al*. 2007). In general, these studies are restricted to crops of commercial interest. The few studies with cultures of cover crops are mainly on LMWOA from extracts of shoot tissue (Franchini *et al*. 1999, 2001; Amaral *et al*. 2004), and information regarding root exudation from cover crops is very limited. The adoption of cover crops in intercrop and crop rotation systems improves both soil organic carbon stocks and lability (Bayer *et al*. 2000, 2001; Diekow *et al*. 2005) and constitutes a great strategy for improving soil quality. Cover crops help increase the agricultural sustainability and profitability (mainly due to reduced N-fertiliser need) and can improve the environment in general, among other benefits. Cover crop species that has the characteristic of Al tolerance through LMWOA exudation by roots have the advantage of being able to grow normally in soils under adverse conditions of acidity and have a great potential for the exploitation of acid subsoil layers. Consequently, the formation of vertical and continuous biopores surrounded by organic matter after senescence of the

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roots of cover crops facilitates the development of the root system of more Al sensitive crops of commercial interests cultivated subsequently (McCallum *et al.* 2004), gradually increasing the levels of organic matter and the Al complexation at these sites.

Because of the high content of organic carbon and relatively easy decomposition, the input of composts to soil represents an interesting strategy for alleviating Al toxicity (Baziramakenga and Simard 1998; Haynes and Mokolobate 2001). Compost production is increasing worldwide and provides an alternative and economical utilisation of the waste products (Stoffella *et al.* 1997; Goldstein 2001). Although studies have demonstrated benefits of compost use in agriculture, the mechanisms by which organic matter alleviates Al toxicity to plants is relatively unknown.

The objectives of this study were to: (1) determine the effect of P depletion and presence of Al on root and shoot growth of representative cover crops, and on their nutrient uptake; (2) characterise the composition of root exudation under P and Al stress in nutrient solution; and (3) evaluate the ability of aqueous extracts of composts in reducing Al phytotoxicity.

Material and methods

Experimental study and design

Three cover crops were used in this study: cowpea (*Vigna unguiculata* subsp. *unguiculata*) var. Brown Crowder, black oat (*Avena strigosa*) var. UPFA 21, and lablab (*Lablab purpureous*), obtained, respectively, from Bay Farm Services (Bay City, MI, USA), Dr Ronald Barnett (Quincy, Fl, USA, acquired from EMBRAPA, Brazil), and Adams-Briscoe Seed Co. (Jackson, GA, USA). These species are used worldwide not only as cover crops, but also for livestock feed and grazing, and human diet.

Seeds were surface sterilised by soaking in sodium hypochlorite 1% (v/v) solution for 5 min, and rinsed three times with deionised water. Seeds were germinated in paper towels in the dark at 25°C. After 4 days, five uniform seedlings (~10 cm length) of each respective crop were suspended through five holes cut into the lid of a 600 mL plastic container. Plants were held in place using sponges. Containers were placed in a controlled environmental chamber with temperature of 25°C and a 16-h photoperiod. Photon flux density at canopy level was $450 \pm 50\,\mu mol/m^2.s$, generated using a combination of fluorescent and incandescent lamps.

Each pot received 500 mL of nutrient solution at pH 4.0, with the following composition (mmol/L): 0.7 K_2SO_4, 0.1 KCl, 2.0 $Ca(NO_3)_2$, 0.5 $MgSO_4$, 0.1 KH_2PO_4, and (in µmol/L): 1.0 H_3BO_3, 0.5 $MnSO_4$, 1.0 $ZnSO_4$, 0.2 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$, and 0.02 $FeCl_3$. This composition is referred to in the present study as the standard nutrient solution (SNS). The solution in the containers was constantly aerated by air pumps and replaced every 2 days, when the containers were randomly rearranged underneath the lights. Solution pH was monitored and adjusted to 4.0 when necessary in such a way that pH never exceeded 5.0. Measurements of pH were conducted using an Orion pH-conductivity meter (Oxford, GA, USA) and 0.2 or 1.0 mol/L HCl solution was used for adjustment. During the experiment, water lost from evapotranspiration was replaced with nanopure water.

All plants were grown in the SNS for 7 days. Following the initial 7 days, the SNS was replaced according to the following treatments, each with three replicates: (1) blank (SNS); (2) SNS minus phosphorus (SNS –P); (3) SNS –P + 20 µmol Al/L; (4) SNS –P + 200 µmol Al/L; (5) SNS –P + 200 µmol Al/L + extract of yard waste (Y); (6) SNS –P + 200 µmol Al/L + extract of GreenEdge (G). Aluminum was added to the solutions as $AlCl_3$ and, according to solution speciation results obtained using the Visual Minteq software program (v.2.40) (Stockholm, Sweden), Al^{3+} activity in treatments (3) and (4) were 2.60 and 27.7 µmol/L, respectively. Yard waste was obtained from West Palm Beach composting facilities (FL, USA). GreenEdge (Gainesville, FL, USA) is a pelleted biosolid produced from sewage sludge and enriched with fertilisers. The extracts were obtained by equilibrating the organic material in water for 12 h at water : solid ratio of 10 : 1 (w/w) and filtering the suspension through Whatman 42 filter paper (Maidstone, UK). Each extract was acidified to pH 4.0 using HCl 1.0 mol/L and was added in a predetermined quantity corresponding to a final DOC (dissolved organic carbon) : Al ratio of 45.

All the containers were arranged in a completely randomised design, with a total of 15 plants per treatment (three containers of five plants each constituted the three replicates per treatment). The experiment was conducted with the three crops being grown simultaneously.

Analysis

After 7, 14, and 21 days of growth in each respective nutrient solution, leaf greenness of each plant was measured using a Spad-502 leaf greenness Meter (Minolta Co. Ltd, Japan). This measurement is a reflection of the chlorophyll content. The exudation of low molecular weight organic acids (LMWOA) from roots was also determined at each interval. Leaf greenness was measured for all the treatments of cowpea and lablab, but the leaves of black oat were too narrow to perform the measurements. LMWOA exudation was analysed for the treatments 1 to 4 (blank, SNS –P, SNS –P + 20 µmol Al/L, and SNS –P + 200 µmol Al/L). Solution samples for LMWOA exudation analysis were collected 24 h after the nutrient solution was replaced, and determined immediately using a high pressure liquid chromatograph (HPLC Waters 2695, Milford, MA, USA) equipped with two Waters C18 columns (25 cm length) (Milford, MA, USA) in series and a Waters 996 Photodiode Array Detector (Milford, MA, USA), under the following chromatographic conditions: phosphoric acid 0.2% (v/v) at pH 2.3 as mobile phase; column temperature of 30°C; diode array detector quantification in $\lambda = 210$ nm, with identification wavelength scans from 200 to 300 nm; injection volume of 50 µL, and flow rate of 0.6 mL/min. The evaluated LMWOA included oxalate, malate, tartarate, acetate, citrate, fumarate, succinate, and propionate. However, only citrate and malate were exuded at concentrations high enough for quantitative analysis, and therefore only the results of these two acids were presented. Detection limits for malate and citrate were 0.5 and 1.15 µmol/L, respectively. Organic acids were identified by comparing retention times of unknowns with those of organic acid standards. LMWOA were quantified by comparing peak areas with an external calibration curve.

After 21 days of cultivation, fresh and dry (70°C for 3 days) weights of root and shoot were determined and root length, specific area, and volume of the roots were analysed using a WinRhizo root scanner (Epson Transparency Unit Model EU-22) and its software (WinRhizo Pro v, Quebec, Canada). The contents of Al, P, K, Ca, and Mg in roots and shoots were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, Ultima, JY Horiba, Edison, NJ, USA) after the samples were digested with nitric acid.

Analysis of dissolved nutrients in the organic extracts after filtration (Whatman 42 + 0.45 μm acetate filter) was performed by the ICP-AES. The results indicate that the extract of yard waste added to the SNS contributed to an increase in nutrient solution concentration in, respectively, 0.05, 0.29, 0.02, and 0.04 mmol/L of Ca, Mg, P, and K. Extract of GreenEdge increased the concentration in, respectively, 0.06, 0.05, 0.03, and 0.03 mmol/L of Ca, Mg, P, and K. During the period of the experiment, precipitates were not observed in any of the compost treatments.

Statistical analysis

Results were statistically analysed by either one-way or two-way analysis of variance (ANOVA) with three replicates. Treatment means were compared using Duncan's Multiple Range Test ($P < 0.05$) in SAS version 8.02 (Statistical Analysis System Institute, Cary, NC). ANOVA for organic acid exudation data was not performed due to the high number of samples below the detection limit.

Results

Organic acid exudation

The presence of Al in nutrient solution enhanced citrate exudation from the roots of the leguminous crops (Table 1). Aluminum at 20 and 200 μmol/L increased citrate exudation at least 8 and 24 times, respectively, for cowpea and 18 and 36 times, respectively, for lablab, as compared with the blank (SNS). No response in organic acid release was observed for black oat under the stress of P deficiency or Al toxicity. For malate release, most of the samples did not have concentrations high enough for detecting by the HPLC. However, most of the samples that had concentrations above the limit of detection were from P supplied plants without Al, suggesting that P deprivation did not enhance malate exudation of the tested plants.

Shoot greenness and shoot and root biomass

Cowpea and lablab leaves had no significant difference in the shoot greenness content during the analysed period (Table 2). Aluminum toxicity symptoms generally manifest first in the root system. Shoot symptoms occurs only when the effect become severe, showing symptoms similar to nutrient deficiency (Foy 1984). Shoots likely would have been significantly different if the experiment continued. As cited above, leaves of black oat were too narrow to allow the chlorophyll meter readings.

Although no significant effect on leaf greenness was detected, shoot biomass (Fig. 1*b* and *d*) decreased due to the presence of Al in solution, showing a tendency similar to the root biomass (Fig. 1*a* and *c*). The absence of P in nutrient solution decreased fresh and dry weights of the three crops, with the most drastic drop being noted for black oat. Gramineous plant species such as oats have less P reserve in seeds than leguminous plants like cowpea and lablab. The absence of P promoted greater decrease in shoot than in root weights. The presence of 200 μmol/L Al without organic extracts led to the smallest fresh and dry weights for the three crops, and biomass yield was decreased by 20, 38, and 25%, respectively, for cowpea, black oat, and lablab, as compared to SNS –P. In contrast, the presence of 20 μmol/L Al had little or no injurious effect on the plants, but was enough to trigger citric acid exudation in the leguminous crops.

Table 1. Exudation of organic acids from roots of three cover crops under P deficiency and Al excess in nutrient solution

Sample collection was performed 24 h after nutrient solution replacement. SNS, Standard nutrient solution; bdl, below detection limit; –P, no phosphorus

Treatment	Citric acid (μmol/L) Days after Al addition in solution			Malic acid (μmol/L) Days after Al addition in solution		
	1	8	15	1	8	15
Cowpea						
1. SNS	1.92 ± 1.37^{A}	bdl	0.53 ± 0.53	bdl	bdl	8.84 ± 3.02
2. SNS –P	0.18 ± 0.18	bdl	bdl	bdl	bdl	bdl
3. SNS –P + 20 μmol/L Al	2.93 ± 0.24	11.60 ± 3.75	1.12 ± 0.23	bdl	bdl	bdl
4. SNS –P + 200 μmol/L Al	25.84 ± 1.55	29.05 ± 18.97	1.48 ± 0.11	bdl	bdl	bdl
Black oat						
1. SNS	1.09 ± 0.69	bdl	bdl	bdl	bdl	bdl
2. SNS –P	0.63 ± 0.16	bdl	bdl	bdl	bdl	bdl
3. SNS –P + 20 μmol/L Al	bdl	bdl	bdl	bdl	bdl	bdl
4. SNS –P + 200 μmol/L Al	1.92 ± 0.46	1.00 ± 1.00	bdl	bdl	bdl	bdl
Lablab						
1. SNS	0.38 ± 0.06	bdl	bdl	52.60 ± 44.09	2539 ± 1696	533 ± 317
2. SNS –P	0.46 ± 0.18	2.62 ± 1.40	bdl	0.90 ± 0.90	bdl	bdl
3. SNS –P + 20 μmol/L Al	1.13 ± 0.26	24.94 ± 6.36	2.36 ± 0.79	76.82 ± 70.05	bdl	bdl
4. SNS –P + 200 μmol/L Al	10.12 ± 1.06	36.00 ± 8.32	5.51 ± 1.40	bdl	bdl	bdl

^{A}Means ± s.e. ($n = 3$).

Table 2. Chlorophyll meter readings (Spad-502) of cowpea and lablab grown in nutrient solution under P-deficiency, Al excess, and compost extract addition

SNS, Standard nutrient solution; –P, no phosphorus; yard, extract from yard waste; GreenEdge, extract from GreenEdge; n.s., not significant with Duncan's multiple range test ($P < 0.05$)

Treatment	Days after add Al in solution 0	7	14
		Cowpea	
1. SNS	38.1 n.s.	39.6 n.s.	43.9 n.s.
2. SNS –P	37.8	38.3	42.3
3. SNS –P + 20 µmol/L Al	41.6	40.7	42.4
4. SNS –P + 200 µmol/L Al	40.2	34.6	38.3
5. SNS –P + 200 µmol/L Al + yard waste	41.0	38.8	40.9
6. SNS –P + 200 µmol/L Al + GreenEdge	40.3	42.0	43.0
		Lablab	
1. SNS	41.8 n.s.	39.3 n.s.	36.4 n.s.
2. SNS –P	40.0	39.9	39.4
3. SNS –P + 20 µmol/L Al	42.7	39.1	36.9
4. SNS –P + 200 µmol/L Al	41.2	41.6	37.6
5. SNS –P + 200 µmol/L Al + yard waste	42.7	39.7	36.2
6. SNS –P + 200 µmol/L Al + GreenEdge	42.5	41.7	39.7

Amendment with organic extracts promoted an increase in root and shoot weights compared to the biomass of plants under treatment 4 (SNS –P + 200 µmol/L). Application of yard waste extract not only alleviated Al toxicity but also increased plant biomass yields to values higher than those from the treatment of SNS –P, having in some cases values similar to or higher than plants under SNS, and this is likely related to the input of extra nutrients from the aqueous extract of yard waste. Extract of GreenEdge was less efficient than that of yard waste, and had a greater effect in alleviating Al toxicity in cowpea than in lablab, and negatively affecting the biomass yield of black oat.

Root scanner results

Aluminum toxicity manifested in root characteristics from root scanner in the three tested species (Fig. 2). Roots of lablab were more sensitive to the presence of 20 µmol/L Al (2.6 µmol/L Al^{3+} activity) than those of cowpea and black oat, as root length decreased by 16, 2, and 3%, respectively. However, the presence of 200 µmol/L Al promoted strong injurious effect on all root measurements in the three species.

Organic extracts alleviated Al toxicity, as their presence resulted in increased length, surface area, volume, and number of tips for both cowpea and lablab (Fig. 2). Similar to plant biomass weights, GreenEdge extract showed less alleviation than yard waste for the leguminous crops, promoting a negative effect on the root growth of black oat.

Aluminum and nutrient content in root and shoot tissue

In general, the concentrations of Al and nutrients in root were more affected by the treatments than those in shoot tissue (Tables 3 and 4). The presence of 20 µmol/L Al in solution increased Al concentration in the root tissue of the three cover crops, but had no effect on shoot Al concentration. Addition of 200 µmol/L Al to the nutrient solution increased Al concentration in root tissue by ~7, 2, and 5 times for cowpea, black oat, and lablab, respectively, relative to the presence of 20 µmol/L Al. Organic extracts were not effective in reducing root Al concentration of black oat. For cowpea and lablab, however, extracts of yard waste decreased their root Al concentrations by 50 and 28%, respectively, and the corresponding values for GreenEdge were 62 and 54%.

Phosphorus supply from SNS improved P content in black oat and lablab roots, but not in the roots of cowpea. The presence of 200 µmol/L Al in solution in treatment 4 (SNS –P + 200 µmol/L Al) increased P concentration in roots of three cover crops, probably due to the smaller root growth and, consequently, smaller P dilution than in plants grown in the absence of Al (treatment 2). Organic extracts improved P content in root tissue of the three plant species. No clear effect of P deprivation and Al stress was found on K and Ca contents in roots, although K content in cowpea root was more concentrated in treatments with smaller root growth. The content of Mg, however, decreased in the roots of black oat and lablab exposed to Al, indicating a possible inhibition in Mg absorption due to Al presence.

Aluminum concentration was significantly lower in shoot tissues than in the root, showing the low translocation of this element in the plant. The presence of Al in solution affected Al concentration in the shoot of cowpea, but had a small or no effect on black oat and lablab shoot. Aluminum supply in solution seemed to have no effect on the concentrations of P, K, Ca, and Mg in shoots for the three species, with the exception of Mg in the shoot of black oat. Although the concentrations were not significantly affected by Al treatment, the total nutrient uptake by plants decreased with the stress of Al, as did the root and shoot weights. Phosphorus concentration in shoots of all crops in the present study was significantly decreased by P deprivation. However, P deprivation did not decrease root P concentration of cowpea.

Similar to the results from root tissue, organic extracts improved nutrient status in shoots, highlighting the effect of

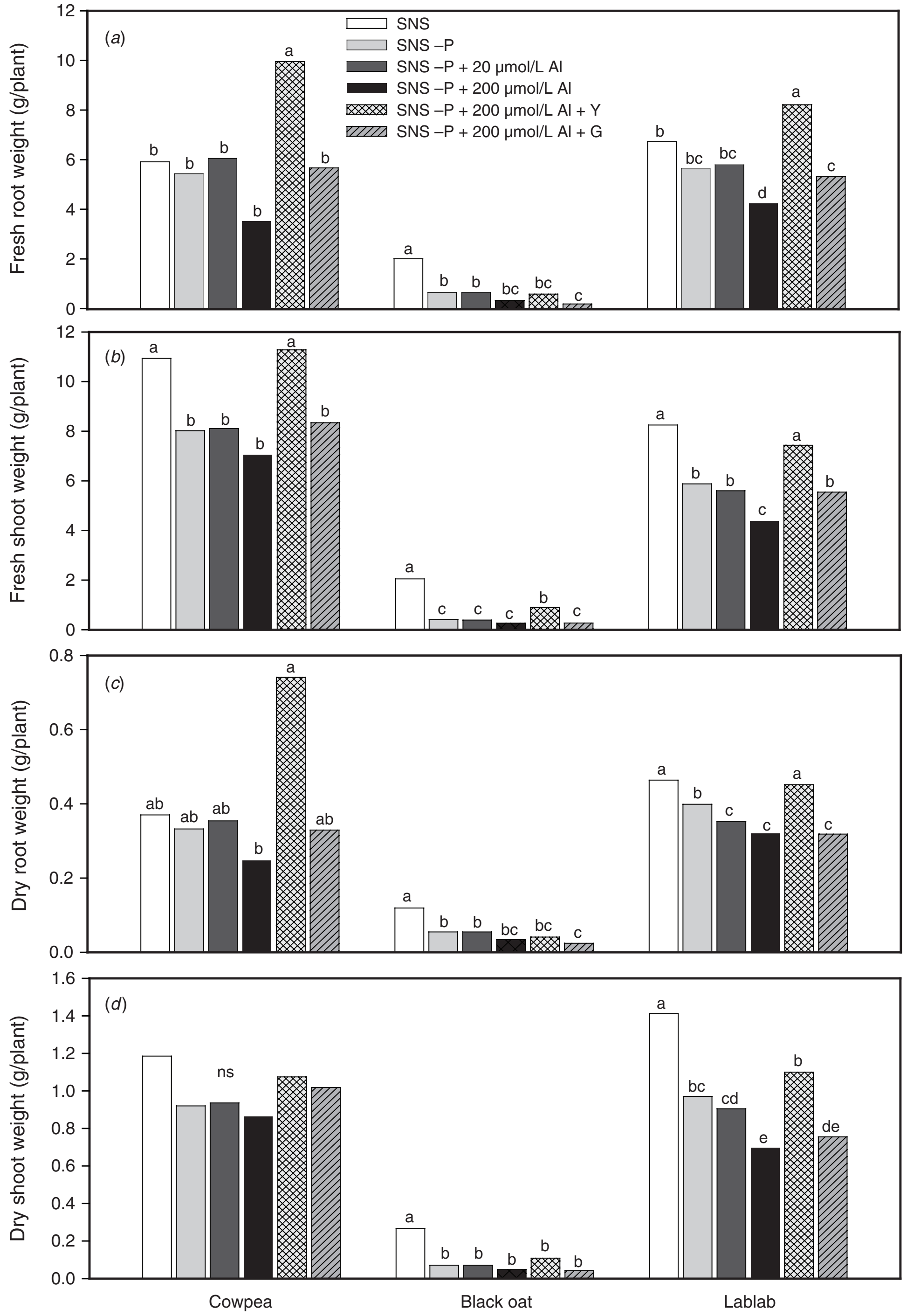

Fig. 1. Biomass weights of cover crops grown in nutrient solution under different treatments. SNS, Standard nutrient solution; –P, no phosphorus; Y, extract from yard waste; G, extract from GreenEdge. Means followed by the same letter, within crop, do not differ with Duncan's multiple range test ($P < 0.05$). n.s., Not significant.

yard waste extract on plant P and K uptake. Between the two organic extracts, yard waste promoted higher Al alleviation than GreenEdge and, interestingly, extract of yard waste also promoted better nutrition level in the plants than GreenEdge (Tables 3 and 4), although the quantity of soluble nutrients added to the nutrient solution by the two organic extracts were similar (except for Mg, that was higher in yard waste than GreenEdge extract).

Discussion

The present study demonstrated the presence of Al tolerance mechanism in plants of cowpea and lablab, which enhanced citric

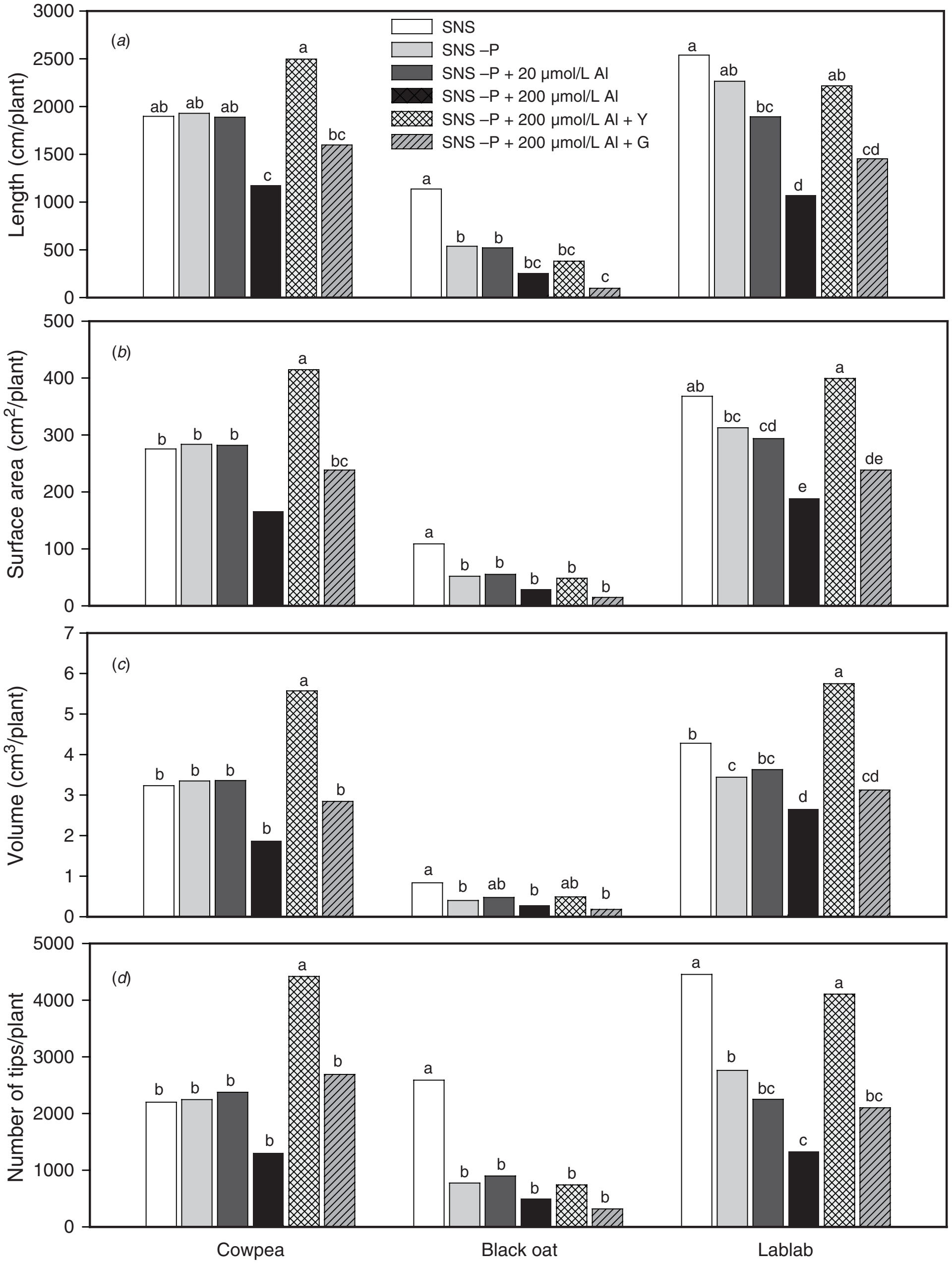

Fig. 2. Root measurements from cover crops grown in nutrient solution under different treatments, obtained from root scanner and analysed by WinRhizo. SNS, Standard nutrient solution; –P, no phosphorus; Y, extract from yard waste; G, extract from GreenEdge. Means followed by the same letter, within crop, do not differ with Duncan's multiple range test ($P < 0.05$).

acid exudation when Al was present in toxic levels in solution (Table 1). The found exudation by roots of the leguminous species seems to be a mechanism of response specific for Al and, to our knowledge, rare studies have demonstrated this mechanism by the used plant species. This is an important finding, as it extends the benefits of cowpea and lablab adoption in a crop rotation system aiming to improve soil quality and decrease production costs in acid soils, indicating great potential for ameliorating Al toxicity in acid subsoils due to the formation of biopores and the addition of organic material in deep layers (McCallum *et al.* 2004).

Jemo *et al.* (2007) evaluated the effects of stress by Al and P on root exudation by cowpea roots. Contrary to the present study, where only citrate exudation was increased in the roots of cowpea, these authors reported an increase in both citrate and malate release due to Al supply in cowpea roots. This discrepancy may be associated with the difference in Al tolerance among the tested varieties of cowpea. They also reported that

Table 3. Aluminum and nutrient concentrations (± s.d.) in root of cover crops grown in nutrient solution treated with P deficiency, Al excess, and compost extracts

SNS, Standard nutrient solution; –P, no phosphorus; yard, extract from yard waste; GreenEdge, extract from GreenEdge; bdl, below detection level. Within columns, means followed by the same letter, within crop, do not differ with Duncan's multiple range test ($P < 0.05$)

Treatment	Al	P	K	Ca	Mg
	(mg/kg)	(g/kg)			
			Cowpea		
1. SNS	bdl	1.004d	16.468d	1.620b	4.108ab
2. SNS –P	bdl	1.120cd	31.156c	2.365a	5.210a
3. SNS –P + 20 μmol/L Al	450.2 ± 107.8	1.097cd	33.464bc	2.466a	5.235a
4. SNS –P + 200 μmol/L Al	3307.0 ± 2180.5	1.428bc	40.592b	1.778ab	1.276bc
5. SNS –P + 200 μmol/L Al + yard waste	1651.1 ± 306.1	1.996b	52.139a	1.856ab	3.371abc
6. SNS –P + 200 μmol/L Al + GreenEdge	1255.2 ± 286.4	1.543a	31.702c	1.517b	0.869c
			Black oat		
1. SNS	bdl	3.470b	34.186b	1.441c	3.602a
2. SNS –P	bdl	1.269c	38.412ab	2.296b	1.194bc
3. SNS –P + 20 μmol/L Al	373.3 ± 100.5	1.249c	41.799ab	2.233b	0.678c
4. SNS –P + 200 μmol/L Al	827.3 ± 37.6	1.888c	37.367ab	2.587b	0.557c
5. SNS –P + 200 μmol/L Al + yard waste	3996.6 ± 695.6	6.560a	45.923a	4.748a	1.880b
6. SNS –P + 200 μmol/L Al + GreenEdge	2908.5 ± 1186.3	3.767b	22.041c	4.506a	0.948c
			Lablab		
1. SNS	19.1 ± 2.9	2.756a	39.864b	7.017ab	3.304a
2. SNS –P	bdl	1.525b	41.404ab	7.412a	1.680b
3. SNS –P + 20 μmol/L Al	636.0 ± 227.7	1.698b	41.547ab	5.462bc	2.075b
4. SNS –P + 200 μmol/L Al	3140.4 ± 709.9	1.964b	34.618b	4.650c	1.277b
5. SNS –P + 200 μmol/L Al + yard waste	2254.2 ± 67.4	2.988a	48.611a	5.393bc	1.848b
6. SNS –P + 200 μmol/L Al + GreenEdge	1434.2 ± 278.0	2.533a	37.445b	4.828c	1.394b

Table 4. Aluminum and nutrient concentrations (± s.d.) in shoot of cover crops grown in nutrient solution treated with P deficiency, Al excess, and compost extracts

SNS, Standard nutrient solution; –P, no phosphorus; yard, extract from yard waste; GreenEdge, extract from GreenEdge; bdl, below detection level. Within columns, means followed by the same letter, within crop, do not differ with Duncan's multiple range test ($P < 0.05$)

Treatment	Al	P	K	Ca	Mg
	(mg/kg)	(g/kg)			
			Cowpea		
1. SNS	bdl	1.899a	27.813 n.s.	17.760a	2.410 n.s.
2. SNS –P	bdl	1.132b	31.726	18.719a	2.773
3. SNS –P + 20 μmol/L Al	bdl	1.000b	27.768	14.176b	2.499
4. SNS –P + 200 μmol/L Al	20.2 ± 6.0	1.037b	31.140	12.056b	2.398
5. SNS –P + 200 μmol/L Al + yard waste	13.3 ± 13.7	1.635a	35.931	13.252b	2.419
6. SNS –P + 200 μmol/L Al + GreenEdge	31.7 ± 2.2	0.916b	29.145	12.446b	2.781
			Black oat		
1. SNS	bdl	4.420b	47.135c	9.377a	2.075a
2. SNS –P	bdl	1.184c	50.311bc	7.176bc	2.005a
3. SNS –P + 20 μmol/L Al	bdl	1.077c	55.421b	5.623cd	1.369b
4. SNS –P + 200 μmol/L Al	bdl	1.268c	55.786b	4.773d	0.909b
5. SNS –P + 200 μmol/L Al + yard waste	bdl	6.814a	64.249a	4.841d	1.345b
6. SNS –P + 200 μmol/L Al + GreenEdge	339.8 ± 158.1	2.018c	43.479c	9.139ab	2.215a
			Lablab		
1. SNS	107.0a	1.892a	21.991d	13.844c	1.765b
2. SNS –P	20.3c	1.300cd	24.638d	21.144a	2.280a
3. SNS –P + 20 μmol/L Al	15.5cd	1.119d	28.666c	20.724a	2.441a
4. SNS –P + 200 μmol/L Al	37.1b	1.317cd	30.084bc	15.558bc	1.956b
5. SNS –P + 200 μmol/L Al + yard waste	10.2d	1.670ab	35.007a	15.179bc	1.853b
6. SNS –P + 200 μmol/L Al + GreenEdge	41.9b	1.546bc	33.131ab	16.904b	2.415a

citrate exudation was primarily enhanced by P deprivation, suggesting that Al treatment further enhanced citrate exudation in P-sufficient, but not in P-deficient plants. In the present study, however, there was no clear evidence of response to exudates from leguminous crops due to P deficiency, although there was a tendency that P deprivation decreased citrate release in cowpea but increased it in lablab. For some plants that have Al-induced exudation of organic acids, P can increase the biosynthesis and reduce metabolism of organic acids, thus enhancing and prolonging their exudation, with a subsequent increase in Al tolerance (Ligaba *et al.* 2004). It is possible, therefore, that the decrease in citrate exudation by cowpea and lablab at the 15th day of Al exposure as compared with the other days of evaluation (1st and 8th), in the present study, was due to damages in the plasma membrane of root cells, which could be alleviated by P supply (Zheng *et al.* 1998*a*; Ligaba *et al.* 2004). These results must be confirmed under soil conditions, since some interactions are overlooked in hydroponic studies, particularly under different P status, although it has been well documented that hydroponic experiments could predict results in soil (Delhaize *et al.* 2004). Besides, the effectiveness of this Al-tolerance mechanism must be tested in experiments with plants cultivated in soil, in order to avoid the dilution effect in hydroponic solution.

In most of the Al-tolerant plant species that release organic acids for Al detoxification in the rhizosphere, exudation occurs mainly from the region of the root apex and nearby (Pellet *et al.* 1995; Ryan *et al.* 1995; Zheng *et al.* 1998*b*; Kochian *et al.* 2005; Jemo *et al.* 2007), although in some species, organic acid release is not tightly spatially regulated and may be not confined to the root apex (Piñeros *et al.* 2002). This has not been confirmed for lablab. It has been predicted that a step gradient of di- and tricarboxilic acids exists in the rhizosphere with an effective sphere of influence in the rhizoplane of between 0.2 and 1.0 mm, depending on soil type, organic acid, and time (Darrah 1991; Jones *et al.* 1996). Considering a radius of 0.5 mm and assuming that the exudation mostly occurs in the last 1 cm of the root tip, with an average diameter of 0.1 mm in this region, in 24 h of exudation, the hypothesised rhizosphere around the tips of cowpea and lablab submitted to 200 μmol/L Al would have a concentration of 720 and 660 μmol/L citrate, respectively, irrespective of elongation and decomposition during this period. In this case, citric acid would not diffuse and dilute as in hydroponic cultivation, being much more effective in forming complexes and alleviating root Al toxicity in soil. It is worth mentioning that the experiment was conducted in non-sterile conditions, and therefore, it is possible that an underestimation of organic acid concentrations may have occurred in this study due to microbial decomposition.

Although the presence of 20 μmol/L Al had little or no injurious effect on the shoot and root biomass of the plants (Fig. 1), it was enough to trigger citric acid exudation in both leguminous crops. The results of root morphological analysis (Fig. 2) revealed that the roots of cowpea were less affected than those of lablab by the presence of 20 μmol/L Al in solution. Besides the non-response to 20 μmol/L Al, cowpea roots were not affected by the absence of P, suggesting that P nutrition status was still being satisfactorily provided from the seed reservoir. This better P status may have improved cowpea tolerance to Al, as once in the root tissue, Al can precipitate P in the apparent free spaces, in addition to Al interfering with several P-dependant physiological mechanisms (Foy 1984; Ligaba *et al.* 2004). This hypothesis is in agreement with the fact that P deprivation did not diminish P concentration in cowpea roots (Table 3), oppositely to the concentration in the other species.

Conceptually, the threshold value used in the literature is the level of Al activity (usually Al^{3+} or the sum of monomeric Al) corresponding to a decrease in root growth equal to 10%. Based on this concept, the threshold for lablab is less than 2.6 μmol/L Al^{3+} activity, as this value of activity decreased root length by 16%. It suggests that this crop is sensitive to Al toxicity, compared to threshold values of some other crops found in literature as corn (*Zea mays*), soybean (*Glycine max*), sunflower (*Helianthus annuus*), coffee (*Coffea arabica*), alfalfa (*Medicago sativa*), and subterranean clover (*Trifolium subterraneum*) seedlings, that ranged from ~4 to 22 μmol/L (Brenes and Pearson 1973; Pavan *et al.* 1982; Alva *et al.* 1986; Comin *et al.* 1999). However, as this crop had the mechanism of citric acid exudation induced by the presence of Al in both levels in nutrient solution, when cultivated in soil, higher threshold value of Al is expected for this species due to accumulation of exuded citric acid in the rhizosphere, as previously discussed.

The presence of Al stress decreased total nutrient uptake by plants [depicted indirectly by the biomass weights (Fig. 1) and element concentrations (Tables 3 and 4)], joined by the decrease in root and shoot weights. These results are similar to those from Fageria (1985) in rice cultivars and from Mariano and Keltjens (2005) in 10 maize genotypes, where the increase in Al concentration in nutrient solution exerted an inhibition effect on the uptake of Ca, Mg, P, K, and others, with concomitant increase in Al concentration in plant tissues. Though it happened in nutrient solution cultivation, an even more accentuated decrease in nutrient uptake would be expected if the plants were grown in soil. In this case, the mechanisms of nutrient supply to root surface would be slower, taking into account that Al toxicity decreased root length, surface area, volume and number of tips (Fig. 2).

As cited above, the input of composts to soil represents an interesting strategy for alleviating Al toxicity (Baziramakenga and Simard 1998; Haynes and Mokolobate 2001) and it has been confirmed by the hydroponic cultivation from this study. Organic extracts alleviated Al phytotoxicity, improving plant growth and ameliorating plant nutrition status. The amendments increased root and shoot weights (Fig. 1), concomitantly to an enhancement in root length, surface area, volume, and number of tips (Fig. 2) for both cowpea and lablab. In addition, extracts of yard waste and GreenEdge decreased root Al concentration (Table 3). The benefits of organic extracts can be due to both Al complexation and nutrient supply from the extracts. Application of yard waste extract not only alleviated Al toxicity but also increased plant biomass weights to values higher than those from the treatment SNS –P, but had in some cases values similar to or higher than plants under SNS. This series of benefits in using organic amendments on acid soils is even enhanced by the fact that the types of amendments herein employed are widely available to farmers, and usually at low costs.

According to the biomass results, extract of GreenEdge was less efficient than that of yard waste, having a greater effect in alleviating Al toxicity in cowpea than in lablab, and negatively affecting the growth of black oat. The growth inhibition in roots of black oat by GreenEdge may indicate a kind of phytotoxicity, which could be due to the presence of, for example, short-chain organic acids resulting from decomposition of organic matter (Sullivan and Miller 2001), or even due to a higher sensitivity of black oat roots to salt concentrations than the roots of the leguminous crops. Although yard waste extracts were more effective in enhancing plant growth than GreenEdge extracts in plants under Al stress in the present study, it is possible that amendment of GreenEdge in soil would have better effects on plant growth than yard waste, because of the enrichment with lime and nutrients in GreenEdge and the kinetic of reactions. Due to the method adopted in this study, this advantage was not displayed.

The better nutrition status and the higher availability of Mg can help explain the greater Al alleviation by yard waste extract than GreenEdge extract, which led to the highest root and shoot weights (Fig. 1) and better root characteristics (Fig. 2). Divalent cations as Ca^{2+} and Mg^{2+} can alleviate Al rhizotoxicity due to competition with Al for binding at sensitive sites in either the symplast or apoplast of root cells, reduction in Al saturation in the root apoplast exchange sites, and decreased Al^{3+} activity at the root cell plasma membrane surface (Kinraide 1998). However, Silva *et al*. (2005) noticed that Mg^{2+} was more effective than Ca^{2+} in alleviating Al toxicity in roots of soybean and that the Mg ameliorative properties could not be accounted for by estimated electrostatic changes in root membrane potential and Al^{3+} activity at the root surface, being the physiological mechanisms of Mg alleviation of Al injury in roots not known. Recently, Yang *et al*. (2006) attested that Mg helped restore Al-reduced plasma membrane H^+-ATPase activity in rice bean roots (*Vigna umbellata*).

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