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Identity of the rhizotoxic aluminium species

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Abstract

The aluminium (III) released from soil minerals to the soil solution under acid conditions may appear as hexaaquaaluminium ($Al(H_2O)_6^{3+}$, or Al^{3+} for convenience) or may react with available ligands to form additional chemical species. That one or more of these species is rhizotoxic (inhibitory to root elongation) has been known for many decades, but the identity of the toxic species remains problematical for the following reasons. 1. Several Al species coexist in solution so individual species cannot be investigated in isolation, even in artificial culture media. 2. The activities of individual species must be calculated from equilibrium data that may be uncertain. 3. The unexpected or undetected appearance of the extremely toxic triskaidekaaluminium ($AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$ or Al_{13}) may cause misattribution of toxicity to other species, especially to mononuclear hydroxy-Al. 4. If H^+ ameliorates Al^{3+} toxicity, or vice versa, then mononuclear hydroxy-Al may appear to be toxic when it is not. 5. The identity and activities of the Al species contacting the cell surfaces are uncertain because of the H^+ currents through the root surface and because of surface charges. This article considers the implications of these problems for good experimental designs and critically evaluates current information regarding the relative toxicities of selected Al species. It is concluded that polycationic Al (charge >2) is rhizotoxic as are other polyvalent cations.

Introduction

Aluminium (III) is a major constituent of mineral soils where it is present in a wide array of primary and secondary minerals (Lindsay, 1979). These hydroxy-rich minerals dissolve to a limited extent under acid-soil conditions, and the Al released to the soil solution reacts with available ligands that may include OH^-, phosphate, F^-, SO_4^{2-}, silicate (Lindsay, 1979) and a large number of organic ligands (Stevenson, 1989). The resulting mononuclear species (one Al atom in the complex) may come into more or less rapid equilibrium, and in some cases equilibrium constants have been estimated (Nordstrom and May, 1989), but continuing reactions may occur as polynuclear species develop and precipitate to form new solid phases (Bertsch, 1989).

The phytotoxicity (more specifically, rhizotoxicity) of Al has been recognized for many decades (Hartwell and Pember, 1918), and for an equal length of time agronomists, soil chemists and others have pondered the identity of the chemical species of Al responsible for the toxicity. A large number of reports contain assertions or assumptions regarding the toxicity or nontoxicity of particular species. However, only a few investigations concerned with species toxicity have incorporated the essential features of experimental design that would allow a reasonable hope of success.

This article sets forth the author's views regarding aspects of experimental design that are essential (though not necessarily sufficient) for the investigation of the relative toxicities of Al species. The article also presents a critical review of current information regarding the relative toxicities of selected Al species and some polyvalent cations (charge >2).

Methods

In this section some important features of good experimental design for Al toxicity experiments are discussed in general terms. Specific examples of implementation are referred to in the Results section. Various designs are illustrated in the tables, but, in general, the solutions listed in the tables are not identical to those used in published experiments.

Speciation of Al solutions

In the investigations under consideration here the common feature is that root growth was measured with reference to the Al species in the rooting medium. Therefore, an accurate computation of Al species activities (speciation) is desirable. An exhaustive speciation is possible only in very simple solutions, and that is the principal reason for the use of hydroponic culture. However, the use of hydroponic culture, in which all of the chemical inputs are known exactly, does not ensure that an accurate speciation is possible. Speciation requires chemical equilibrium among species whose identity must be known. In the case of solutions containing both Al and phoshpate, for example, neither the identity of the species nor the attainment of equilibrium can be assumed (Nordstrom and May, 1989). The best chance of an accurate speciation occurs when the aerated basal (Al-free) culture medium contains only $CaCl_2$ and HCl for pH adjustment. In most cases seedling root elongation is vigorous for several days in this simple medium. Other solutes should be added only if essential to the particular study.

Even with simple solutions, speciation can be fraught with difficulties. All chemical reactions occurring in the solution must be known, and the reactions must attain equilibrium or near equilibrium. Correct equilibrium constants must be available and must be used in iterative computations for which comprehensive computer programs are often used. The available versions of the programs may have been copied and edited many times by previous users. Some versions contain errors in theory, data base, and programming (Parker *et al.*, 1987 and errata in Soil Sci. Soc. Am. J. 51, 1680; Sposito, 1986). Data bases must be edited to incorporate selected equilibrium constants. Output must be checked qualitatively to see that the products of all expected reactions appear, then the quantitative output must be checked, in detail, by hand. Finally, published reports must contain all relevant information so that the reader can check the speciation. Selected equilibrium reactions and sample speciations are presented in Tables 1–4.

Multiple variables

In the time-honored tradition of experimental science, each Al species under investigation should be the only variable in the culture media of the experiment. That is impossible in Al solutions which always contain more than one Al species (Fig. 1). Any attempt to vary the activity of one of the species usually causes variations in the acivities of other Al species. Collinearity among Al species is a regular problem in studies of Al toxicity, and toxicity has sometimes been

Table 1. Equilibrium reactions used in the computation of species activities in Tables 2, 3 and 4 and Figures 1 and 2. Constants for the formation of mononuclear hydroxy-Al species were compiled by Nordstrom and May (1989). Other constants were taken from Lindsay (1979) who probably intended 5.1 for the formation of $Al(SO_4)_2^-$ (see discussion in Kinraide and Parker [1987b])

Equilibrium reactions	Equilibrium constants (log K)
$Al^{3+} + H_2O = AlOH^{2+} + H^+$	−5.00
$Al^{3+} + 2H_2O = Al(OH)_2^+ + 2H^+$	−10.1
$Al^{3+} + 3H_2O = Al(OH)_3^0 + 3H^+$	−16.8
$Al^{3+} + 4H_2O = Al(OH)_4^- + 4H^+$	−22.7
$Al^{3+} + SO_4^{2-} = AlSO_4^+$	3.2
$Al^{3+} + 2SO_4^{2-} = Al(SO_4)_2^-$	5.1
$Ca^{2+} + SO_4^{2-} = CaSO_4^0$	2.3
$CO_{2(g)}{}^a + H_2O = H_2CO_3^0$	−7.46
$H_2CO_3^0 = H^+ + HCO_3^-$	−6.36
$H_2CO_3^0 = 2H^+ + CO_3^{2-}$	−16.69
$Ca^{2+} + H_2CO_3^0 = CaHCO_3^+ + H^+$	−5.24
$Ca^{2+} + H_2CO_3^0 = CaCO_3^0 + 2H^+$	−13.55
$CaCO_{3(calcite)} = CaCO_3^0$	−5.27

[a] $CO_{2(g)}$ expressed as μL CO_2 per L atmosphere.

Table 2. Composition of culture media constant in $\{Al^{3+}\}$ and variable in $\{AlSO_4^+\}$ and $\{Al(SO_4)_2^-\}$. $\{Ca^{2+}\} = 0.15$ m*M*, $\{Na^+\} = 17.2 \pm 0.2$ m*M* and pH = 4.3 throughout. In solutions 1 through 6 $\{Al^{3+}\} = 1.0$ μ*M*, and $\{Cl^-\}$ and $\{SO_4^{2-}\}$ were similar to values in solutions 7 through 12 which, together with solution 13, were used for the computation of relative root length.

Solution No.	$[CaCl_2]$ (μ*M*)	$[AlCl_3]$	$[NaCl]$	$[Na_2SO_4]$	$\{AlSO_4^+\}$	$\{Al(SO_4)_2^-\}$
1	260	4.0	20000	0	0.0	0.0
2	300	6.0	16600	1700	1.5	0.1
3	330	8.3	13000	3500	3.0	0.5
4	360	10.9	9400	5300	4.5	1.0
5	390	13.8	6000	7000	6.0	1.8
6	430	17.0	1400	9300	7.5	2.9
					$\{Cl^-\}$	$\{SO_4^{2-}\}$
7	260	0.0	20000	0	17900	0
8	300	0.0	16600	1700	14900	940
9	330	0.0	13000	3500	11800	1890
10	360	0.0	9400	5300	8700	2830
11	390	0.0	6000	7000	5800	3670
12	430	0.0	1400	9300	1970	4800
13	260	50.0	20000	0	18000	0

ascribed to an Al species that was correlated with another species to which toxicity could have been ascribed (Kinraide and Parker, 1989a, b). (See Tables 2, 3, and 4.)

In addition, other variables, such as pH (Tables 3 and 4), ionic strength, or the activities of counter ions (Table 2), may change with the attempt to vary the Al species of interest. This problem necessitates the use of multiple control (zero-Al) solutions which, nevertheless, may fail to reveal interactions between the Al species of interest and the other variables (Kinraide, 1990; Kinraide and Parker, 1987b). Consequently, the true effect of the Al species upon root elongation

Table 3. Composition of culture media factorial in $[AlCl_3]$ and pH. Each solution contained 0.4 m*M* $CaCl_2$; $\{Ca^{2+}\} =$ 0.34 m*M* and $\{Cl^-\} = 0.81 \pm 0.03$ m*M* throughout. Solutions 10 through 15 were used for the computation of relative root length

Solution No.	pH	$[AlCl_3]$ (μ*M*)	$\{Al^{3+}\}$	$\{AlOH^{2+}\}$	$\{Al(OH)_2^+\}$
1	4.60	0.70	0.35	0.14	0.04
2	4.80	0.70	0.28	0.18	0.09
3	5.00	0.70	0.20	0.20	0.16
4	4.60	1.40	0.69	0.28	0.09
5	4.80	1.40	0.56	0.35	0.18
6	5.00	1.40	0.41	0.41	0.32
7	4.60	2.10	1.04	0.41	0.13
8	4.80	2.10	0.84	0.53	0.27
9	5.00	2.10	0.61	0.61	0.49
10	4.60	0.00	0.00	0.00	0.00
11	4.70	0.00	0.00	0.00	0.00
12	4.80	0.00	0.00	0.00	0.00
13	4.90	0.00	0.00	0.00	0.00
14	5.00	0.00	0.00	0.00	0.00
15	4.60	20.00	9.79	3.90	1.23

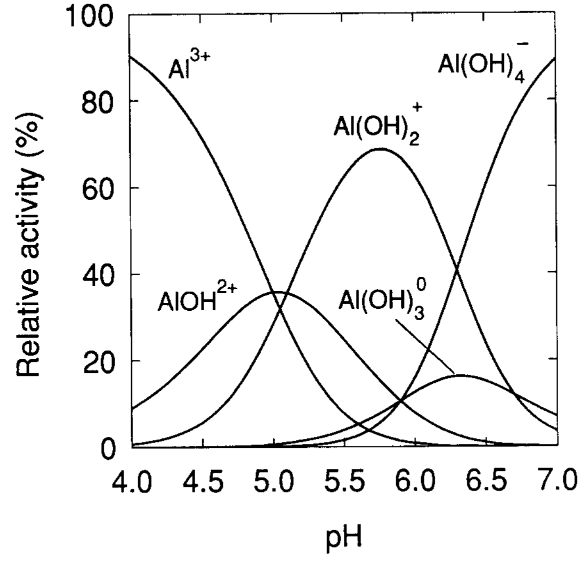

Fig. 1. Relative activities of mononuclear Al species assuming the absence of polynuclear Al and Al ligands other than OH^-. Note that the curve for $Al(OH)_3^0$ is closely related to the ratio $\{Al^{3+}\}/\{H^+\}^3$ and to the inverse of the solubility of solid-phase $Al(OH)_3$. The curve is a plot of $\{Al(OH)_3^0\}/$ $\mathrm{sum}\{Al_{mono}\}$ which equals $(K_3/\mathrm{sum}\{Al_{mono}\})(\{Al^{3+}\}/\{H^+\}^3)$ where K_3 is the third hydrolysis constant in Table 1.

Table 4. Composition of culture media constant in $\{Al^{3+}\}$ and variable in sum $\{Al\text{-}OH_{mono}\}$ activities, or constant in sum $\{Al\text{-}OH_{mono}\}$ and variable in $\{Al^{3+}\}$. Each solution contained 0.4 m*M* $CaCl_2$; $\{Ca^{2+}\} = 0.34$ m*M* and $\{Cl^-\} = 0.79 \pm 0.01$ m*M* throughout. Additional solutions, similar to those in Table 3, would be needed for the computation of relative root length

Solution No.	pH	$[AlCl_3]$	$\{Al^{3+}\}$	$\{AlOH^{2+}\}$	$\{Al(OH)_2^+\}$	sum $\{Al\text{-}OH_{mono}\}$
		(µ*M*)				
1	5.07	0.60	0.15	0.18	0.17	0.35
2	4.92	0.75	0.25	0.21	0.14	0.35
3	4.82	0.89	0.35	0.23	0.12	0.35
4	4.73	1.04	0.45	0.24	0.10	0.35
5	4.67	1.18	0.55	0.26	0.10	0.35
6	4.61	1.33	0.65	0.27	0.09	0.35
7	4.60	0.70	0.35	0.14	0.04	0.18
8	4.78	0.85	0.35	0.21	0.10	0.31
9	4.89	0.99	0.35	0.27	0.17	0.44
10	4.97	1.13	0.35	0.33	0.24	0.57
11	5.03	1.27	0.35	0.37	0.32	0.70
12	5.08	1.42	0.35	0.42	0.40	0.83

may be hidden. In any case, the problem of multiple variables must be considered in the experimental designs and the subsequent data analyses.

Polynuclear Al

Polynuclear Al is defined here as any species, complex, or aggregation (including solid-phase $Al(OH)_3$) that contains more than one Al atom. The appearance of polynuclear Al creates problems in two ways. If appreciable amounts of mononuclear Al aggregate unexpectedly, then the activities of the mononuclear species will be overestimated. These solutions could be much less toxic than expected if the polynuclear Al occurs as nontoxic solid phases. The much more serious problem is the unexpected development of the exceedingly toxic triskaidekaaluminium ($AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$) referred to as Al_{13} (Bertsch, 1989; Parker *et al.*, 1989a). Al_{13} is insidious because it can inhibit root elongation before it is likely to be detected in the bulk phases of culture media. Submicromolar concentrations can be inhibitory, and formation in the root free space is a possibility (Kinraide, 1990).

Al_{13}, or other polynuclear species, can arise if the [Al] increases or if the pH rises in acidic solutions or falls in basic solutions (Fig. 2) (Bertsch, 1989; Kinraide, 1990; Parker *et al.*, 1989a). The status of Al_{13} as a metastable intermediate in the formation of $Al(OH)_{3(s)}$, and the conditions fostering its formation and disappearance are subjects of ongoing research. Fortunately, a detailed knowledge of the chemistry of polynuclear Al is not required in order to predict the intermediate-term (days to months) stability of mononuclear solutions. Acidic solutions, lacking ligands other than OH^-, and prepared from

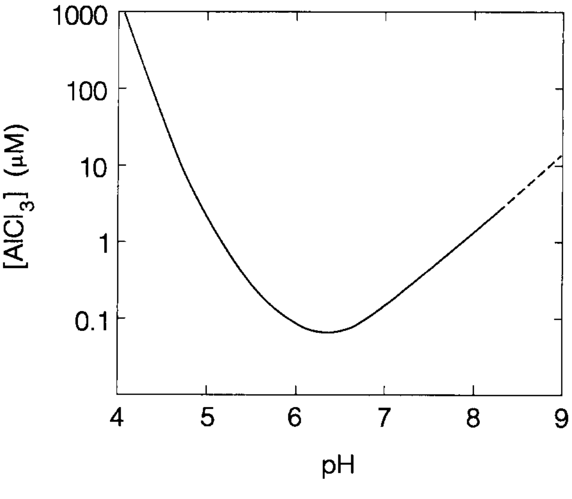

Fig. 2. Stability of Al solutions composed of $AlCl_3$ and 0.4 m*M* $CaCl_2$ at various pH values (adjusted with HCl or NaOH) in equilibrium with 350 µL CO_2 per L atmosphere. The curve delineates the combinations of $[AlCl_3]$ and pH at which $\{Al^{3+}\}/\{H^+\}^3 = 10^{8.8}$. Al undergoes rapid polynuclear aggregation in acidic solutions at values $>10^{8.8}$ (area above the curve). Alkaline solutions will tolerate a ratio of 10^{10} (an [Al] about 16 times higher) before the onset of rapid aggregation. $CaCO_3$ may precipitate at pH values >8.35.

mononuclear Al stock solutions, are stable for many days if $\{Al^{3+}\}/\{H^+\}^3 < 10^{8.8}$ (*i.e.*, $[Al(OH)_3^0] < 10^{-8}\ M$) (braces denote activities in mol/L). This criterion was derived from a recomputation of data from solution assays presented in a variety of publications (Kinraide and Parker, 1989a). Basic solutions, similarly prepared, are likewise stable provided $\{Al^{3+}\}/\{H^+\}^3 < 10^{10.0}$ (*i.e.*, $[Al(OH)_3^0] < 10^{-6.8}\ M$) (Kinraide, 1990).

If possible, the ratio $\{Al^{3+}\}/\{H^+\}^3$ should not exceed $10^{8.1}$, which defines the solubility of synthetic gibbsite (May *et al.*, 1979). That was possible for the solutions in Table 2, but in solution no. 9 in Table 3 the ratio equalled $10^{8.79}$. The important point is that the unexpected appearance of polynuclear Al must be avoided in the culture media. Investigators would be wise to test their own solutions if the conditions are marginal for the stability of mononuclear Al. At present, the ferron assay appears to be preferred for the detection of Al_{13} (Parker *et al.*, 1988a,b, 1989a). Unfortunately, there are no adequate means of detecting Al_{13} confined to the root free space, and investigators can only be warned of its possible presence (see *Aluminate* in the Results section).

Cation amelioration

Divalent cations are effectve ameliorants of Al toxicity beyond ionic-strength reductions of Al activities (Ali, 1973; Kinraide and Parker, 1987a). Monovalent cations are ameliorative at higher concentrations for some plants but not others (preliminary studies indicate that Na^+ does not reduce red clover sensitivity to Al). Consequently, cation activities must be carefully controlled in Al-toxicity experiments (Table 2). Occasionally it is desirable to reduce root sensitivity to Al, and that can be done with cations. Parker *et al.* (1988a) used relatively high $[CaCl_2]$ so that Al could be raised to an easily assayable but not-too-toxic range. H^+ is a problematical cation. In certain experiments pH is used to adjust the distribution of Al species (Tables 3 and 4). If H^+ ameliorates Al toxicity, or vice versa, then the results of these experiments may be ambiguous (see Discussion).

Relative root length (RRL)

The required use of multiple control solutions necessitates the expression of root elongation in the test solutions in relative terms. One suitable expression is the following: $RRL = 100\,(L_{Al} - L_S)/(L_C - L_S)$ in which L_{Al} is the root length in the Al solution, L_C is the root length in the corresponding Al-free control, and L_S is the root length in saturating levels of Al. The quantity L_S is mainly the root length at the time of transfer of the seedling to the culture media, but there is some additional, mainly initial, root elongation even at saturating levels of Al. This elongation can cause a large, false increase in RRL if it is ignored and if L_{Al} is low. The solutions used for the measurement of L_C (*i.e.*, the control solutions) must be considered carefully.

Results

In this section the rhizotoxicity of individual Al species will be considered. For convenience the hexaaquaaluminium species, $(Al(H_2O)_6^{3+}$, and most other species, will be written without the coordinated water. In all cases considered here Al is in the 3+ oxidation state and when referred to without reference to a particular ligand may be written Al(III), but for convenience Al will be written in place of Al(III). The practice of referring to all soluble Al(III) as Al^{3+} is less common in agriculture than it is in medicine (Ganrot, 1986) but is unfortunate wherever it occurs.

Al^{3+}

The widespread, but mainly unverified, assumption that Al^{3+} is toxic may have arisen in part because of the repeated misuse of Al^{3+} to refer to all soluble Al(III). For example, Hue *et al.* (1986) cited Adams and Lund (1966), Brenes and Pearson (1973) and Pavan *et al.* (1982) to support the statement that phytotoxicity is correlated with $\{Al^{3+}\}$ in soil solutions. In fact, Al^{3+} was not distinguished from total solution Al in the two older articles. Nevertheless, Hue *et al.* (1986) are correct; toxicity is generally correlated with $\{Al^{3+}\}$ in soil solutions (Wright *et al.*, 1987). More solid-phase Al dissolves at lower pH so that total soluble Al increases (Wright *et al.*, 1987). Also, the relative abundance of Al^{3+} increases with decreasing pH (Fig. 1). This does not prove the toxicity of Al^{3+}, however, and in virtually every investigation with soils, toxicity could have been attributed to other Al species which increased with Al^{3+}.

Hydroponic experiments have also generally failed to address the problem of collinearity among Al species. Some experiments purported to address the issue of relative toxicity among Al^{3+} and the hydroxy-Al species have been conducted at a single pH and variable total [Al] (Al_T) (Noble and Sumner, 1988; Pavan and Bingham, 1982), but because of the perfect collinearity among these species no conclusions can be drawn regarding relative toxicities. The converse design – constant Al_T and variable pH – can yield useful data. As the pH declines below 5, $\{Al^{3+}\}$ increases and the activities of all other Al species decline (Fig. 1). If the relative root length also declines one could logically attribute toxicity to Al^{3+} if a few precautions had been taken and if a few problems can be ignored (see Discussion).

Surprisingly, there are very few experiments that have used this simple design to confirm the toxicity of Al^{3+}, and it was not until 1988 that a convincing study was published (Parker *et al.*, 1988a). That study clearly demonstrated the increasing toxicity to wheat roots of solutions at constant Al_T and decreasing pH, provided that polynuclear Al was absent. When polynuclear Al did form the solutions became very toxic as the pH rose above 4.8. The toxicity of Al^{3+} was confirmed in a subsequent study (Kinraide and Parker, 1989a) that employed several experimental designs including one in which $\{Al^{3+}\}$ was held constant across a series of solutions of variable pH (Table 4). As the pH rose the activities of the mononuclear hydroxy-Al species also rose, but the solutions remained uniformly toxic to wheat. Apparently, mononuclear hydroxy-Al was not toxic to wheat.

The rhizotoxicity of Al^{3+} has not been confirmed for any other species, but not for want of an effort to do so. Kinraide and Parker (1990) failed to detect Al^{3+} toxicity against red clover, lettuce and turnip. Instead, mononuclear hydroxy-Al appeared to be toxic according to experiments described in the next section. In addition, these authors (Kinraide and Parker, 1989a; 1990) reanalyzed published experiments with soybean (Alva *et al.*, 1986; Noble *et al.*, 1988). The experiments were difficult to analyze. The culture media contained complete nutrient media including the ligands phosphate, EDTA, and variable $[SO_4^{2-}]$ or $[F^{-1}]$. There was a copious production of polynuclear Al in some of the solutions. The experimental designs generally entailed a factorial array of Al_T and pH that generated a continuous variation of Al species and high collinearity among some species (Table 3). Nonlinear multiple regression analysis appeared to be the only means of detecting Al^{3+} toxicity in the face of possible toxicity from other species (Kinraide and Parker, 1989a; 1990). According to these analyses mononuclear hydroxy-Al appeared to be more toxic than Al^{3+}, which may have been toxic also.

Mononuclear hydroxy-Al

These species include $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^0$ and $Al(OH)_4^-$. The last-named species, aluminate, is not significant below pH 5 (Fig. 1) and will be discussed in a separate section. $Al(OH)_3^0$, always a minority species, probably never exceeds submicromolar concentrations before the onset of polynuclear aggregation (Kinraide and Parker, 1989a); in addition, the first two species are highly correlated with one another or with Al^{3+} for any experiment that can be designed over the pH 4 to 5 range (Tables 3 and 4). It is nearly impossible to extend the pH beyond these limits. Below pH 4 growth is usually very poor, and Al^{3+} dominates in any case; beyond pH 5 Al_T must be exceedingly low to avoid polynuclear aggregation (Fig. 2). Consequently, the toxicity of $AlOH^{2+}$, $Al(OH)_2^+$ and $Al(OH)_3^0$ will be considered as a group.

Mononuclear hydroxy-Al (excluding aluminate) appears to be toxic to the dicotyledonous plants that have been studied. (That this appearance may be deceptive will be discussed later.) Experiments were designed in which $\{Al^{3+}\}$ remained constant but the activity of mononuclear hydroxy-Al rose as the pH increased (see Table

4). The relative root lengths decreased with these increases, indicating an increasing toxicity against the dicots (Kinraide and Parker, 1990), but similar experiments with wheat yielded contrary results (see previous section). Complementary experiments were performed in which $\{AlOH^{2+}\}$ was held constant but $\{Al^{3+}\}$ increased. In these experiments with dicots the relative root lengths remained constant, indicating no response to Al^{3+} (Kinraide and Parker, 1990).

The previous section referred to the reanalysis of published experiments in which solutions were factorial in Al_T and pH. These experiments indicate that soybean is more sensitive to mononuclear hydroxy-Al than to Al^{3+}. Similar designs were used by Kinraide and Parker (1989a and 1990; see Table 3), but simple culture media were used, and precautions were taken to avoid polynuclear Al in the bulk solution. Nonlinear multiple regression analysis indicated mononuclear hydroxy-Al toxicity against red clover, lettuce and turnip but not against wheat.

Aluminate

At $pH \geq 7.9$ aluminate ($Al(OH)_4^-$) constitutes over 99% of the mononuclear Al activity in the absence of powerful ligands other than OH^- (Fig. 1). Alkaline Al solutions and substrates can be toxic (Fuller and Richardson, 1986; Jones, 1961; Rees and Sidrak, 1955), and for that reason toxicity has been ascribed to aluminate. Aluminate is not always toxic, however; solutions containing 25 μM Al became progressively less Al toxic to both wheat and red clover as the pH rose from 8.0 to 8.9 (Kinraide, 1990). Al_{13} was absent from the bulk phases of these solutions, but the Al at the lower pH was on the verge of polynuclear aggregation because $\{Al^{3+}\}/\{H^+\}^3 > 10^{10}$. (Note that this ratio increases with increasing pH below 6.3 and with decreasing pH above pH 6.3; see Fig. 2.). Because the root free space was acidic the conditions there were likely to be conducive to the formation of Al_{13}, especially at the lower bulk-phase pH. Consequently, the author concluded that aluminate is not toxic, and that Al toxicity in alkaline solutions is attributable to Al_{13} in the root free space.

Sulfate complexes

The case against the toxicity of sulfate complexes ($AlSO_4^+$ and $Al(SO_4)_2^-$) is good and is relatively easy to develop. Thus it is surprising that convincing experiments were not reported until 1986. Cameron *et al.* (1986), using simple media of constant [Ca] and ionic strength, effectively showed that the variation in barley root length was dictated by Al^{3+} (or mononuclear hydroxy-Al species, since the pH was constant in the experiments) and not by sulfate complexes. Kinraide and Parker (1987b) demonstrated the same for wheat and red clover in experiments in which $\{Ca^{2+}\}$, $\{Na^+\}$, $\{Al^{3+}\}$ and mononuclear hydroxy-Al activities were held constant (Table 2). That study also confirmed the absence of polynuclear species in the sulfate solutions. A recent report also confirmed the nontoxicity of sulfate complexes of Al in soil solutions (Wright *et al.*, 1989).

Fluoride complexes

The case against the toxicity of the fluoride complexes (AlF^{2+}, AlF_2^+, AlF_3^0, AlF_4^-, AlF_5^{2-} and AlF_6^{3-}) is also good. Experimentally, one may adjust $[AlCl_3]$ and [NaF] at constant pH in order to hold all nonfluoride species of Al constant while varying the fluoride species. The solutions should then be aged at least overnight because of slow complexation between Al and F^- (Plankey *et al.*, 1986). Because both salts are added at submillimolar levels there is no chance of significant Na^+ amelioration of Al toxicity. Alternatively, one could provide a uniform background level of Na^+ with, say, 10 mM NaCl. F^-, at the levels used in these experiments, is unlikely to be significantly rhizotoxic, but appropriate control solutions are, of course, necessary. As with every other experiment with Al, one can never be sure that F^- and Al^{3+} (or other Al species) do not interact physiologically.

Definitive experiments with Al and F are surprisingly recent. Konishi and Miyamoto (1983) reported that NaF alleviated Al toxicity against pollen tube elongation, and Kinraide *et al.* (1985) reported that 2 or 3 μM NaF eliminated the severe toxicity of 1 or 2 μM $AlCl_3$ to clover roots. More detailed experiments were reported by Cameron *et al.* (1986) and by subsequent authors (Noble *et al.*, 1988; Parker *et al.*, 1989b). Soil solutions with high levels of Al-fluoride complexes may also be nontoxic (Wright *et al.*, 1987).

Organic complexes

The detoxification of Al-toxic soils by additions of organic matter is well-documented (Ahmad and Tan, 1986; Hoyt and Turner, 1975). Hydroponic culture media can also be detoxified by additions of organic ligands of Al (Bartlett and Riego, 1972a; Hue *et al.*, 1986). This experience has led to the general conviction that organic complexes of Al are not rhizotoxic. The most comprehensive investigation to use chemically defined media was reported by Hue *et al.* (1986). Root elongation into a medium of 0.25 m*M* $CaCl_2$ and various levels of $AlCl_3$ and selected organic acids at pH 4.75 was measured after two days. One can see from that study the ameliorative capacity of the organic acids (with the exception of salicylic acid) correlated positively with the log of the stability constants of the Al-organic acid complexes tabulated by Nordstrom and May (1989). It may be that the salicylic acid did not complex Al as expected at pH 4.75, as suggested by the authors. The logical inference from these detoxification studies is that organic complexes are nontoxic – a failure to ameliorate reflects noncomplexation. The structural requirement for strong binding (and ameliorative capacity) appears to be an arrangement of OH or COOH groups so that Al may be bound between two O atoms in a 5- or 6-bond ring structure (Hue *et al.*, 1986; Motekaitis and Martel, 1984).

$AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$ (Al_{13})

In a pioneering study Bartlett and Riego (1972b) attributed toxicity to polynuclear hydroxy-Al. Although they performed no assays, they did prepare a solution almost certainly dominated by soluble hydroxy-Al polynuclear species. Corn plants were transferred, with washes, at two-day intervals, between a complete nutrient solution and the Al solution. This solution was very toxic, and it is reasonable to attribute toxicity to polynuclear Al. That article, with its discussion of the potential behavior of polynuclear Al in soils, appears to have been ahead of its time because more than ten years elapsed before work on polynuclear Al toxicity resumed.

Wagatsuma and Ezoe (1985) described experiments in which root elongation in several plant species was more inhibited by 370 μM Al at pH 4.5 than pH 4.1. According to assays, the solutions contained 83% polynuclear Al at the higher pH and 32% at the lower pH so the authors concluded that polynuclear Al may be more toxic to plant roots than mononuclear Al. Two years later Wagatsuma and Kaneko (1987) presented more detailed experiments leading to the same conclusions. Additional experiments illustrated a peak loss of K^+ and a peak accumulation of Al at pH 4.7 – the pH of highest polynuclear Al in the 220 μM Al solutions. These authors did not refer to Al_{13} explicitly, but they did state the chemical formula of the polynuclear-Al ions had been identified by Akitt *et al.* (1972) and Akitt and Farthing (1981).

Parker *et al.* (1988a) reported experiments in which the sum of mononuclear-Al activities was adjusted to prescribed values and in which polynuclear hydroxy-Al was carefully monitored. The rhizotoxicity of the latter was clearly demonstrated in solutions free of toxic levels of mononuclear Al. In a second study (Parker *et al.*, 1989a) the toxic polynuclear Al was identified as Al_{13}, and response curves for soybean and two wheat cultivars were presented. Al_{13} was ten-fold more toxic than Al^{3+} when expressed as [Al], and the Al-tolerant wheat cultivar Seneca was just as sensitive to Al_{13} as the Al-sensitive cultivar Tyler. Ultrafiltration studies and estimates of OH/Al ratios, as well as the NMR analyses, indicate that the ferron-assayed reactive polynuclear species were composed principally (and perhaps exclusively) of Al_{13}.

Polyvalent cations

Because polycationic Al (charge >2) appears to be toxic, evidence for the general rhizotoxicity of polyvalent cations is presented here. Clarkson (1965) found the potentially trivalent cations Al, gallium, indium, and lanthanum to be similarly toxic to onion roots. Scandium was also rhizotoxic (Clarkson and Sanderson, 1969). New data reported here show that Sc is about ten-fold more toxic to wheat roots than mononuclear Al on a concentration basis. Wheat seeds (*Triticum aestivum* cv. Tyler) were germinated for 2 d then transferred to 0.4 m*M* $CaCl_2$ at pH 4.3 containing 0 to 0.8 μM $ScCl_3$ for 2 d of additional growth. At 0.2 μM Sc relative root length was 42 ± 2% (mean and SE), and at 0.4 μM inhibition was complete. The speciation of Sc in this representative experiment was unknown, but if polynuclear hydroxy-Sc species were present they were about as toxic as Al_{13}.

Organic polycations such as poly-L-lysine and polymyxin B are also very toxic (Reuveni *et al.*, 1985 and references therein). In an experiment similar to that described for Sc the tetravalent polyamine spermine was added to 0.4 m*M* $CaCl_2$ at pH 4.3 at 0 to 16 μM. At 4 μM the relative root lengths of wheat were 58 ± 4% (mean and SE), and inhibition was complete at 16 μM. Thus polyvalent cations appear to be generally rhizotoxic, though not necessarily all by the same mechanism.

Discussion

Several problems prevent the unqualified attribution of rhizotoxicity to any particular Al species with the possible exception of Al_{13}. One class of problems pertains to the bulk solutions used in the experiments and includes uncertainties regarding speciation and the design of experiments to isolate the variables of interest. Another class of problems pertains to uncertainties regarding the chemistry of the root free space and the identity and activity of the Al species actually making contact with cell membranes or other points of critical contact. There is even a semantic problem. One could designate as rhizotoxic any Al species that, when present in the bulk phase of the rooting medium, contributes, directly or indirectly, to root toxicity. According to that view aluminate was rhizotoxic at pH 8.0 but not at 8.9 in the experiments referred to earlier (Kinraide, 1990). A less liberal view would designate aluminate as nontoxic if, in fact, toxicity were the consequence of Al_{13} formation in the free space.

The apparent toxicity of mononuclear hydroxy-Al to dicotyledonous plants is very problematical. The confirmation of mononuclear hydroxy-Al toxicity is confounded by three alternative hypotheses. The first alternative hypothesis is that the apparent sensitivity of the dicots to these species may be a real sensitivity to Al^{3+} that increases as pH increases. Because of hydrolysis equilibria (Table 1), $\{AlOH^{2+}\} = \{Al^{3+}\}K_1/\{H^+\}$ and $\{Al(OH)_2^+\} = \{Al^{3+}\}K_2/\{H^+\}^2$ where K_1 and K_2 are the first and second hydrolysis constants. Thus, any expression of root elongation as a function of mononuclear hydroxy-Al can be alternatively expressed as a function of $\{Al^{3+}\}$ and $\{H^+\}$. That is, Al^{3+} toxicity may be ameliorated by H^+ – a possibility that has been proposed (Grauer and Horst, 1990; Kinraide and Parker, 1990; Parker *et al.*, 1988) but not confirmed.

Amelioration by H^+ may be another instance of cation amelioration (Ali, 1973; Kinraide and Parker, 1987a), but if that were the case then H^+ is effective at much lower activities than the >1 m*M* activities required for divalent cations and the >10 m*M* activities required for other monovalent cations. This higher effectiveness could be accounted for by high-affinity binding sites (pK_a values >4) that, when occupied by H^+, cannot bind Al^{3+} or interfere with Al^{3+} binding somewhere else. In addition, H^+ binding would reduce negative surface charges, thereby reducing Al enrichment of the cell surface. The difference between wheat and the dicots may reflect a difference in the pK_a values of these supposed binding sites. If the pK_a value for the dicots were in the pH 4 to 5 range then sensitivity to Al^{3+} could increase as pH increases from 4 to 5. This would give the appearance of sensitivity to mononuclear hydroxy-Al. If similarly critical binding sites were absent in wheat or if the pK_a were less than 4 then amelioration of Al^{3+} would not occur between pH 4 and 5. That these differences could occur is suggested by the already-known differences between the grasses and the dicots in cation exchange properties (Marschner, 1986).

The second alternative hypothesis for the apparent toxicity of mononuclear hydroxy-Al is that Al^{3+}, the toxic Al species, ameliorates H^+ toxicity. All of the plant species considered here (wheat, red clover, lettuce, turnip and soybean) were sensitive to the pH of the control solutions. Turnip was the extreme case with elongation at pH 4.6 only 34% that at pH 5.0 (Kinraide and Parker, 1990), and soybean was the next most sensitive with elongation at pH 4.2 equal to 53% that at pH 4.8 (data of Noble *et al.*, 1988, analyzed by Kinraide and Parker, 1990). At low pH some of the toxic effect of H^+ may be ameliorated by Al^{3+} thus offsetting some of the toxic effect of that ion, but at higher pH the full toxic effect of Al^{3+} would be expressed. In the control solutions the full toxic effect of H^+ would be expressed with the net effect that solution toxicities expressed in terms of RRL would give the false impression that solutions of constant $\{Al^{3+}\}$ increase in Al toxicity as pH rises. In truth, the Al toxicity of the solutions remains constant but the H^+ toxicity of the Al-containing solutions decreases, relative to the controls, as the pH declines. It is suggestive that low levels of Al greatly stimulate turnip root elongation at low pH. The difference between wheat and the dicots could be explained by a lower sensitivity to H^+ by wheat (which appears to be the case) or by a reduced capacity of Al^{3+} to ameliorate H^+ toxicity in wheat.

The third alternative hypothesis for the apparent toxicity of mononuclear hydroxy-Al is that Al_{13} forms in the root free space at higher bulk-solution pH values. Examination of many plant species demonstrates a general pattern of H^+ influx (and external alkalinization) into the expanding cells of the root tips and a pattern of H^+ extrusion (and external acidification) from fully expanded cells of more mature regions (Miller and Gow, 1989). Consequently, both the pH and the distribution of Al species must be different in the root free space than in the bulk medium. It is possible that wheat and the dicots are both sensitive to Al^{3+} but that the conditions in the free space of dicot roots are more conducive to the formation of Al_{13} as the external pH rises between 4 and 5. This is likely the case because the higher cation exchange capacity of dicots (Marschner, 1986) means that $\{Al^{3+}\}$ will be higher in the free space. Equal pH values there would mean higher values for $\{Al^{3+}\}/\{H^+\}^3$ for the dicots. This third alternative hypothesis could be tested if it were possible to prevent the synthesis of Al_{13}, perhaps with a specific ligand.

The case for mononuclear hydroxy-Al is not good because of the plausible alternative hypotheses presented above. One wonders whether the same arguments could be reversed in order to explain away the apparent toxicity of Al^{3+} to wheat. For example, could protonation of surface sites render roots more sensitive to mononuclear hydroxy-Al so that solutions become more toxic at lower pH even as the toxic species decrease? That seems less likely, and confirmation of the nontoxicity of Al^{3+} would be a remarkable result given the otherwise general rhizotoxicity of polyvalent cations.

For the present, it may be reasonable to conclude that Al_{13} and Al^{3+} are rhizotoxic even though the evidence for the latter is confined to wheat. Mononuclear hydroxy-Al (excluding aluminate) appears to be rhizotoxic to dicotyledonous plants, but that apparent toxicity may be attributable to Al^{3+} or Al_{13}. Aluminate is not rhizotoxic, and the occasional apparent toxicity is probably attributable to Al_{13}. Mononuclear Al complexes of SO_4^{2-}, F^-, and organic ligands are not rhizotoxic. A unifying hypothesis is that polycationic Al species are rhizotoxic (as are other polyvalent cations) and that ligands reduce rhizotoxicity by reducing charge. Another hypothesis is that the differential toxicity of Al species may simply reflect their activities at the negatively charged surface of the cell. The concentrating effect of negative charge (which can lie both in the cell wall and on the plasma membrane) is greater for more highly charged cations.

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