

**Speciation and Phytotoxicity of Mono- and Polynuclear Aluminum in Dilute  
Hydroxy-Aluminum Solutions**

by

David R. Parker

Dissertation submitted to the Faculty of the  
Virginia Polytechnic Institute and State University  
in partial fulfillment of the requirements for the degree of  
Doctor of Philosophy  
in  
Agronomy

APPROVED:

---

L. W. Zelazny, Co-chairman

---

D. C. Martens, Co-chairman

---

T. B. Kinraide

---

D. R. Bevan

---

J. G. Dillard

February 15, 1988  
Blacksburg, Virginia

**Speciation and Phytotoxicity of Mono- and Polynuclear Aluminum in Dilute Hydroxy-  
Aluminum Solutions**

by

David R. Parker

L. W. Zelazny, Co-chairman

D. C. Martens, Co-chairman

Agronomy

(ABSTRACT)

Despite long-standing interest in the toxicity of aluminum (Al) to plants, relationships between phytotoxicity and the chemistry of Al in hydroxyl-dominated systems remain unresolved. First, the ferron (8-hydroxy-7-iodo-5-quinoline-sulfonic acid), aluminon (aurintricarboxylic acid, triammonium salt), and 8-hydroxyquinoline methods were evaluated and compared for their ability to yield estimates of the mononuclear fraction,  $f_m$ , in Al solutions of varying basicity. Reactions of ferron or aluminon with Al were kinetically modeled as two parallel irreversible reactions, while 8-hydroxyquinoline required use of an arbitrary cutoff time. Estimates of  $f_m$  were in the order 8-hydroxyquinoline > ferron > aluminon at basicities  $\leq 1$ , while at a basicity of 2.25 the general order was aluminon  $\geq$  8-hydroxyquinoline > ferron. Aging solutions up to 32 days resulted in decreased estimates of  $f_m$  using aluminon, but estimates using ferron and 8-hydroxyquinoline were only minimally affected. All three methods yielded results of adequate precision for most purposes, although the ferron procedure was only about one-half as sensitive than the other two. Additional studies using ferron demonstrated its utility for characterizing the nonmononuclear Al fraction using kinetic analyses. The ferron method was selected for continued use based on its simplicity, level of precision, and moderate reaction rate with Al.

Next, 2-day old wheat (*Triticum aestivum* L.) seedlings were transferred to 500-mL test solutions for 2 days of additional growth at 298 K in the dark, and final root lengths used as the measure of Al toxicity. Quantities of polynuclear hydroxy-Al were estimated using ferron, and activities of mononuclear species were calculated using the computer program GEOCHEM. In the absence of polynuclear hydroxy-Al, raising solution pH consistently ameliorated Al toxicity suggesting that  $Al^{3+}$  is the primary toxic species. The results did not confirm other reports that the sum of the mononuclear species activities is a reliable predictor of Al phytotoxicity. Polynuclear hydroxy-Al was also demonstrably toxic, supporting isolated reports of the same finding.

Last, the toxicity of the polynuclear species as a function of experimental conditions was investigated. Increasing solution age, rate of base addition, or levels of added phosphate decreased the quantity of reactive polymers ( $[Al_b]$ ) as measured by ferron. Inhibition of root growth of both wheat and soybean (*Glycine max* (L.) Merr.) was well-correlated with  $[Al_b]$ , and these polymers were considerably more toxic than  $Al^{3+}$ . The wheat cultivars 'Tyler' and 'Seneca' exhibited differential tolerance to  $Al^{3+}$ , but not to  $[Al_b]$ , further suggesting differences in the physiological bases of mono- and polynuclear Al toxicity. Characterization of  $Al_b$  by several methods indicated that the toxic species are relatively small, highly charged, and principally the so-called  $Al_{13}$  polymer. These findings have practical implications for the accurate prediction of toxic Al levels in both solution culture media and soil solutions.

## Acknowledgements

First, I would like to thank my co-advisors, Drs. Lucian Zelazny and Dave Martens, for their many ideas, their encouragement, and their friendship over the course of my studies. Dr. Tom Kinraide has my special gratitude for the many hours spent sharing ideas on this research, and for the great pleasure I have derived from our other cooperative efforts. It is my sincere hope that he also found these endeavors to be stimulating and rewarding. I am also grateful to Drs. Dave Bevan and John Dillard for their valued insights as members of my advisory committee.

This research could not have been completed without the assistance of a number of individuals. Specifically, I am indebted to Dr. Phil Jardine, for sharing his expertise and thoughts on the ferron method; to Dr. Walter Baethgen, for his thoughtful advice on statistical analyses; to Ms. Nancy Phillips, for performing the ICPES analyses; to Drs. Tom Starling and Glenn Buss, for providing wheat and soybean seeds, respectively; to Mr. Bob Arnold of the USDA lab in Beckley, for conducting a number of plant growth experiments; and to Dr. Paul Bertsch, for arranging for the  $^{27}\text{Al}$ -NMR analysis. In addition, I would like to thank Mike Anderson, Mark Stolt, Steve Nagle, Paul Gassman,

Barry Stewart, John Chermak, Dennis Dove, Mike Goatley, Keith Edmisten, and Daryoosh Nabati, as well as many other fellow graduate students and softball team members, for making my stay at Virginia Tech such an enjoyable and enriching experience.

I would like to thank my parents, Mary and Ren, whose love and patronage over the years has made most of my achievements possible. Last, I would like to express my deepest love and gratitude to Pam Padgett, and to our three 'children' -- Bert, Spot, and Chinook -- for the immeasurable joy they have brought me over the past two years, and without whose forbearance and support this research could not have been completed.

# Table of Contents

<b>Introduction</b> .....	<b>1</b>
<b>References</b> .....	<b>5</b>
<b>Comparison of Three Spectrophotometric Methods for Differentiating Mono- and Polynuclear Hydroxy-Aluminum Complexes</b> .....	<b>8</b>
<b>Abstract</b> .....	<b>8</b>
<b>Introduction</b> .....	<b>9</b>
<b>Materials and Methods</b> .....	<b>13</b>
<b>Solution Preparation</b> .....	<b>13</b>
<b>Speciation Procedures</b> .....	<b>14</b>
<b>Theoretical</b> .....	<b>16</b>
<b>Results and Discussion</b> .....	<b>18</b>
<b>Method Optimization</b> .....	<b>18</b>
<b>Method Comparisons</b> .....	<b>24</b>
<b>Effects of Solution Age</b> .....	<b>28</b>
<b>Sensitivity and Precision</b> .....	<b>30</b>
<b>Additional Ferron Investigations</b> .....	<b>31</b>

Conclusions .....	35
References .....	39
<b>Aluminum Speciation and Phytotoxicity in Dilute Hydroxy-Aluminum Solutions .....</b>	<b>42</b>
Abstract .....	42
Introduction .....	43
Materials and Methods .....	46
Solution Culture Methodology .....	46
Solution Preparation and Speciation .....	47
Results .....	50
Initial Al Experiments .....	50
Improved Al Experiments .....	53
Lanthanum Experiments .....	58
Discussion .....	59
References .....	67
<b>Phytotoxicity and Characterization of Polynuclear Hydroxy-Aluminum Complexes .....</b>	<b>70</b>
Abstract .....	70
Introduction .....	71
Materials and Methods .....	73
Seedling Culture .....	73
Solution Preparation and Speciation .....	74
Characterization of Al <sub>b</sub> .....	76
Results and Discussion .....	78
Wheat Experiments .....	78
Soybean Experiments .....	85
Characterization of Al <sub>b</sub> .....	89
Summary and Conclusions .....	93
<b>Table of Contents</b>	<b>vii</b>

<b>References</b> .....	<b>94</b>
<b>Summary and Conclusions</b> .....	<b>97</b>
<b>Appendix A. Additional Data for Chapter II</b> .....	<b>103</b>
<b>Appendix B. Additional Data for Chapter III</b> .....	<b>116</b>
<b>Appendix C. Additional Data for Chapter IV</b> .....	<b>125</b>
<b>Vita</b> .....	<b>134</b>



## List of Illustrations

Figure 1.	Typical absorbance vs. time plots for the reaction of ferron and aluminon with Al solutions of varying basicity	20
Figure 2.	Effect of fitted second order mononuclear rate coefficients on the computed fraction of mononuclear Al by ferron and aluminon	23
Figure 3.	Fraction of mononuclear Al vs. basicity for the three methods for two sets of solutions at differing ages	26
Figure 4.	Computed fraction of mononuclear Al vs. solution age at three basicities for the three methods	29
Figure 5.	Absorbance vs. time plots for the reaction of ferron with Al solutions of varying basicity carried out to 1 hour	34
Figure 6.	Unreacted Al vs. time for the partially-neutralized solutions depicted in Fig. 5	36
Figure 7.	Root length of Tyler wheat vs. solution pH at three levels of total Al	51
Figure 8.	Data from Fig. 7 plotted as root length vs. activities of $Al^{3+}$ (a) or sum of mononuclear species (b)	52
Figure 9.	Distribution of Al species (a) and root lengths of Seneca wheat (b) vs. pH at constant sum of mononuclear species activities	54
Figure 10.	Distribution of Al species and relative root growth (RRG) of Tyler wheat vs. pH in the absence of polynuclear Al	56
Figure 11.	Fraction of polynuclear Al and relative root growth (RRG) of Tyler wheat vs. basicity	57
Figure 12.	Root length of Tyler wheat as a function of pH in the presence or absence of added La	60
Figure 13.	Relative root growth of Tyler and Seneca wheat vs. concentration of $Al_0$	84

Figure 14. Relative root growth of Tyler and Seneca wheat vs. concentration of $Al^{3+}$ at pH 4.3	86
Figure 15. Relative root growth of Stafford soybeans vs. concentration of $Al_0$ or concentration of $Al^{3+}$ at pH 4.3.	88
Figure 16. Fraction of $Al_0$ retained vs. nominal molecular weight cutoff of several membrane filters	91
Figure 17. Linearized second-order plot for the reaction of ferron and mononuclear Al	107
Figure 18. Linearized second-order plot for the reaction of aluminon and mononuclear Al	108
Figure 19. Fraction of mononuclear Al vs. basicity by the three methods for the 0.4 mM $CaCl_2$ solutions after 30 d aging	110
Figure 20. Fraction of mononuclear Al vs. basicity by the three methods for the 40 mM NaCl solutions after 3 d aging	111
Figure 21. Linearized first-order plots for the reaction of ferron and polynuclear Al	114
Figure 22. Linearized second-order plots for the reaction of ferron and polynuclear $Al^+$	115
Figure 23. Distribution of Al species (a) and root lengths of Tyler wheat (b) vs. pH at constant sum of mononuclear species activities	118
Figure 24. Fraction of polynuclear Al and relative root growth (RRG) of Tyler wheat vs. basicity	119
Figure 25. Fraction of polynuclear Al and relative root growth (RRG) of Seneca wheat vs. basicity	121
Figure 26. Root length of Tyler and Seneca wheat vs. $Al^{3+}$ or $La^{3+}$ activity at pH 4.3	124
Figure 27. Root length of Seneca wheat as a function of pH in the presence or absence of added La	125
Figure 28. Fraction of $Al_0$ retained vs. nominal molecular weight cutoff of several membrane filters	132
Figure 29. Fraction of $Al_0$ retained vs. nominal molecular weight cutoff of several membrane filters	133

## List of Tables

Table 1.	Typical variations in fitted second-order mononuclear rate coefficients using ferron and aluminon	22
Table 2.	Quadruplicate analyses by the three speciation methods for solutions of varying basicity aged 21 d	32
Table 3.	Computed fraction of mononuclear Al and coefficients of determination for the regression of the reactions depicted in Fig. 5 on Eq. II	37
Table 4.	Thermodynamic constants used for computed speciation of growth solutions.	49
Table 5.	Coefficients of determination for the regression of root length vs. various species activities using two sets of thermodynamic constants	64
Table 6.	Influence of rate of base addition to Al stock solutions on speciation and root growth of Tyler and Seneca wheat.	80
Table 7.	Influence of stock solution age on Al speciation and root growth of Tyler and Seneca wheat.	81
Table 8.	Influence of added phosphate on Al speciation and root growth of Tyler and Seneca wheat.	82
Table 9.	Effect of calcium on absorbance of Al monomer-aluminon mixtures	105
Table 10.	Effect of reaction time on absorbance of Al monomer-8-hydroxyquinoline mixtures	109
Table 11.	Fraction of mononuclear Al by ferron and the 'instantaneous' 8-hydroxyquinoline methods	113
Table 12.	Computed fraction of mononuclear Al and coefficients of determination for the solutions from Table 3 after 35 d aging	116
Table 13.	Computed species activities and pH for the experiments depicted in Figs. 11 and 25	122

Table 14. Final root lengths of Stafford soybeans in solutions containing only mononuclear Al .....	129
Table 15. Influence of stock solution age and rate of base injection on Al speciation and root growth of Stafford soybeans .....	130
Table 16. Ferron-based speciation of partially neutralized Al solutions as a function of dilution .....	131
Table 17. Data for computations of the bound OH/Al ratio in the $Al_6$ polymers .	134

# Chapter I

## Introduction

Although it has been some 70 years since Hartwell and Pember (1918) first postulated that soluble aluminum (Al) is a major limitation to plant growth in acid soils, the relationships between the aqueous chemistry of Al and phytotoxicity are still not fully understood. That efforts to relate toxicity to levels of Al in soil solution have met with variable degrees of success (Adams and Lund, 1966; Brenes and Pearson, 1973; Pavan et al., 1982; Adams and Moore, 1983; Adams and Hathcock, 1984; Wright and Wright, 1987; Wright et al., 1987) may be partly ascribable to an insufficient understanding of the importance of speciation to biological toxicity. Recent concerns about the possible effects of acidic deposition on the biogeochemistry of soil Al has broadened the scope of interest in Al toxicity to both terrestrial and aquatic organisms (Driscoll et al., 1980; Ulrich et al., 1980; Baker and Schofield, 1982).

The chemistry of Al is quite complex, its high ionic charge and small crystalline radius yield a level of reactivity unmatched by any other soluble metal found in soil solution,

and a variety of hydrolysis, complexation, and chelation reactions have been reported. As solution pH is raised above 4.0,  $\text{Al}^{3+}$  gives way to the mononuclear species  $\text{AlOH}^{2+}$ ,  $\text{Al}(\text{OH})_2^+$ ,  $\text{Al}(\text{OH})_3^0$ , and  $\text{Al}(\text{OH})_4^-$  (waters of hydration are omitted here and elsewhere for clarity) (Baes and Mesmer, 1976). In addition, larger polynuclear hydroxy-Al species may form as metastable intermediates in the precipitation of solid phase  $\text{Al}(\text{OH})_3$  (Smith, 1971; Hsu, 1977). Inorganic ligands such as sulfate and fluoride, as well as a host of organic compounds, may also form soluble complexes with Al (Lindsay, 1979; Haug, 1984; Hue et al., 1986). In general, polynuclear and chelated forms of Al have been considered to be nontoxic to plants, but agreement is not uniform on this point.

While the exact molecular basis for Al toxicity remains elusive (Foy, 1984; Haug and Caldwell, 1985), the symptoms are well-known. Roots of Al-affected plants are short, stubby, distorted, and discolored (Moore, 1974; Foy, 1984). The overall stunting of the root system greatly limits the plants' ability to explore the soil for needed water and nutrients (Foy, 1984). Consequently, Al phytotoxicity is often studied in the laboratory using solution culture techniques wherein root elongation is used as the primary measure of Al stress (Moore, 1974). In addition to the types of studies reported here, this approach is useful for examining the basic physiology of Al toxicity, and for rapidly screening genetic material for differential Al tolerance (Kinraide et al. 1985; Moore et al., 1976).

In nutrient solution studies, amelioration of Al toxicity has been achieved by the addition of various solutes to the media. The effective ameliorative treatments can be divided into two broad categories. The first is the addition of Al-complexing ligands, including hydroxyl ions, which may reduce the concentration of toxic Al species. The

second is the addition of simple salts of the alkaline or alkaline earth metals (e.g.,  $\text{CaCl}_2$ ), wherein the anion is not a significant ligand for Al. In such cases, the concentrations of toxic Al are not reduced, but activities are, and there are both theoretical and empirical bases for using the latter in studies of ionic toxicities (Khasawneh, 1971; Cramer and Lauchli, 1986). Alternatively, salt additions may relieve Al toxicity through a direct physiological effect of the cation, perhaps, as an example, through competition for external binding sites on root cells (Kinraide and Parker, 1987a). Recent studies have indicated that both ionic strength effects and physiological effects play roles in cation amelioration of Al toxicity (Kinraide and Parker, 1987a).

At the initiation of these and other studies, information on the relative phytotoxicities of inorganic mononuclear complexes, including the hydrolysis products, was either sparse or conflicting. Since that time, studies have demonstrated the nontoxicity of Al-sulfate (Cameron et al., 1986; Kinraide and Parker, 1987b) and Al-fluoride (Cameron et al., 1986; Parker et al., 1988) complexes. There is also general agreement that organo-Al complexes are nontoxic (see Hue et al., 1986, and references contained therein).

With hydroxy-Al species, the situation was far less clear. That raising the solution pH will eventually relieve Al-toxicity is well-documented; it remains unclear whether this should be ascribed to (i) differential toxicity of the mononuclear hydrolysis products, (ii) formation of nontoxic polynuclear hydroxy-Al complexes or solid phase  $\text{Al}(\text{OH})_3$ , or (iii) a physiological response whereby increasing pH reduces sensitivity to Al. Among the mononuclear species, toxicity has variously been ascribed to  $\text{Al}^{3+}$  (Adams and Lund, 1966; Brenes and Pearson, 1973; Pavan and Bingham, 1982; Pavan et al., 1982), to  $\text{AlOH}^{2+}$  (Moore, 1974; Alva et al., 1986), to  $\text{Al}(\text{OH})_2^+$  (Alva et al., 1986), and to the sum (expressed as activities) of all mononuclear species (Blamey et al., 1983; Alva et al.,

1986a, 1986b). Polynuclear hydroxy-Al has generally been viewed as nontoxic (Blamey et al., 1983; Alva et al., 1986b), but several reports have suggested that these species can be highly toxic (Bartlett and Riego, 1972; Wagatsuma and Ezo, 1985; Wagatsuma and Kaneko, 1987). That these conflicting views exist may, in part, be due to difficulties in complete and accurate speciation of the growth media, especially where chemically complex nutrient solutions have been used. Moreover, the polynuclear species can be quite variable, their composition and extent depending on Al concentration, pH, ionic strength, ligands present, temperature, and the age and method of preparation of the solution, as well as other factors (Smith, 1971; Hsu 1968, 1977; Turner, 1976; Bertsch, 1987).

Thus, it is not presently possible to make definitive generalizations concerning the relationships between Al speciation and biological responses in systems where hydroxyl is the dominant Al-complexing ligand. In order for such relationships to be evaluated under 'field' conditions -- i.e., soil solutions and natural waters -- they must first be established under controlled chemical environments in the laboratory. Whenever possible, Al speciation is most readily accomplished computationally, using known formation and solubility constants. However, metastable polynuclear complexes render a computational approach untenable, and a convenient empirical method is required to characterize and quantify these species. Consequently, the objectives of this research were (i) to refine and evaluate several empirical methods for differentiating mono- and polynuclear Al, (ii) to clarify the relationships between speciation and toxicity in hydroxy-Al solutions where a variety of mononuclear, polynuclear, and solid-phase species may form, and (iii) to more fully investigate the phytotoxicity of polynuclear hydroxy-Al.



## References

- Adams, F., and P. J. Hathcock. 1984. Aluminum toxicity and calcium deficiency in acid subsoil horizons of two Coastal Plains soil series. *Soil Sci. Soc. Am. J.* 48:1305-1309.
- Adams, F., and Z. F. Lund. 1966. Effect of chemical activity of soil solution aluminum on cotton root penetration of acid subsoils. *Soil Sci.* 101:193-198.
- Adams, F., and B. L. Moore. 1983. Chemical factors affecting root growth in subsoil horizons of Coastal Plain soils. *Soil Sci. Soc. Am. J.* 47:99-102.
- Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986. Effects of phosphorus/aluminum molar ratio and calcium concentration on plant response to aluminum toxicity. *Soil Sci. Soc. Am. J.* 50:133-137.
- Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986. Relationships between root length of soybean and calculated activities of aluminum monomers in solution. *Soil Sci. Soc. Am. J.* 50:959-962.
- Baes, C. F., Jr., and R. E. Mesmer. 1976. *The hydrolysis of cations.* John Wiley and Sons, New York.
- Baker, J. P., and C. Schofield. 1982. Aluminum toxicity to fish in acidic waters. *Water Air Soil Pollut.* 18:289-309.
- Bartlett, R. J., and D. C. Riego. 1972. Toxicity of aluminum in relation to pH and phosphorus. *Soil Sci.* 114:194-201.
- Bertsch, P. M. 1987. Conditions for  $Al_13$  polymer formation in partially neutralized aluminum solutions. *Soil Sci. Soc. Am. J.* 51:825-828.
- Blamey, F. P. C., D. G. Edwards, and C. J. Asher. 1983. Effects of aluminum, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. *Soil Sci.* 136:197-207.
- Cameron, R. S., G. S. P. Ritchie, and A. D. Robson. 1986. Relative toxicities of inorganic aluminum complexes to barley. *Soil Sci. Soc. Am. J.* 50:1231-1236.
- Cramer, G. R., and A. Lauchli. 1986. Ion activities in solution in relation to Na-Ca interactions at the plasmalemma. *J. Expt. Bot.* 37:321-330.
- Driscoll, C. T., Jr., J. P. Baker, J. J. Bisogni, Jr., and C. L. Schofield. 1980. Effect of aluminum speciation on fish in dilute acidified waters. *Nature* 284:161-164.
- Foy, C. D. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. *In* F. Adams (ed.) *Soil acidity and liming.* 2nd ed. *Agronomy* 12:57-97.

- Hartwell, B. L., and F. R. Pember. 1918. The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *Soil Sci.* 6:259-279.
- Haug, A. 1984. Molecular aspects of aluminum toxicity. *CRC Critical Reviews in Plant Sciences.* 1:345-373.
- Haug, A., and C. R. Caldwell. 1985. Aluminum toxicity in plants: The role of the root plasma membrane and calmodulin. p. 359-381. *In* J. B. St. John et al. (ed.) *Frontiers of membrane research in agriculture.* Rowman and Allanheld, Totowa, NJ.
- Hsu, P. H. 1968. Interactions between aluminum and phosphate in aqueous solution. *Adv. Chem. Ser.* 73:115-127.
- Hsu, P. H. 1977. Aluminum hydroxides and oxyhydroxides. p. 99-143. *In* J. B. Dixon and S. B. Weed (ed.) *Minerals in soil environments.* Soil Science Society of America, Madison, WI.
- Hue, N. V., G. R. Craddock, and F. Adams. 1986. Effect of organic acids on aluminum toxicity in subsoils. *Soil Sci. Soc. Am. J.* 50:28-34.
- Khasawneh, F. E. 1971. Solution ion activity and plant growth. *Soil Sci. Soc. Am. Proc.* 35:426-436.
- Kinraide, T. B., R. C. Arnold, and V. C. Baligar. 1985. A rapid assay for aluminum phytotoxicity at submicromolar concentrations. *Physiol. Plant.* 65:245-250.
- Kinraide, T. B., and D. R. Parker. 1987a. Cation amelioration of aluminum toxicity in wheat. *Plant Phys.* 83:546-551.
- Kinraide, T. B., and D. R. Parker. 1987b. Nonphytotoxicity of the aluminum sulfate ion,  $AlSO_4^-$ . *Physiol. Plant.* 71:207-212.
- Lindsay, W. L. 1979. *Chemical equilibria in soils.* John Wiley and Sons, New York.
- Moore, D. P. 1974. Physiological effects of pH on roots. p. 135-151. *In* E. W. Carson (ed.) *The plant root and its environment.* Univ. Press of Virginia, Charlottesville.
- Moore, D. P., W. E. Kronstad, and R. J. Metzger. 1976. Screening wheat for aluminum tolerance. p. 287-295. *In* M. J. Wright (ed.) *Plant adaptation to mineral stress in problem soils.* Cornell Univ. Agric. Exp. Sta., Ithaca, NY.
- Parker, D. R., L. W. Zelazny, and T. B. Kinraide. 1988. Chemical speciation and plant toxicity of aqueous aluminum. *Adv. Chem. Ser.* (in review).
- Pavan, M. A., and F. T. Bingham. 1982. Toxicity of aluminum to coffee seedlings grown in nutrient solutions. *Soil Sci. Soc. Am. J.* 46:993-997.
- Pavan, M. A., F. T. Bingham, and P. F. Pratt. 1982. Toxicity of aluminum to coffee in ultisols and oxisols amended with  $CaCO_3$ ,  $MgCO_3$ , and  $CaSO_4 \cdot 2H_2O$ . *Soil Sci. Soc. Am. J.* 46:1201-1207.

- Smith, R. W. 1971. Relations among equilibrium and nonequilibrium aqueous species of aluminum hydroxy complexes. *Adv. Chem Ser.* 106:250-279.
- Turner, R.C. 1976. Effect of aging on properties of polynuclear hydroxyaluminum cations. *Can. J. Chem.* 54: 1528-1534.
- Wagatsuma, T., and Y. Ezoe. 1985. Effect of pH on ionic species of aluminum in medium and on aluminum toxicity under solution culture. *Soil Sci. Plant Nutr.* 31:547-561.
- Wagatsuma, T., and M. Kaneko. 1987. High toxicity of hydroxy-aluminum polymer ions to plant roots. *Soil Sci. Plant Nutr.* 33:57-67.

# Chapter II

## Comparison of Three Spectrophotometric Methods for Differentiating Mono- and Polynuclear Hydroxy-Aluminum Complexes

### *Abstract*

Increasing interest in the relationships between aluminum (Al) speciation and biological toxicity has created a need for accurate methods for speciating aqueous Al. Polynuclear hydroxy-Al complexes are generally viewed as metastable species, and are thus more amenable to empirical than to computational approaches to speciation. The objectives of this study were to evaluate and compare the ferron (8-hydroxy-7-iodo-5-quinoline-sulfonic acid), aluminon (aurintricarboxylic acid, triammonium salt), and 8-hydroxyquinoline methods for their ability to differentiate

mono- and polynuclear Al in solutions typical of those used in related phytotoxicity studies. Test solutions were 20  $\mu\text{M}$  in  $\text{AlCl}_3$ , 0.4 mM in  $\text{CaCl}_2$ , or 40 mM in  $\text{NaCl}$ , and had basicities (molar  $\text{OH}/\text{Al}$  ratios) ranging from -1 to 2.25. Reactions between test solutions and ferron or aluminon were kinetically modeled as two parallel irreversible reactions to yield estimates of the mononuclear fraction,  $f_m$ . The reaction of 8-hydroxyquinoline with mononuclear Al was essentially instantaneous, and use of an arbitrary cutoff was unavoidable. Estimates of  $f_m$  were in the order 8-hydroxyquinoline > ferron > aluminon at basicities  $\leq 1$ , while at a basicity of 2.25 the general order was aluminon  $\geq$  8-hydroxyquinoline > ferron. Increasing ionic strength of test solutions increased estimates of  $f_m$  by all methods. Significant quantities of polynuclear Al were detected even in solutions without added base or with equimolar levels of  $\text{HCl}$ . Aging solutions up to 32 days resulted in decreased estimates of  $f_m$  using aluminon, but estimates using ferron and 8-hydroxyquinoline were only minimally affected. All three methods yield results of adequate precision for most purposes, although the ferron procedure is somewhat less sensitive than the other two. Additional studies using ferron demonstrated its utility for characterizing the nonmononuclear Al fraction using kinetic analyses. Ferron may be the preferred method based on its simplicity, level of precision, and moderate reaction rate with Al.

## *Introduction*

Despite ever-increasing interest in the soil chemical factors governing aluminum (Al) toxicity to agronomic crops, many questions regarding the relationships between Al speciation and toxicity remain unanswered. Possible mobilization of soil Al by acid de-

position, and consequent adverse effects on a variety of organisms, provide an additional impetus for discerning the basic relationships between Al chemistry and biological responses. Whenever possible, Al speciation is most readily accomplished computationally, using known formation and solubility constants. Although some authors have treated polynuclear hydroxy-Al species thermodynamically (see, e.g., Baes and Mesmer, 1976), it is more generally accepted that they are metastable intermediates in the precipitation of  $\text{Al}(\text{OH})_3$  (Smith, 1971; Smith and Hem, 1972; Turner, 1976; Hsu, 1977). The presence of these polynuclear complexes, as well as uncharacterized organic ligands, renders a computational approach untenable in many systems. Consequently there is need for well-defined empirical techniques that can assist in providing reasonable estimates of the distribution of Al species.

A variety of methods have been employed to characterize and quantify polynuclear hydroxy-Al. These include nuclear magnetic resonance spectroscopy (Bertsch et al., 1986); gel chromatography and ultrafiltration (Rengasamy and Oades, 1978); use of ion exchange (Bersillon et al., 1980) and chelating resins (Campbell et al., 1983); kinetics of basic Al-sulfate precipitation (Bersillon et al., 1980); and use of the fluoride ion selective electrode (Hodges, 1987). Perhaps more common is the use of a variety of modified spectrophotometric techniques originally developed for quantitative analysis of Al (see Barnhisel and Bertsch, 1982). Spectrophotometric methods are appealing for their relative speed and simplicity -- important criteria for routine speciation applications where quantification, as opposed to characterization, of the Al species is the primary objective. Three such methods -- the ferron, aluminon, and 8-hydroxyquinoline procedures -- have been prominently featured in the soils and environmental literature.

The ferron reagent (8-hydroxy-7-iodo-5-quinoline-sulfonic acid) was first introduced by Davenport (1949) for quantitative analysis of Al. Smith (1971), and Smith and Hem (1972) employed ferron to distinguish mono- and polynuclear Al in partially neutralized solutions with the assumption that mononuclear Al and ferron react instantaneously. Bersillon et al. (1980) proposed a 30 s reaction time before reading absorbance to quantify mononuclear Al. Smith and Hem (1972), Tsai and Hsu (1984), and Bertsch et al. (1986) observed that the reaction of polynuclear hydroxy-Al with ferron conforms to pseudo-first order kinetics. Jardine and Zelazny (1986) described the reaction of ferron and partially neutralized Al solutions as a series of three first-order reactions -- a rapid mononuclear reaction, a slower reaction with reactive polynuclear species, and a third, very slow reaction with larger polymers and/or colloidal phases. Because the first two reactions overlap, Jardine and Zelazny (1986) employed nonlinear least squares analyses to fit two simultaneous first-order reactions to absorbance-time functions to quantify mononuclear Al, and thus avoided the use of arbitrary cutoff times. Subsequently, Jardine and Zelazny (1987) reported that the first 250 s of the reaction could be better described by two simultaneous pseudo second-order reactions, independent of whether the chloride, nitrate, perchlorate, or sulfate salt was used to prepare the Al solutions.

The aluminon reagent (aurintricarboxylic acid, triammonium salt) has been widely used for the determination of total solution Al (Hsu, 1963; Barnhisel and Bertsch, 1982). Hsu (1968) suggested that, by omitting the acidification and heating steps used to hydrolyze complexed Al, that the method could be employed to differentiate uncomplexed Al in solutions containing phosphate. Blamey et al. (1983) utilized this approach to measure mononuclear Al in nutrient solutions containing added hydroxyl and phosphate, and reported little change in absorbance between 20 and 60 min after mixing. They selected a 30 min reaction time to define mononuclear Al. This method was subsequently em-

ployed by Alva et al. (1986a,b) in Al phytotoxicity studies, but aside from the cursory investigation by Blamey et al.(1983), it has not been evaluated.

The 8-hydroxyquinoline method was first proposed by Okura et al. (1962) to distinguish mono- and polynuclear Al in partially neutralized solutions. After a 10-s reaction, the Al-quinolate complex was extracted into chloroform and measured spectrophotometrically. Turner (1969,1976) utilized a similar method but reported that lower-order polymers may react within very short times. He also noted a pseudo first-order reaction between the reagent and polymeric Al. May et al. (1979) modified the method to utilize toluene as the extracting solvent, and to eliminate interferences due to fluoride and various metals. Bloom et al. (1978), using butyl acetate as the solvent for total Al analysis, recommended a 15-min reaction time to allow elimination of Fe interference, but noted that an instantaneous reaction might provide a better estimate of uncomplexed, mononuclear Al. James et al. (1983) employed butyl acetate extraction and a 15-s reaction time to estimate 'labile' Al. They obtained qualitative agreement between measured and predicted values for solutions containing hydroxyl, fluoride, or citrate, but conceded that uncertainties in thermochemical constants and in the attainment of equilibrium should instill caution in interpreting their results. They suggested that the 15-s reaction measured uncomplexed Al plus a kinetically-labile portion of the complexed Al, and thus provided only an operational definition of labile Al, not an absolute measure. The method of James et al. (1983) has been used for estimating phytotoxic Al in soil solution (Adams and Hathcock, 1984).

Despite the ubiquity of these three speciation methods, few comparisons between them have been made. Hodges (1987) included the ferron and 8-hydroxyquinoline methods in a comparison of five speciation techniques, but did not address the problem of



hydroxy-Al polymers *per se*. Wright et al. (1987) found that aluminon and 8-hydroxyquinoline yielded different values for reactive Al in soil solution, but that correlations with Al phytotoxicity were equally good for the two methods. Ferron was of limited utility due to interferences from manganese in solution. Further characterization of the reactions between these reagents and various forms of complexed Al is needed in order to ascertain what fraction of Al is measured by each. In related research on Al phytotoxicity (see Chapter III), a convenient method was needed for routine speciation of dilute, partially-neutralized Al solutions containing no other Al-complexing ligands. Consequently, the objectives of this research were (i) to compare speciation results from the ferron, aluminon, and 8-hydroxyquinoline methods for dilute, partially neutralized Al solutions typical of those used in phytotoxicity studies, (ii) to evaluate the kinetics of the reactions between the three reagents and mononuclear and polynuclear Al, and (iii) to optimize the three methods and to evaluate their relative sensitivity and precision.

## ***Materials and Methods***

### **Solution Preparation**

All test solutions were 20  $\mu\text{M}$  in Al, prepared from a 1  $\text{mM}$   $\text{AlCl}_3$  stock solution, and contained either 0.4  $\text{mM}$   $\text{CaCl}_2$  or 40  $\text{mM}$   $\text{NaCl}$ . Solutions were made to *ca.* 90% of their final volume of 200 mL, and purged of  $\text{CO}_2$  by bubbling with prepurified  $\text{N}_2$  gas for 2 h. Appropriate basicities (added molar OH/Al ratio,  $n_0$ , where a negative value indicates added  $\text{H}^+$ ) were achieved by the slow (0.05  $\text{mL min}^{-1}$ ) injection of standardized

CO<sub>2</sub>-free 0.005 M HCl or NaOH using a Radiometer ABU-80 auto-burette, and brought to volume. The solutions were vigorously stirred during acid/base addition and maintained under an N<sub>2</sub> atmosphere. Solutions were stored in sealed polymethylpentane jars in a reduced-CO<sub>2</sub> atmosphere at ambient laboratory temperatures (298 ± 3 K) until just prior to analysis. Mononuclear standards, ranging from 0 to 20 μM in Al, were prepared from the same stock and made to 0.2 mM in HCl.

All reagents were of analytical grade or higher purity. Double deionized water was used throughout, and all glassware was acid-washed as needed to prevent contamination. Total Al content (Al<sub>t</sub>) of the stock solution was determined using inductively-coupled plasma emission spectroscopy with standards prepared from Al wire.

## Speciation Procedures

All spectrophotometric measurements were made with a Beckman DU-7 UV-VIS spectrophotometer using matched quartz cuvettes with a 1-cm path length. For the ferron and aluminon methods, the instrument's kinetics mode was used, and the raw absorbance data were collected and converted to concentrations by a microcomputer for subsequent kinetic analyses. All solutions and reagents were maintained at 298 K throughout the analyses. Assays were performed in duplicate unless otherwise indicated. Absorbance spectra of appropriate mixtures of mononuclear standards and the three analytical reagents were periodically measured to determine the optimum wavelength for each method. The pH of all test solutions was measured using a Radiometer PHM84 pH meter equipped with glass and calomel electrodes.

*Ferron* - The ferron procedure used was similar to that described by Jardine and Zelazny (1986, 1987). The composition of the final mixed reagent was thus 1.58 mM ferron, 0.31 mM *o*-phenanthroline, 0.95 M sodium acetate (NaOAc), 0.32 M NH<sub>2</sub>OH • HCl, and 0.74 M HCl, with a pH of 5.2. The mixed reagent was used between 5 and 7 d after preparation as recommended by Jardine and Zelazny (1987). One milliliter of sample was pipeted into a cuvette and placed in the spectrophotometer, and 0.8 mL of the mixed reagent injected using an autopipet. The injection of the ferron reagent provided adequate mixing of the sample and reagent (Jardine and Zelazny, 1986). Absorbance was monitored as a function of time (generally 60 readings min<sup>-1</sup>) at 363 nm.

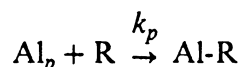
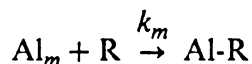
*Aluminon* - A modified version of the method of Blamey et al. (1983) was utilized. The composition of the aluminon reagent was 0.74 mM aluminon (Aldrich Chemical Co., No. A3,688-3), 0.60 M NaOAc, and 2.09 M acetic acid (HOAc), with a pH of 4.2 (Barnhisel and Bertsch, 1982). In the absence of any solution Fe, ascorbic acid was omitted. One-half milliliter of sample and 1.0 mL of reagent were mixed as for the ferron method, and absorbance monitored (10 readings min<sup>-1</sup>) at 545 nm.

*8-hydroxyquinoline* - The procedure given by James et al. (1983) was employed, except that larger culture tubes (25 x 150 mm) were used to accommodate a sample volume of 10 mL. Samples were rapidly injected with an autopipet into the reagent mixture (4 mL 0.069 M 8-hydroxyquinoline-0.44 M HOAc plus 10 mL water plus 5 mL 1.0 M NaOAc) and shaken for 15 s before stopping the reaction by rapid injection of 5 mL butyl acetate followed by 15 s additional shaking. After separation, the absorbance of the organic phase was measured at 385 nm. For investigations of the kinetics of the quinolate-Al reaction, a similar procedure was used except that the reaction was conducted in a 50 mL polyethylene beaker with a magnetic stir bar to achieve constant

mixing. The reaction was stopped at varying times after sample addition by rapid injection of butyl acetate using a repipet.

## Theoretical

The reaction of any analytical reagent, R, with a mixture of mono- and polynuclear Al can be expressed as two simultaneous, irreversible reactions (Smith and Hem, 1972; Jardine and Zelazny, 1986, 1987)



where:  $\text{Al}_m$  = mononuclear Al species  
 $\text{Al}_p$  = reactive polynuclear Al species  
 $k_m$  = the mononuclear rate coefficient  
 $k_p$  = the polynuclear rate coefficient  
 $\text{Al-R}$  = a common product, i.e., the Al-reagent complex formed

and the exact stoichiometries of the reactions are ignored. Although degradation of  $\text{Al}_p$  to  $\text{Al}_m$  may be a prerequisite for the formation of Al-R from the polynuclear species,  $k_p$  includes this rate-limiting step and thus accurately describes the reaction of  $\text{Al}_p$  and R (Jardine and Zelazny, 1986). As long as the molar ratio of R to Al exceeds about 50, the rates of reaction depend only on the quantities of Al present (Mark and Rechnitz, 1968; Jardine and Zelazny, 1986, 1987). Thus, two general rate expressions can be written:

$$\frac{-d[Al_m]}{dt} = k_m [Al_m]^n \quad [II.1]$$

$$\frac{-d[Al_p]}{dt} = k_p [Al_p]^{n'} \quad [II.2]$$

where  $n$  and  $n'$  are the respective apparent reaction orders, which may or may not be the same for both reactions. Combining and integrating Eqs. 1 and 2 yields a general-order rate expression for any  $n$  and  $n' \neq 1$  (Jardine and Zelazny, 1987)

$$[Al]_{un} = \sqrt[n-1]{\frac{1}{k_m t (n-1) + \frac{1}{[Al_m^0]^{n-1}}}} + \sqrt[n'-1]{\frac{1}{k_p t (n'-1) + \frac{1}{[Al_p^0]^{n'-1}}}} \quad [II.3]$$

where:  $[Al]_{un}$  = the total Al left unreacted at any time,  $t$   
 $[Al_m^0]$  = the initial concentration of mononuclear Al  
 $[Al_p^0]$  = the initial concentration of polynuclear Al

Except where noted, calculation of  $[Al]_{un}$  is based on the known total Al content of the test solution and comparison of absorbance with a standard curve for mononuclear solutions. Equation 3 holds except when either  $n$  or  $n' = 1$ . However, if a first order reaction is needed, a value very close to 1 (e.g., 1.01) can be used and Eq. II.3 will correctly describe the reaction (Jardine and Zelazny, 1987). The mononuclear rate coefficient,  $k_m$ , is determined by application of a single-species version of Eq. II.3 to data for mononuclear Al standards. For polynuclear solutions,  $k_m$ ,  $n$ , and  $n'$  are fixed, while  $[Al_m]$ ,  $[Al_p]$ , and  $k_p$  are computed, yielding an estimate of the fraction of mononuclear Al,  $f_m$ . All computations were performed using a general-purpose nonlinear least squares inversion regression program (Jardine and Zelazny, 1986, 1987).

## *Results and Discussion*

### **Method Optimization**

In preliminary studies, various parameters for each method were evaluated to optimize sensitivity and reproducibility. All three methods were found to be sensitive to temperature, especially aluminon, so care was taken to equilibrate and maintain all solutions at 298 K. Frink and Peech (1962) reported that solution Ca interfered with the aluminon determination of Al, but at high molar ratios (*ca.* 100:1) of Ca to aluminon. No interference from up to 10 mM Ca in test solutions was observed with the version of the method employed here.

The relative volumes of reagents and solutions were also adjusted. For ferron, the ratio yields a ferron/Al molar ratio of 63, which is high enough to ensure an excess of reagent during the reaction (Mark and Rechnitz, 1968; Jardine and Zelazny, 1986, 1987). Higher ratios decreased sensitivity as indicated by the final absorbance for mononuclear standards. Similarly, the aluminon/Al ratio was adjusted to *ca.* 100, the lowest ratio that yielded a linear calibration plot for mononuclear standards. Higher ratios also decreased sensitivity. For 8-hydroxyquinoline, it was found that, at 20  $\mu$ M Al, a sample volume of 10 mL in conjunction with the reagent volumes specified by James et al. (1983) resulted in linear standard curves and good sensitivity.

A series of preliminary studies was conducted to evaluate the kinetics of the reactions between the three reagents and Al, and the suitability of the kinetic approach to speciation proposed by Jardine and Zelazny (1986,1987). The reaction of ferron with

mononuclear Al was complete within about 2 min as indicated by the plateau in absorbance for a 20  $\mu\text{M}$  mononuclear standard (Fig. 1). Analysis of this reaction by nonlinear regression techniques, or by constructing a linear second-order plot ( $1/[\text{Al}]_{\text{un}}$  vs.  $t$  [Moore and Pearson, 1981]) indicated that it is well-approximated by second order kinetics with respect to Al, confirming the findings of Jardine and Zelazny (1987). The reaction could also be modelled as 3/2 order, but the fit to the observed data was no better than for second order. Differential analyses (Froment and Bischoff, 1979, p. 46-47) were performed by estimating tangents over the initial 60 s of the reaction and consistently yielded fitted reaction orders of between 1.5 and 2. It was thus concluded that a second order reaction was a suitable model for the reaction of mononuclear Al and ferron.

Polynuclear Al can react appreciably with ferron in the first 2 min, thus overlapping the mononuclear reaction (Fig. 1; Jardine and Zelazny, 1986), and the polynuclear reaction continued out to *ca.* 30 min. A 5-min reaction time was selected for regressing the data using the two-species, second-order model (Eq. II.3) proposed by Jardine and Zelazny (1987). The fit of the model to the observed data was generally quite good, as  $R^2$  values almost always exceeded 0.95.

With aluminon, the kinetics of reaction even with mononuclear solutions was rather slow (Fig. 1). In contrast to the findings of Blamey et al. (1983), small but significant increases in absorbance were observed out to 1 h and beyond. Hsu (1963) noted that absorbance increased for as long as two days, albeit quite slowly. The first 15 to 20 min of the reaction with mononuclear Al could be fit quite well to a second-order reaction, although differential analyses indicated that a 5/2 order with respect to Al was also a plausible model. However, as with ferron there was no improvement in fit using the

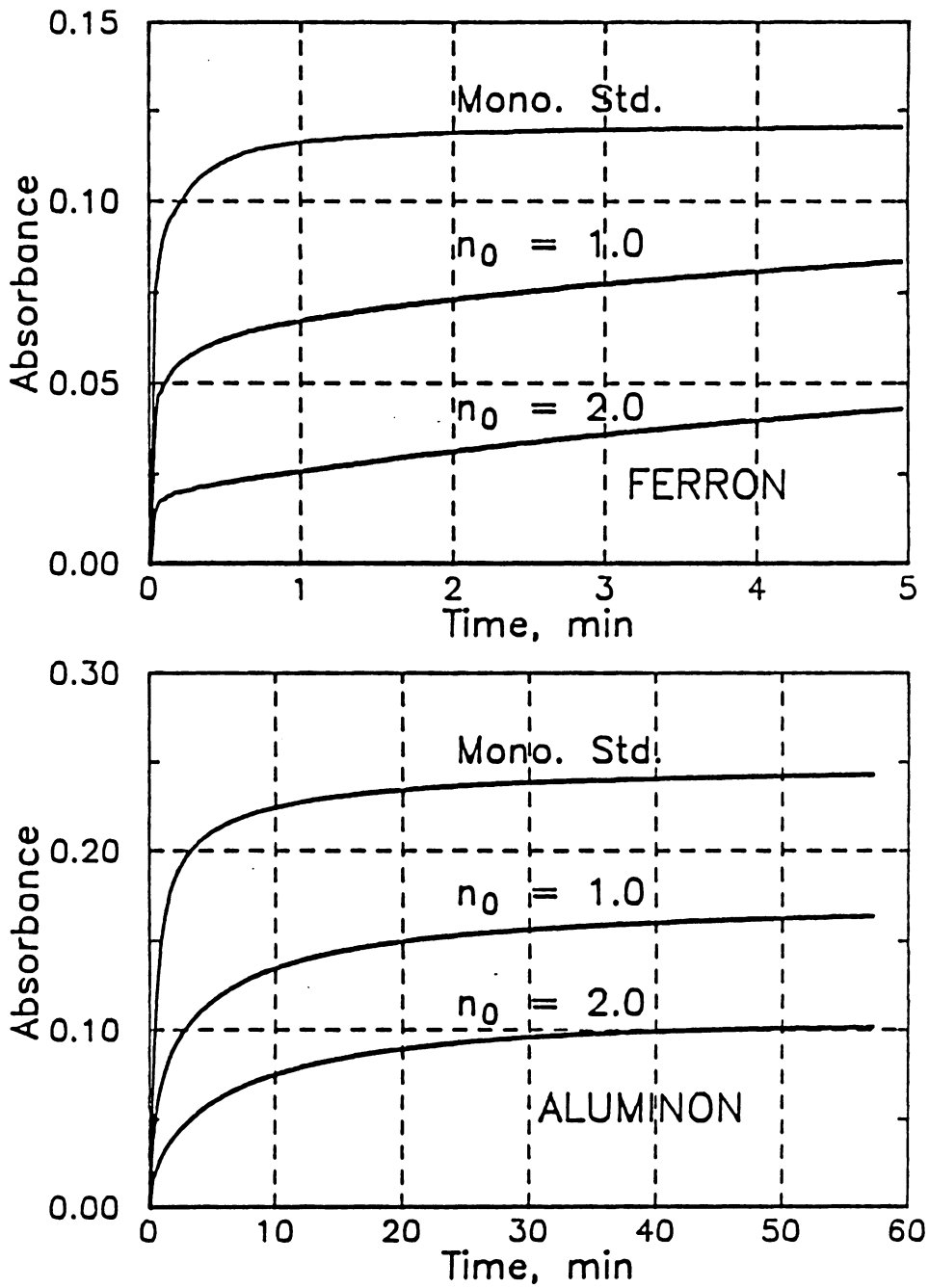


Figure 1. Typical absorbance vs. time plots for the reaction of ferron and aluminon with Al solutions of varying basicity: Solutions contained  $20 \mu M$   $Al_3$  and  $0.4 mM$   $CaCl_2$ , and were aged 4 d before analysis.



nonintegral reaction order. Thus, a second-order reaction was selected for modeling the mononuclear reaction.

As with ferron, the reaction of polynuclear Al with aluminon overlapped that of the mononuclear species, and continued out to long times (Fig. 1). However, the lack of a clear termination point for the mononuclear reaction rendered it difficult to isolate the polymer reaction with aluminon. For the sake of consistency with the ferron method, the first 24 min of this reaction was modeled as two parallel pseudo second-order reactions to yield estimates of  $f_m$ . The fit of the model to the observed data was consistently very good --  $R^2$  values of less than 0.99 were rarely observed. Use of reaction times up to 1 h did not improve the fit, nor result in significantly different estimates of  $f_m$ .

Despite the good fit of the ferron and aluminon reactions with mononuclear Al to second order kinetics, it was observed that the fitted rate coefficients,  $k_m$ , varied for standards differing in Al<sub>i</sub> (Table 1). This indicated that the kinetics of the reactions are nonideal, not an unexpected result for association reactions in high-ionic strength solutions (Moore and Pearson, 1981). This led to concern that computed values of  $f_m$  for test solutions could be confounded by this variation in  $k_m$ . Sensitivity analyses were conducted to evaluate the effect of varying  $k_m$  over the observed range (Table 1) on computed  $f_m$  for selected solutions. The results (Fig. 2) indicated that computed  $f_m$  is not particularly sensitive to moderate differences in  $k_m$  used to analyze the data. It was thus concluded that the average fitted value of  $k_m$  for 10, 14, and 20  $\mu M$  mononuclear Al standards obtained during each analysis could be used to accurately model the kinetics for the test solutions.

Using the beaker-stir bar variation of the 8-hydroxyquinoline procedure, no change in absorbance was observed when the reaction time for mononuclear standards was varied

Table 1. Typical variations in fitted second-order mononuclear rate coefficients using ferron and aluminon

$Al_i$ $\mu M$	Ferron -----L $\mu mol^{-1} min^{-1}$ -----	Aluminon
10	2.63	0.118
14	2.03	0.108
20	1.30	0.071

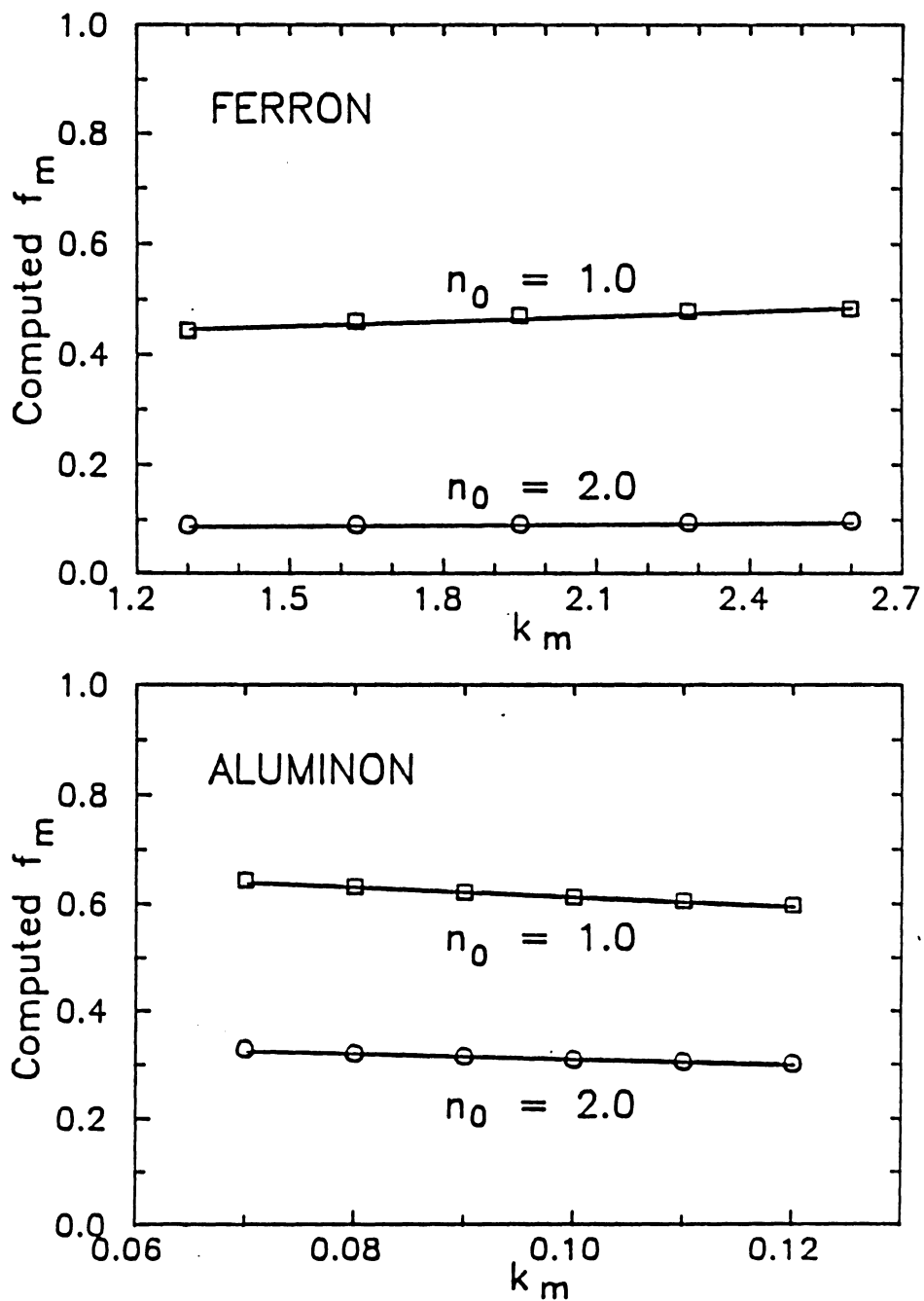


Figure 2. Effect of fitted second order mononuclear rate coefficients on the computed fraction of mononuclear Al by ferron and aluminon: All solutions contained  $20 \mu M$   $Al_t$  and  $0.4 mM$   $CaCl_2$ , and were aged 2 d before analysis.

from 2 to 60 s (data not shown). It was concluded that the reaction of 8-hydroxyquinoline with mononuclear Al is essentially instantaneous, in agreement with the findings of Turner (1969). Thus, a kinetic approach was obviated, and the 15-s reaction proposed by James et al. (1983) was used for most of the following results.

## Method Comparisons

Two series of solutions with basicities ranging from -1.0 to 2.25 were prepared. One series contained 0.4 mM CaCl<sub>2</sub>, the other 40 mM NaCl, corresponding to the range in ionic strength employed in some related phytotoxicity studies (Kinraide and Parker, 1987). Each series was assayed twice -- after aging for 3 and 30 days. Representative results are presented in Fig. 3; the other two sets of results were quite similar and are omitted here. Increasing ionic strength from *ca.* 1.2 to 40 mM increased values of  $f_m$  by approximately 0.1 to 0.3 in solutions of  $n_0 > 0$ , and the increase was observed for all three methods (Fig. 3). Surprisingly, there seems to be little literature addressing this phenomenon. Hsu (1977) noted that increasing concentrations of a relatively indifferent anion such as Cl<sup>-</sup> will have little effect on the uptake of added hydroxyl by Al, but will suppress spontaneous hydrolysis and polymer growth, presumably via anion shielding of positively-charged, bound waters. Alternatively, these results might be explained by ionic strength effects on activity coefficients, from which one would predict greater solubility of solid phase Al(OH)<sub>3</sub>, and thus reduced formation of the metastable polynuclear species, at the higher ionic strength. However, all results followed the same general pattern, regardless of ionic strength. Estimates of  $f_m$  by ferron and 8-hydroxyquinoline closely paralleled one another over the entire range of  $n_0$ , the latter method yielding estimates about 0.1 higher (Fig. 3). These differences suggest that 8-hydroxyquinoline

often measures a kinetically-reactive portion of the polynuclear Al, in agreement with the findings of Turner (1969), and inferable from those of James et al. (1983).

A supplementary study was conducted in an attempt to achieve better agreement in  $f_m$  between the ferron and 8-hydroxyquinoline methods by employing an instantaneous reaction for the latter. Solutions with  $n_0$  ranging from 0 to 2.25 were assayed after 7 d aging. The reaction was conducted in a beaker with a stir-bar as described above, and stopped via injection of butyl acetate immediately after sample injection. Assays by this method yielded estimates of  $f_m$  comparable to those obtained by ferron at  $n_0 \geq 1.5$ , but estimates *ca.* 0.11 greater were obtained at lower basicities (data not shown). These results suggest that, at low basicities, small polymers are included in  $f_m$  when using 8-hydroxyquinoline, regardless of reaction time. At basicities  $\geq 1.5$ , degradation of polynuclear Al by this reagent appears to depend on the duration of reaction. In addition, precision with 8-hydroxyquinoline was somewhat reduced using the instantaneous reaction, but it seems likely that this could be overcome with practice.

Results using the aluminon method showed a consistent pattern wherein lower estimates of  $f_m$  were obtained at low values of  $n_0$  as compared to the other two methods (Fig. 3). As  $n_0$  increased, a crossover occurred such that aluminon yielded estimates of  $f_m$  as high or higher than 8-hydroxyquinoline at  $n_0 = 2.25$  (Fig. 3). Although these results suggest that aluminon may not be as sensitive to changes in Al speciation as the other two methods, the cause of this discrepancy remains obscure. At low values of  $n_0$ , a differential reaction rate with the monomeric hydrolysis products seems unlikely, given the generally accepted notion that equilibrium between these Al species is virtually instantaneous (Hsu, 1977). It is possible that very small polynuclear units exist at low basicities, and that these species were included in the mononuclear fraction as deter-

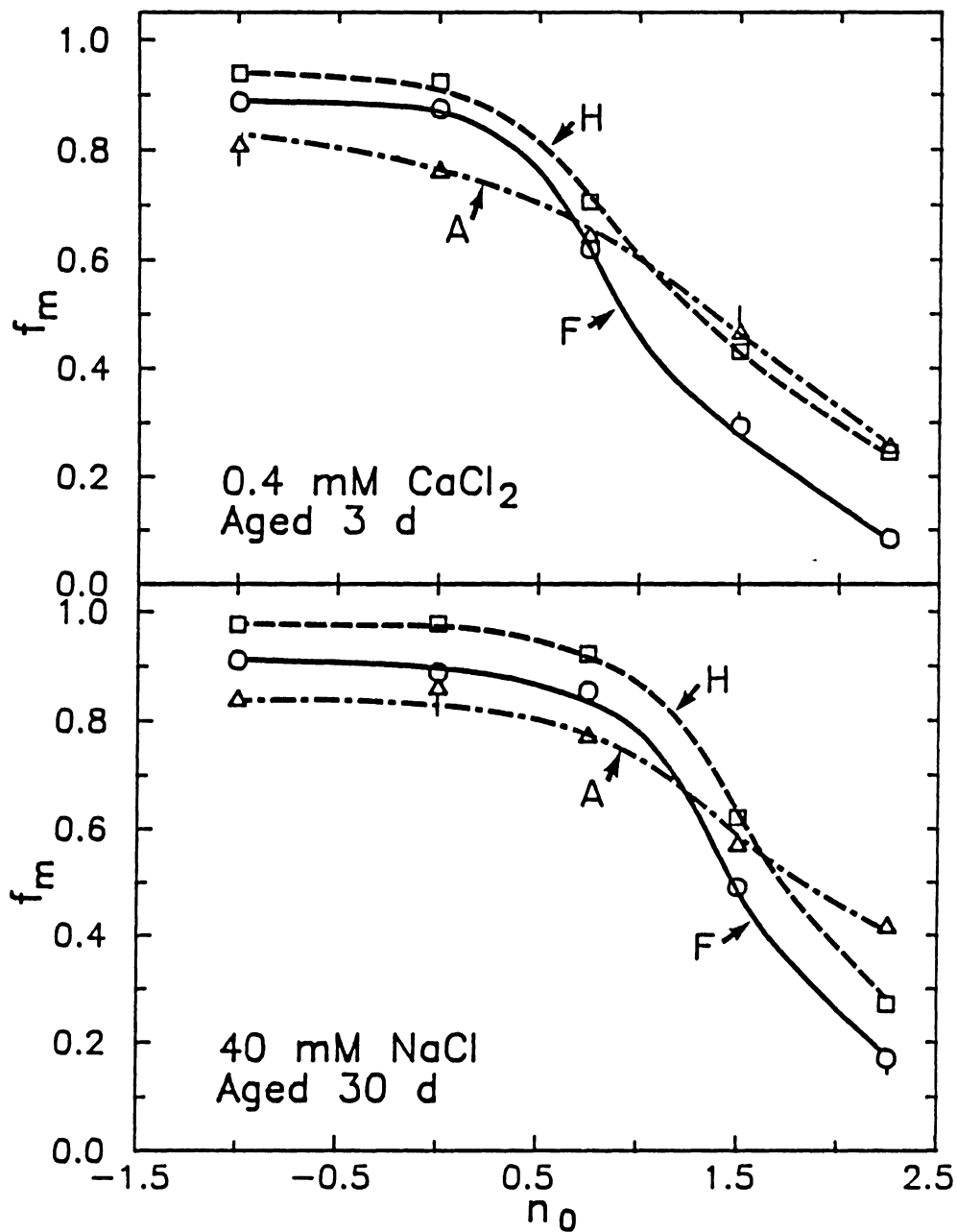


Figure 3. Fraction of mononuclear Al vs. basicity for the three methods for two sets of solutions at differing ages: F = ferron, A = aluminon, and H = 8-hydroxyquinoline. Solutions contained  $20 \mu\text{M}$   $\text{Al}_T$ . Vertical bars indicate standard errors of duplicate determinations where  $1 \text{ SE} > \text{symbol size}$ .

mined by ferron and 8-hydroxyquinoline, but not by aluminon. Bertsch et al. (1986) attributed discrepancies between ferron and  $^{27}\text{Al}$  NMR analyses to the presence of small polymers, perhaps dimers and trimers, that were included in  $f_m$  by ferron. At present, it is uncertain which method yields the most accurate estimate of  $f_m$  at low values of  $n_0$ . At higher basicities, it would appear that aluminon more aggressively degrades polynuclear Al, leading to the highest estimates of  $f_m$ . Alternatively, complexes may have formed between aluminon and polynuclear Al that increased absorbance (Hsu, 1963), thus yielding overestimates of  $f_m$ .

All three methods detected some polynuclear Al at  $n_0 = -1$  and 0. In general, Al salts dissolved in water without added base are thought to be essentially 100% mononuclear (Hsu, 1977). Exceptions include the findings of Smith and Hem (1972), although it is necessary to extrapolate their data to  $n_0 = 0$ , and those of Bersillon et al. (1980) and Blamey et al. (1983), although none of these studies reported  $f_m$  values below 0.9 for  $n_0 = 0$  solutions. At  $n_0 = 0$ ,  $f_m$  values as low as 0.81, 0.73, and 0.92 were observed with ferron, aluminon, and 8-hydroxyquinoline, respectively, and only slightly higher minimum values at  $n_0 = -1$ . The concentrations of Al used in the present study are rather low in comparison to most speciation research. The quantity of polymers observed at low basicities here are most likely due to oversaturation with respect to solid phase  $\text{Al}(\text{OH})_3$  in very dilute Al solutions without added acid. Frink and Peech (1963) observed increased apparent degrees of supersaturation with respect to  $\text{Al}(\text{OH})_3$  with increasing dilution of simple Al salts. Decreasing solution pH with aging indicated that hydrolysis was proceeding. Although they did not attempt to quantify the polynuclear species, these trends are consistent with the observation that  $f_m$  is consistently  $< 1$  for very dilute solutions with  $n_0 = 0$ .

With ferron, use of either the 30 s cutoff proposed by Bersillon et al. (1980) or the kinetic approach resulted in very similar values of  $f_m$  at low values of  $n_0$  ( $< 1.0$ ). However, as  $n_0$  increased, use of the arbitrary cutoff yielded estimates of  $f_m$  up to 0.12 greater than those obtained via kinetic analysis. Similarly, Jardine and Zelazny (1987) reported that agreement between a 30 s cutoff and the kinetic approach was quite good at  $n_0 < 1.5$ , but that differences increased with further increases in basicity. Using the 30 min cutoff proposed by Blamey et al. (1983), aluminon yielded estimates of  $f_m$  up to 0.17 greater than those obtained using the kinetic model. It was thus concluded that the kinetic approach is generally superior for these two methods. It was observed, however, that differences between the two approaches diminish with increasing solution age, presumably because the quantity of reactive polymers decreases (Smith and Hem, 1972; Turner, 1976). When only mononuclear Al and very unreactive polynuclear complexes or colloidal  $\text{Al}(\text{OH})_3$  are present, use of an arbitrary cutoff or the kinetic model should yield similar estimates of  $f_m$ , regardless of  $n_0$ .

## Effects of Solution Age

A single series (0.4 mM in  $\text{CaCl}_2$ ) of solutions with  $n_0$  ranging from 0 to 2 was assayed at 2, 4, 8, 16, and 32 days after preparation. With ferron and 8-hydroxyquinoline, estimates of  $f_m$  showed no consistent, substantial effect of solution age (Fig. 4), consistent with the findings of Smith and Hem (1972), Tsai and Hsu (1984,1985), and Jardine and Zelazny (1986). Turner (1976) observed increases in  $f_m$  of about 0.15 from 4 to 790 d aging using 8-hydroxyquinoline; over the first 30 d, however,  $f_m$  increased very little.



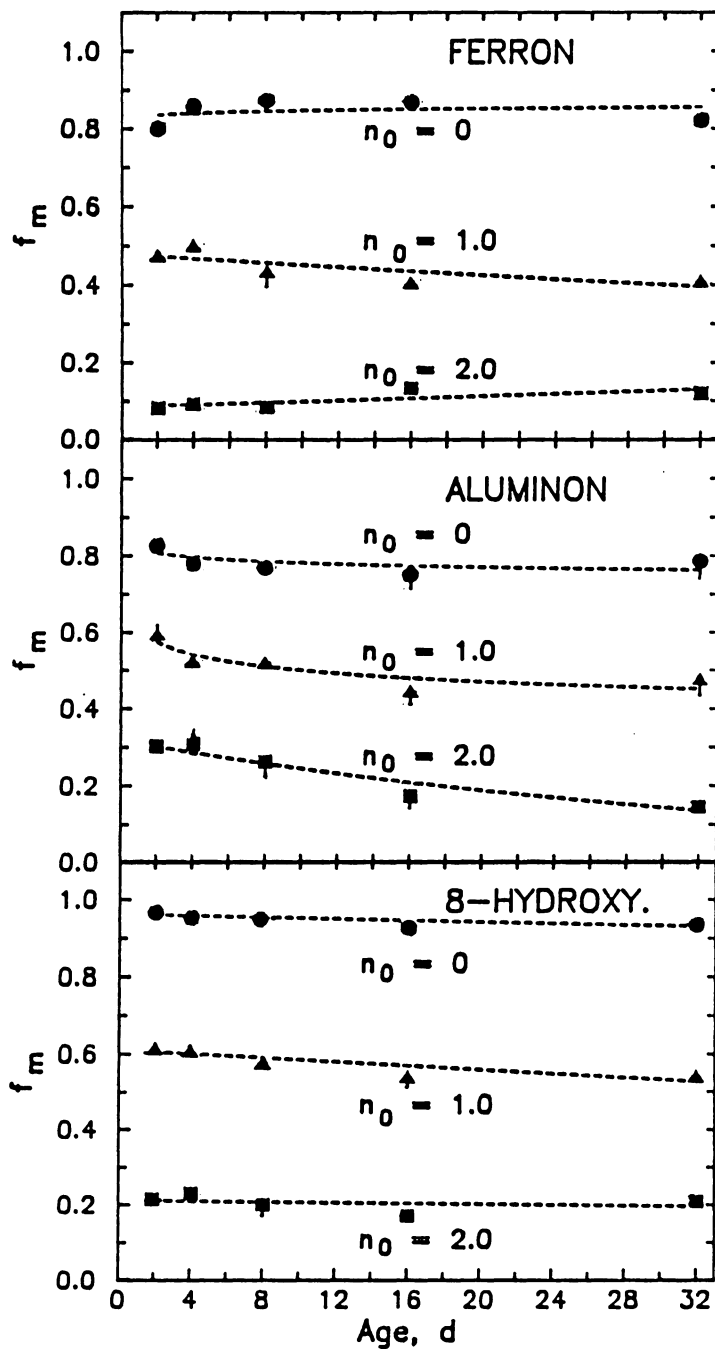


Figure 4. Computed fraction of mononuclear Al vs. solution age at three basicities for the three methods: All solutions contained  $20 \mu\text{M Al}_t$  and  $0.4 \text{ mM CaCl}_2$ . Vertical bars indicate standard errors of duplicate determinations where  $1 \text{ SE} > \text{symbol size}$ .

Estimates of  $f_m$  by aluminon showed more consistent decreases with increasing solution age (Fig. 4). Thus, with aging, estimates of  $f_m$  at high values of  $n_0$  were more in agreement with those obtained by ferron and 8-hydroxyquinoline. This may be ascribed to structural and/or size changes of polymeric Al that decrease its reactivity with aluminon, and thus reduce its inclusion in the monomeric fraction by this method. However, at low basicities, aluminon still yielded the lowest estimates of  $f_m$  regardless of solution age. It remains unclear whether this is a strength or a weakness of this method (see above).

## Sensitivity and Precision

As an indicator of the sensitivity of the three methods, typical absorbances for a 20  $\mu M$  mononuclear Al standard combined with the appropriate reagent mixture are as follows: 0.12, 0.24, and 0.26 absorbance units (a.u.) for ferron, aluminon, and 8-hydroxyquinoline, respectively. Thus, ferron is only about one-half as sensitive as the other two methods, and at Al<sub>i</sub> much less than 10  $\mu M$ , instrument resolution (about 0.003 a.u.) will limit accuracy. However, it was found that by adjusting the molar ratio of ferron/Al to 50, sensitivity can be enhanced somewhat at lower values of Al<sub>i</sub>. For example, a 10  $\mu M$  Al solution at a volumetric reagent/sample ratio of 0.4 yields an absorbance of about 0.075, as opposed the value of 0.06 obtained with the 0.8 ratio normally used in this study.

A single series (0.4 mM in CaCl<sub>2</sub>) of solutions with  $n_0$  ranging from -1 to 2 was assayed in quadruplicate 21 days after preparation. The results (Table 2) indicate that all three methods yield estimates of  $f_m$  with adequate precision for most purposes. The standard errors for the quadruplicate determinations were greatest for aluminon and least with

8-hydroxyquinoline. However, day-to-day experience suggested that the aluminon method is the most erratic of the three, and to a greater extent than is indicated by the data in Table 2. This may be ascribable to the fact that aluminon forms a color lake with Al (Frink and Peech, 1962), rather than a true complex. Greater problems with baseline drift were also noted with aluminon, especially if assays were conducted over several hours, as well as an extreme sensitivity to any deviations in temperature.

### **Additional Ferron Investigations**

Based on the above findings, the ferron method is preferred for routine estimation of mononuclear Al, at least in the simple chemical media employed for studying Al phytotoxicity (Kinraide and Parker, 1987). The method may not be suitable for soil solutions (Wright et al., 1987), and its lack of sensitivity may limit its use with crops that are sensitive to very low levels ( $< 1 \mu M$ ) of Al (Kinraide et al., 1985). Otherwise, it demonstrates good precision, and offers the further advantage that the nature and reactivity of the polynuclear Al species can be readily studied kinetically, and within reasonable times (Smith, 1971; Smith and Hem, 1972; Jardine and Zelazny, 1986). In contrast, the aluminon method is hampered by very slow kinetics, which precludes convenient study of polymer reactivity, and suffers from some lack of precision. The 8-hydroxyquinoline method can readily provide precise estimates of  $f_m$ , but may tend to overestimate this quantity. Moreover, although 8-hydroxyquinoline has been employed to kinetically study polynuclear and solid-phase Al (Turner, 1969, 1976), a large number of solvent extractions is required for a single analysis. Such data are more readily acquired using ferron and a spectrophotometer with kinetics capabilities.

**Table 2. Quadruplicate analyses by the three speciation methods for solutions of varying basicity aged 21 d**

$n_0$	Ferron		Aluminon		8-hydroxyquinoline	
	$f_m$	SE†	$f_m$	SE	$f_m$	SE
-1	0.903	0.009	0.807	0.039	0.980	0.013
0	0.869	0.020	0.731	0.025	0.932	0.008
1	0.502	0.028	0.496	0.021	0.605	0.011
2	0.153	0.022	0.202	0.032	0.199	0.008

†standard error

At the initiation of this study, the primary goal was to obtain reliable estimates of  $f_m$ , but evidence that the polynuclear hydroxy-Al complexes can be phytotoxic (see Chapter III) warranted a closer examination of the reactions between these species and ferron. Jardine and Zelazny (1987) reported that the Al-ferron reaction could be modeled as two simultaneous second order reactions for the initial 250 s of the reaction. For the above results, that scheme was followed and good fits to the data were obtained. However, others have reported that the reaction with polynuclear Al is first order (Smith and Hem, 1972; Tsai and Hsu, 1984; Bertsch et al., 1986). A model wherein a first order reaction is used for the polymers could also describe my data quite well for the first 5 min of the reaction, and in fact, computed values of  $f_m$  never differed by more than 0.02 for the two models. This agreement can be ascribed to the fact that little polynuclear Al reacts within 5 min, such that the polymer reaction can be fit to either a first or second order reaction. Only by monitoring the reaction for longer times (e.g., 1 hour) was it possible to discern the true nature of the ferron-polynuclear Al reaction.

Another set of solutions with  $[\text{CaCl}_2] = 0.4 \text{ mM}$  and  $n_0 = 0.75, 1.50, \text{ and } 2.25$ , was prepared and then assayed after 3 and 35 d aging. A flattening of the absorbance-time function occurred at *ca.* 24 to 30 min (Fig. 5), corresponding to the completion of the reaction of ferron and reactive polymers (Smith and Hem, 1972). Subsequent increases in absorbance were small or negligible, and corresponded to unreactive polymers or colloidal hydroxy-Al ( $\text{Al}_c$  in the parlance of Smith and Hem [1972]). Linearized first order plots ( $\log [\text{Al}]_{\text{un}}$  vs.  $t$  [Moore and Pearson, 1981]) and second order plots were constructed for the reaction from 90 s to 24 min, under the assumption that  $\text{Al}_c$  did not contribute significantly to absorbance over this period. Conformity to first order kinetics for the reactive polymers was confirmed by the greater linearity observed for those plots (data not shown).

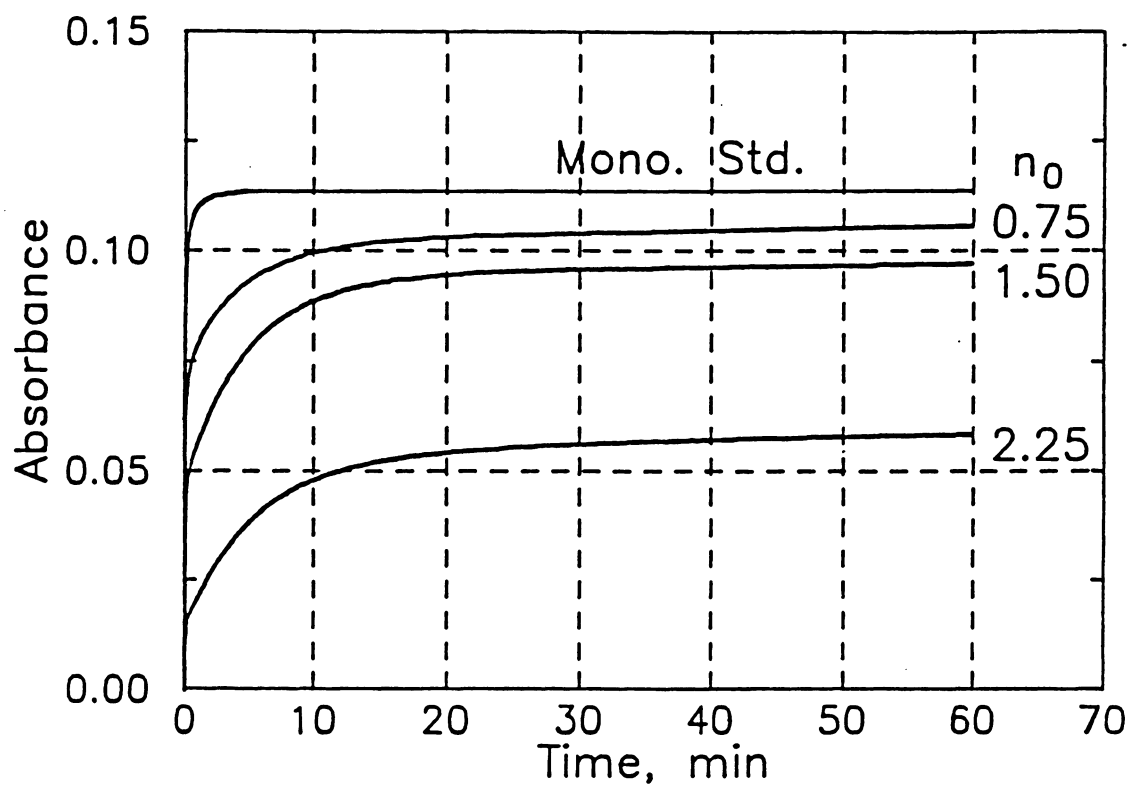


Figure 5. Absorbance vs. time plots for the reaction of ferron with Al solutions of varying basicity carried out to 1 hour: All solutions contained  $20 \mu M$   $Al_1$  and  $0.4 mM$   $CaCl_2$ , and were aged 3 d before analysis.

In order to properly apply Eq. II.3, the system must contain a mixture of just two species, or two types of species. However, at high basicities and over long reaction times,  $Al_2$  becomes a significant component, and a three-species model would be required. Consequently, application of Eq. II.3 to the first 24 min of the ferron reaction resulted in poor fits and high estimates of  $f_m$  (Fig. 6a and Table 3). These errors can be corrected by subtracting the  $Al_2$  fraction from  $Al_1$  to obtain the *effective* concentration of Al available to react with ferron, and computing  $[Al]_{un}$  accordingly. The Al unreacted at 30 min was used to estimate  $Al_2$ , under the assumption that its reaction with ferron was negligible during this period. This assumption seemed reasonable given the very slow reaction of  $Al_2$  observed after 30 min (Fig. 5), but more exact corrections can be made on a point-by-point basis using an assumed reaction order for  $Al_2$  (Smith, 1971; Tsai and Hsu, 1984). Use of data corrected for  $Al_2$  resulted in much better fits to Eq. II.3 (Fig. 6b and Table 3). Agreement between computed values of  $f_m$  and those obtained using only the first 5 min of the reaction was remarkably good, regardless of the choice of  $n'$  (Table 3). Similar results were obtained for these solutions after 35 d aging (data not shown). Thus, when only the first 5 min of the reaction is monitored, correction for the  $Al_2$  fraction is unnecessary, and this time-saving approach is adequate when  $f_m$  is the quantity of interest. If the nature and reactivity of the polynuclear species are of interest, the reaction should be monitored for longer times so that  $Al_2$  can be estimated.

## *Conclusions*

1. Reactions of ferron and aluminon with mixtures of mono- and polynuclear Al-hydroxide species can be kinetically modeled as two simultaneous reactions, yielding

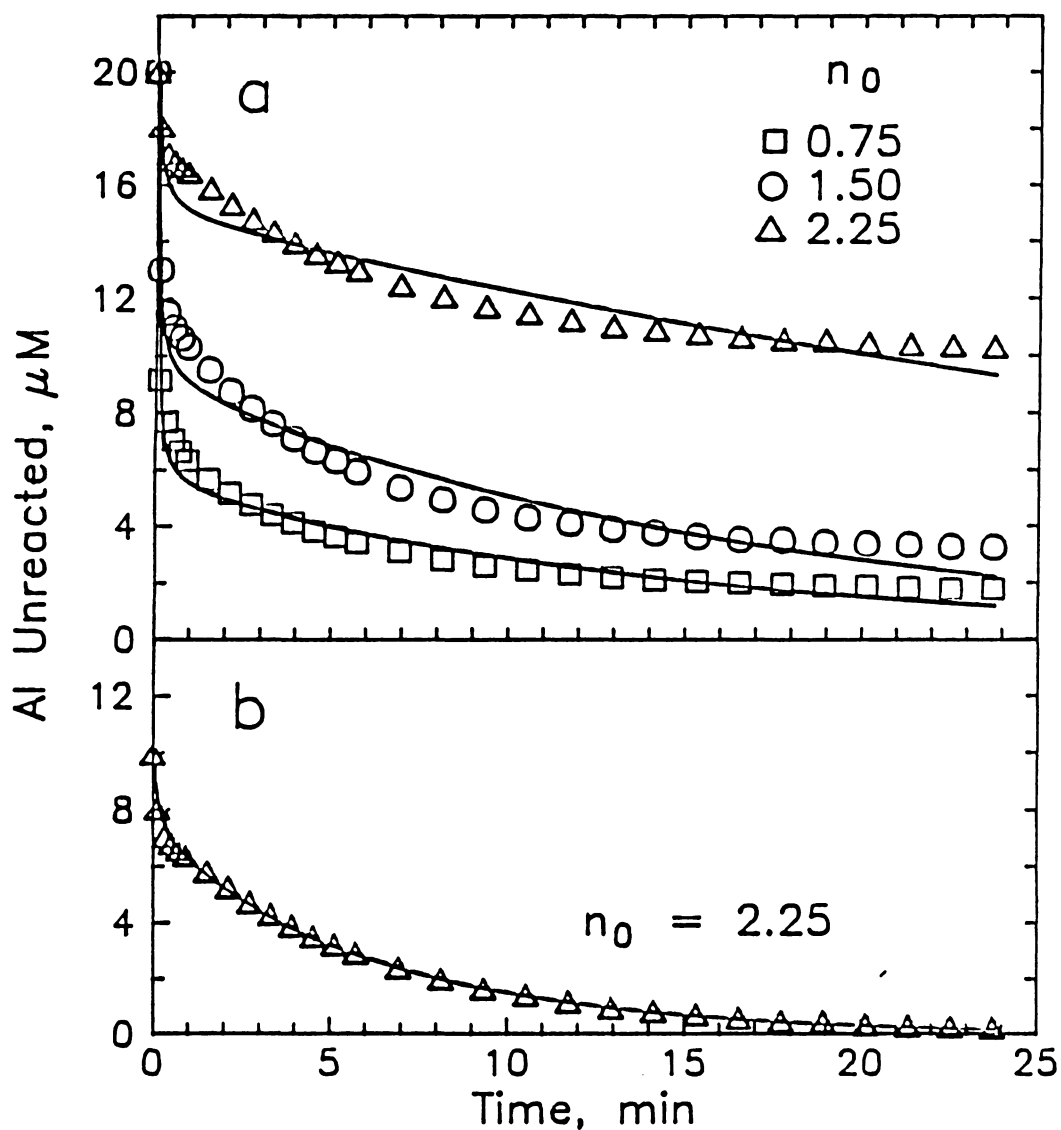


Figure 6. Unreacted Al vs. time for the partially-neutralized solutions depicted in Fig. 5: Symbols are for observed data, solid lines for best-fit regression lines. (a) Modeled as second-order monomeric reaction, first-order polymeric reaction, and not corrected for unreactive Al ( $\text{Al}_c$ ). (b) Modeled as in (a), but corrected for  $\text{Al}_c$ . Solutions with  $n_0 = 0.75$  and 1.5 are omitted for clarity, but showed equally good agreement between observed and fitted values.



Table 3. Computed fraction of mononuclear Al and coefficients of determination for the regression of the reactions depicted in Fig. 5 on Eq. II.3. The alternative kinetic models are all based on the same data representing single determinations at each value of  $n_0$ .

Model	Rxn. orders		Duration	Corrected for Al <sub>c</sub> ?	$n_0 = 0.75$		$n_0 = 1.50$		$n_0 = 2.25$	
	$n$	$n'$			$f_m$	$R^2$	$f_m$	$R^2$	$f_m$	$R^2$
			min							
1	2	1	24	no	0.721	0.957	0.542	0.936	0.283	0.905
2	2	2	24	no	0.661	0.988	0.446	0.985	0.256	0.933
3	2	1	24	yes	0.652	0.995	0.419	0.997	0.142	0.999
4	2	1	5	no	0.656	0.988	0.437	0.989	0.164	0.996
5	2	2	5	no	0.649	0.987	0.425	0.985	0.159	0.996

estimates of the mononuclear fraction. The reaction of 8-hydroxyquinoline with mononuclear Al is essentially instantaneous, and the use of an arbitrary cutoff time is unavoidable.

2. Although each method will provide a consistent, reproducible estimate of the mononuclear fraction, differences between methods occur. At low basicities, the estimates of  $f_m$  are in the order 8-hydroxyquinoline > ferron > aluminon, while at higher basicities the general order is aluminon  $\geq$  8-hydroxyquinoline > ferron. In comparison to the other methods, aluminon appears to be somewhat insensitive to changes in speciation.
3. Aging solutions up to 32 days results in decreased estimates of  $f_m$  using aluminon, but estimates using ferron and 8-hydroxyquinoline are only minimally affected.
4. At  $Al_i = 20 \mu M$ , all three methods yield results of adequate precision for most applications. The order of precision is 8-hydroxyquinoline > ferron > aluminon. Ferron is only one-half as sensitive as the other two methods, and may be of limited use at Al concentrations much below  $10 \mu M$ , although careful adjustment of the molar ferron/Al ratio can improve sensitivity somewhat.
5. Ferron may be the preferred method, at least in chemically simple media, due to its simplicity, precision, and moderately rapid kinetics. Additional information regarding the nature and reactivity of polynuclear and solid phase Al can be readily obtained using a kinetic approach. The best kinetic model is one where monomers obey a pseudo second-order rate law, reactive polymers a pseudo first-order law, and the data is corrected for the unreactive polynuclear and/or solid phase fraction.

## References

- Adams, F., and P. J. Hathcock. 1984. Aluminum toxicity and calcium deficiency in acid subsoil horizons of two Coastal Plains soil series. *Soil Sci. Soc. Am. J.* 48:1305-1309.
- Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986a. Effects of phosphorus/aluminum molar ratio and calcium concentration on plant response to aluminum toxicity. *Soil Sci. Soc. Am. J.* 50:133-137.
- Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986b. Relationships between root length of soybean and calculated activities of aluminum monomers in solution. *Soil Sci. Soc. Am. J.* 50:959-962.
- Baes, C. F., Jr., and R. E. Mesmer. 1976. *The hydrolysis of cations.* John Wiley and Sons, New York.
- Barnhisel, R., and P. M. Bertsch. 1982. Aluminum. *In* A. L. Page et al. (ed.) *Methods of soil analysis, Part 2.* 2nd ed. *Agronomy* 9:275-300.
- Bersillon, J.L., P.H. Hsu, and F. Fiessinger. 1980. Characterization of hydroxy-aluminum solutions. *Soil Sci. Soc. Am. J.* 44:630-634.
- Bertsch, P. M., W. J. Layton, and R. I. Barnhisel. 1986. Speciation of hydroxy-aluminum solutions by wet chemical and aluminum-27 NMR methods. *Soil Sci. Soc. Am. J.* 50:1449-1454.
- Blamey, F. P. C., D. G. Edwards, and C. J. Asher. 1983. Effects of aluminum, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. *Soil Sci.* 136:197-207.
- Bloom, P.R., R.M. Weaver, and M.B. McBride. 1978. The spectrophotometric and fluorometric determination of aluminum with 8-hydroxyquinoline and butyl acetate extraction. *Soil Sci. Soc. Am. J.* 42:713-716.
- Campbell, P. G. C., M. Bisson, R. Bougie, A. Tessier, and J. Villeneuve. 1983. Speciation of aluminum in acidic freshwaters. *Anal. Chem.* 55:2246-2252.
- Davenport, W. H. 1949. Determination of aluminum in the presence of iron. *Anal. Chem.* 21:710-711.
- Frink, C. R., and M. Peech. 1962. Determination of aluminum in soil extracts. *Soil Sci.* 93:317-324.
- Frink, C.R. and M. Peech. 1963. Hydrolysis of the aluminum ion in dilute aqueous solutions. *Inorg. Chem.* 2:473-478.

- Froment, G. F., and K. B. Bischoff. 1979. Chemical reactor analysis and design. John Wiley and Sons, New York.
- Hodges, S. C. 1987. Aluminum speciation: A comparison of five methods. *Soil Sci. Soc. Am. J.* 51:57-64.
- Hsu, P. H. 1963. Effect of initial pH, phosphate, and silicate on the determination of aluminum with aluminon. *Soil Sci.* 96:230-238.
- Hsu, P. H. 1968. Interactions between aluminum and phosphate in aqueous solution. *Adv. Chem. Ser.* 73:115-127.
- Hsu, P. H. 1977. Aluminum hydroxides and oxyhydroxides. p. 99-143. *In* J. B. Dixon and S. B. Weed (ed.) *Minerals in soil environments*. Soil Science Society of America, Madison, WI.
- James, B. R., C. J. Clark, and S. J. Riha. 1983. An 8-hydroxyquinoline method for labile and total aluminum. *Soil Sci. Soc. Am. J.* 47:893-897.
- Jardine, P. M., and L. W. Zelazny. 1986. Mononuclear and polynuclear aluminum speciation through differential kinetic reactions with ferron. *Soil Sci. Soc. Am. J.* 50:895-900.
- Jardine, P. M., and L. W. Zelazny. 1987. Influence of inorganic anions on the speciation of mononuclear and polynuclear aluminum by ferron. *Soil Sci. Soc. Am. J.* 51:889-892.
- Kinraide, T. B., R. C. Arnold, and V. C. Baligar. 1985. A rapid assay for aluminium phytotoxicity at submicromolar concentrations. *Physiol. Plant.* 65:245-250.
- Kinraide, T. B., and D. R. Parker. 1987. Cation amelioration of aluminum toxicity in wheat. *Plant Phys.* 83:546-555.
- Mark, H. B., Jr., and G. A. Rechnitz. 1968. *Kinetics in analytical chemistry*. John Wiley and Sons, New York.
- May, H. M., P. A. Helmke, and M. L. Jackson. 1979. Determination of mononuclear dissolved aluminum in near-neutral waters. *Chem. Geol.* 24:259-269.
- Moore, J. W., and R. G. Pearson. 1981. *Kinetics and mechanisms*. 3rd ed. John Wiley and Sons, New York.
- Okura, T., K. Goto, and T. Yotuyanagi. 1962. Forms of aluminum determined by an 8-quinolinolate extraction method. *Anal. Chem.* 34:581-582.
- Rengasamy, P. and J. M. Oades. 1978. Interaction of monomeric and polymeric species of metal ions with clay surfaces. III. Aluminum (III) and chromium (III). *Aust. J. Soil Res.* 16:53-66.
- Smith, R. W. 1971. Relations among equilibrium and nonequilibrium aqueous species of aluminum hydroxy complexes. *Adv. Chem Ser.* 106:250-279.

- Smith, R. W., and J. D. Hem. 1972. Effect of aging on aluminum hydroxide complexes in dilute aqueous solutions. U.S. Geol. Surv. Water-Supply Paper 1827-D.
- Tsai, P.P., and P.H. Hsu. 1984. Studies of aged OH-Al solutions using kinetics of Al-ferrous reactions and sulfate precipitation. *Soil Sci. Soc. Am. J.* 48:59-65.
- Tsai, P.P., and P.H. Hsu. 1985. Aging of partially neutralized aluminum solutions of sodium hydroxide/aluminum molar ratio = 2.2. *Soil Sci. Soc. Am. J.* 49:1060-1065.
- Turner, R.C. 1969. Three forms of aluminum in aqueous systems determined by 8-quinolinolate extraction methods. *Can. J. Chem.* 47:2521-2527.
- Turner, R.C. 1976. Effect of aging on properties of polynuclear hydroxyaluminum cations. *Can. J. Chem.* 54: 1528-1534.
- Wright, R. J., V. C. Baligar, and S. F. Wright. 1987. Estimation of phytotoxic aluminum in soil solution using three spectrophotometric methods. *Soil Sci.* 144:224-232.

# Chapter III

## Aluminum Speciation and Phytotoxicity in Dilute Hydroxy-Aluminum Solutions

### *Abstract*

Although it has long been recognized that toxicity of aluminum (Al) to plants is a major growth-limiting factor in acid soils, relationships between the aqueous chemistry of Al and phytotoxicity are still not fully understood. The effect of added hydroxyl ions remains particularly problematic in that, although increasing solution pH generally relieves Al toxicity, it is unclear whether this should be ascribed to differential toxicity of the mononuclear hydrolysis products, or to formation of nontoxic polynuclear hydroxy-Al complexes or solid phase  $\text{Al}(\text{OH})_3$ . Solution culture techniques were used to examine the relationships between Al speciation and phytotoxicity in dilute, chemically well-defined systems wherein  $\text{OH}^-$  was the only Al-complexing ligand. Two-

day old wheat (*Triticum aestivum* L.) seedlings were transferred to 500-mL test solutions for 2 days of additional growth at 298 K in the dark, and final root lengths used as the measure of Al toxicity. Quantities of polynuclear hydroxy-Al were estimated with a modified version of the ferron procedure, and activities of mononuclear species were calculated using the computer program GEOCHEM. In the absence of polynuclear hydroxy-Al, increasing solution pH consistently ameliorated Al toxicity suggesting that, although  $\text{Al}^{3+}$  is not necessarily the sole toxic species, its activity is the best single indicator of Al stress. The results did not confirm other reports that the sum of the mononuclear species activities is a reliable predictor of Al phytotoxicity. Polynuclear hydroxy-Al was also demonstrably toxic, supporting isolated reports of the same finding. The common practice of excluding the polynuclear fraction from estimates of toxic Al should thus be reevaluated. These findings have practical implications for the accurate prediction of toxic Al levels in both solution culture media and soil solutions.

## ***Introduction***

Soil scientists have long been interested in the chemical factors that govern Al toxicity to plants in acid soils, and efforts to relate toxicity to speciation in soil solution have met with variable degrees of success (Adams and Lund, 1966; Brenes and Pearson, 1973; Pavan et al., 1982; Adams and Moore, 1983; Adams and Hathcock, 1984; Wright et al., 1987). In addition, recent concerns about the possible mobilization of soil Al by acid deposition, and its deleterious effects on terrestrial vegetation (Ulrich et al., 1980) and aquatic biota (Driscoll et al., 1980), have prompted further interest in Al speciation-toxicity relationships. However, because soil solution and natural waters are inherently

complex, basic principles need to be firmly established under controlled chemical conditions. To that end, Al phytotoxicity has often been studied using solution culture techniques wherein root elongation is used as the primary measure of Al stress.

In nutrient solution studies, amelioration of Al toxicity has been achieved by the addition of various solutes to the media. It is now apparent that additions of simple salts such as CaCl<sub>2</sub> and NaCl alleviate toxicity through both a reduction in the activity of toxic Al (ionic strength effect) as well as a direct physiological effect of the cation (Alva et al., 1986a; Kinraide and Parker, 1987a). Ligands such as sulfate (Kinraide and Parker, 1987b), fluoride (Cameron et al., 1986), and organic acids (Hue et al., 1986) seem to ameliorate toxicity in direct relation to the extent to which they complex Al. However, the effect of hydroxyl ions remains problematic. That raising solution pH generally relieves Al toxicity is well-documented; it is unclear whether this effect should be ascribed to differential toxicity of the mononuclear hydrolysis products, or to formation of non-toxic polynuclear hydroxy-Al complexes or solid phase Al(OH)<sub>3</sub>. A third, previously untested hypothesis is that there may be a physiological response whereby low pH predisposes the plant to Al injury, and that improved root growth with increasing pH is to some degree independent of changes in Al speciation. Low pH, especially in conjunction with low concentrations of Ca, may disrupt root cell membrane structure and function (Moore, 1974; Foy, 1984), and membranes may be critical in Al toxicity -- either as a barrier to cytoplasmic accumulation of Al (Moore, 1974) or as a site of primary lesion (Foy, 1984; Haug and Caldwell, 1985).

Although recent articles (Adams and Hathcock, 1984; Alva et al., 1986b; Hue et al., 1986) have cited reports that purport a correlation between phytotoxicity and the activity of the Al<sup>3+</sup> species, close inspection of the original reports (Adams and Lund, 1966;



Brenes and Pearson, 1973; Pavan and Bingham, 1982; Pavan et al., 1982) raises questions as to the validity of those correlations. Working with both nutrient solutions and displaced soil solutions, Adams and Lund (1966) assumed that all Al was in the  $Al^{3+}$  form when calculating the activity of this species, despite a pH range of 4.9 to 5.7. Brenes and Pearson (1973) apparently followed the same procedure in their studies of displaced soil solutions. Although Pavan and Bingham (1982) accounted for Al hydrolysis in their nutrient solutions, pH was fixed at  $4.0 \pm 0.2$ . Thus, the ratio of  $Al^{3+}$  activity to those of all other species was essentially constant, rendering it impossible to draw any inferences about differential toxicity of species.

Moore (1974) observed that inhibition of wheat (*Triticum aestivum* L.) root growth by Al went through a maximum at pH 4.5, and concluded that one of the monomeric hydrolysis products, probably  $AlOH^{2+}$ , was the primary toxic species. Despite seemingly high basicities for many solutions, however, no attempt was made to account for polynuclear Al. Blamey et al. (1983) studied soybean (*Glycine max* (L.) Merr.) root elongation in nutrient solutions varying in pH, P, and Al. Having accounted for polynuclear Al via a modified aluminon method, they obtained a high correlation between relative root length and the sum of the activities of all mononuclear species. But, they noted that there was a high degree of collinearity among the various mononuclear species, and that it was thus difficult to infer whether some were more toxic than others. Subsequently, Alva et al. (1986b) reported higher correlations between toxicity to several crops and the activity of  $AlOH^{2+}$  or  $Al(OH)_2^+$  than with any other species activity. However, the correlations were generally no better than those obtained using the sum of the mononuclear species, and suffered from similar complications due to collinearity.

Many of the reports cited above suggest that phytotoxicity should be ascribed only to mononuclear Al. In contrast, Bartlett and Riego (1972) reported that polynuclear hydroxy-Al was quite toxic. They were able to prepare stable hydroxy-Al solutions with pH levels as high as 6.8 that were highly inhibitory to corn (*Zea mays* L.) root growth, but noted that additions of phosphate induced precipitation of Al and thus ameliorated toxicity. Although limited to a few observations, data presented by Wagatsuma and Ezoe (1985) also indicated that polynuclear Al can be equally as toxic as the mononuclear forms.

Clearly, Al toxicity cannot at this time be attributed solely to mononuclear Al, nor to any one (or more) of the individual mononuclear species. Consequently, the objectives of this research were to use solution culture techniques to clarify the relationships between speciation and phytotoxicity in dilute hydroxy-Al solutions containing no other Al-complexing ligands. Lanthanum (La), a nonhydrolyzing trivalent metal that is also phytotoxic, was used in an attempt to evaluate any speciation-independent effects of pH on Al toxicity.

## ***Materials and Methods***

### **Solution Culture Methodology**

Wheat seeds were germinated on moist filter paper in the dark for 2 d. Five seedlings were selected for a uniform primary root length of  $15 \pm 2$  mm and transferred to each 500-mL test solution for 2 d of additional growth at  $298 \pm 1$  K in the dark. The seedlings

were floated in the solutions on slabs of closed-cell polyethylene foam. Beakers were loosely covered to minimize evaporation and the solutions gently aerated. At termination, the lengths of the longest two roots of each seedling were recorded, and the means for each beaker used as the measure of Al toxicity. For selected experiments, root growths relative to the control (0 Al) treatments were computed by subtracting the initial 15 mm from all final root lengths. All test solutions contained a minimum of 0.4 mM CaCl<sub>2</sub>, but this concentration was varied as needed to manipulate sensitivity to Al (Kinraide and Parker, 1987a). The only other additions were Al or La from stocks described below, and HCl for pH adjustment. Despite the absence of other nutrients, root elongation in the absence of Al or La was vigorous ( $\approx 1 \text{ mm hr}^{-1}$ ). Previous reports provide additional details of the culture method and demonstrate its utility as a sensitive assay for Al toxicity (Kinraide et al., 1985; Kinraide and Parker, 1987a, 1987b). For most experiments the Al-sensitive variety 'Tyler' was used, but the more tolerant 'Seneca' was used in selected experiments as well.

### **Solution Preparation and Speciation**

All reagents were of analytical or higher purity. Double deionized water was used throughout, and all glassware was acid-washed. All growth solutions were prepared from stock solutions of AlCl<sub>3</sub> • 6H<sub>2</sub>O or LaCl<sub>3</sub> • 7H<sub>2</sub>O (0.5 or 1.0 mM). Total Al or La content of stock solutions was determined using inductively-coupled plasma emission spectroscopy (ICPES) and standards prepared from Al wire or La<sub>2</sub>O<sub>3</sub>. Where it was necessary to add base to the Al solutions, 0.5 mM Al stocks were preadjusted by slow injection (0.5 mL min<sup>-1</sup>) of standardized 0.05 M NaOH using an autoburette.

The pH of all growth solutions was measured with a Radiometer PHM84 meter equipped with glass and calomel electrodes. In selected experiments, a modified version of the spectrophotometric ferron procedure was used to estimate the fraction of mononuclear Al (Chapter II). A 5-min reaction was employed, and the data fit to a simultaneous kinetic model using a pseudo second-order rate law for monomers and a pseudo first-order law for reactive polymers. Polynuclear Al was computed as the difference between total Al (based on the ICPEES analysis of the stock solution) and the ferron-based estimate of mononuclear Al. Activities of mononuclear species were computed with a modified version (Parker et al., 1987 and errata in Soil Sci. Soc. Am. J., 51:1092 and 1680) of the program GEOCHEM (Sposito and Mattigod, 1980). Although disparate values for the Al hydrolysis constants have been reported (see Baes and Mesmer, 1976; Sadiq and Lindsay, 1979), the values given by Lindsay (1979) were selected for these computations (Table 4). Hydrolysis constants for La were taken from Baes and Mesmer (1976); all solutions were computed to contain essentially only the  $\text{La}^{3+}$  ion. Similarly, reported formation constants for La-Cl complexes were judged to be small enough that these species could be ignored.

Root growths were related to the concentrations of total Al ( $[\text{Al}]$ ) and mononuclear Al ( $[\text{Al}_m]$ ); to the fraction of polynuclear Al ( $f_p$ ); to the activity of  $\text{Al}^{3+}$  ( $\{\text{Al}^{3+}\}$ ); and to the sum of the mononuclear species activities ( $\{\text{Al}_m\}$ ). In this study, all nonmononuclear Al was included in  $f_p$ ; no distinction was made between reactive polymers, unreactive polymers, or precipitated hydroxy-Al (Smith, 1971; Turner, 1976).

**Table 4. Thermodynamic constants used for computed speciation of growth solutions.**

Reaction	log <i>K</i>
$\text{Ca}^{2+} + \text{Cl}^- = \text{CaCl}^+$	-1.00
$\text{Ca}^{2+} + 2\text{Cl}^- = \text{CaCl}_2^0$	0.00
$\text{Al}^{3+} + \text{H}_2\text{O} = \text{AlOH}^{2+} + \text{H}^+$	-5.02
$\text{Al}^{3+} + 2\text{H}_2\text{O} = \text{Al}(\text{OH})_2^+ + 2\text{H}^+$	-9.30
$\text{Al}^{3+} + 3\text{H}_2\text{O} = \text{Al}(\text{OH})_3^0 + 3\text{H}^+$	-14.99
$\text{Al}^{3+} + 4\text{H}_2\text{O} = \text{Al}(\text{OH})_4^- + 4\text{H}^+$	-23.33
$\text{La}^{3+} + \text{H}_2\text{O} = \text{LaOH}^{2+} + \text{H}^+$	-8.5

## *Results*

### **Initial Al Experiments**

Initial experiments using Tyler wheat were factorial in pH (4.2 to 5.0) and  $Al_t$  (0 to 12  $\mu M$ ). Root growth improved as pH increased up to 4.8 (Fig. 7), but consistently declined at the highest pH where  $Al_t = 8$  or 12  $\mu M$  (filled symbols in Fig. 7). Plotting the results as root length vs. computed  $\{Al^{3+}\}$  resulted in a close fit to an exponential function (Fig. 8a), with the exception of the above-mentioned two points (filled symbols in Fig. 8a). In contrast, root length was not well-correlated with  $\{Al_m\}$  and, within a given  $Al_t$ , the correlation was positive rather than negative (Fig. 8b).

These results suggest that, in contrast to other recent reports (Blamey et al., 1983; Alva et al., 1986b),  $\{Al_m\}$  is not a reliable predictor of Al toxicity. However, the experiments depicted in Figs. 7 and 8 were imperfect in that the solutions were not assayed for polynuclear Al, and it was assumed that none was present in the absence of added base. Subsequent research suggested that small but significant quantities of polynuclear Al may be present in solutions with no added base, and even in slightly acidified solutions (Chapter II). Moreover, where  $Al_t$  is varied widely, interpretation of data can be confounded by collinearity among the various Al species (Blamey et al., 1983). Consequently, new experiments were designed to avoid these complications.

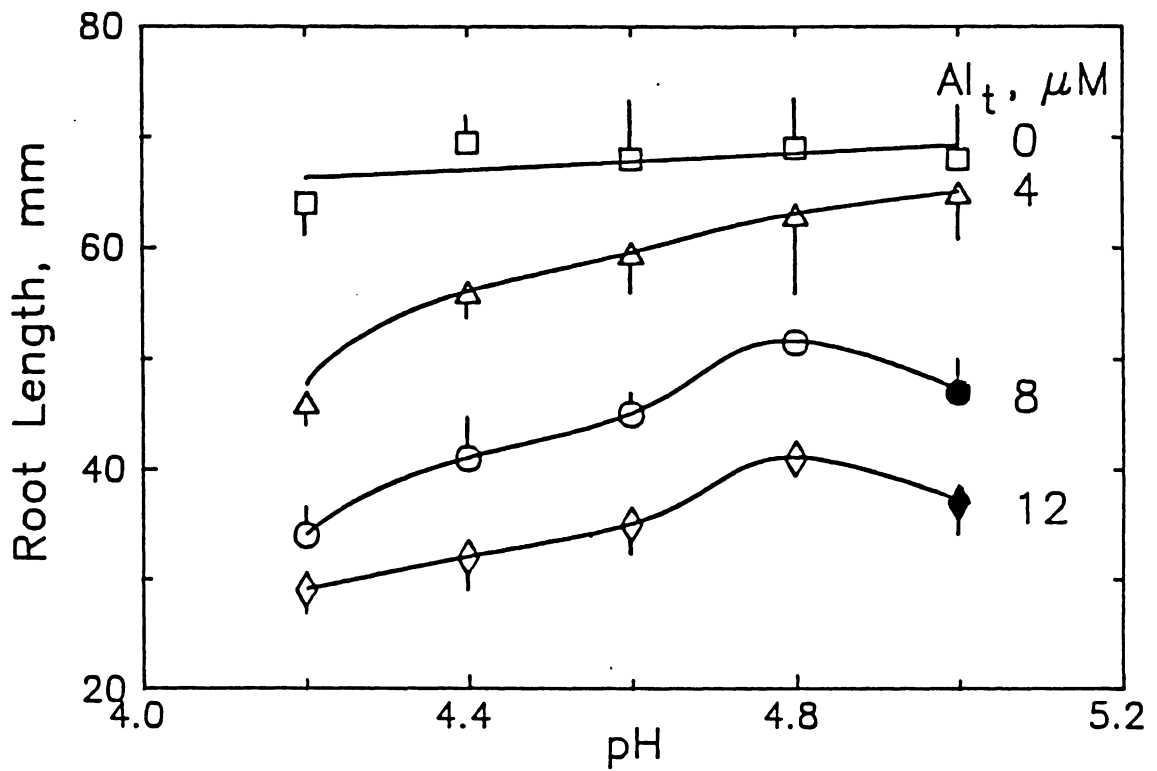


Figure 7. Root length of Tyler wheat vs. solution pH at three levels of total Al:  $[CaCl_2] = 1.5$  mM. Points represent means of four identical, unreplicated experiments. Vertical bars indicate standard error of the mean where it exceeds symbol size, and lines are fitted by hand.

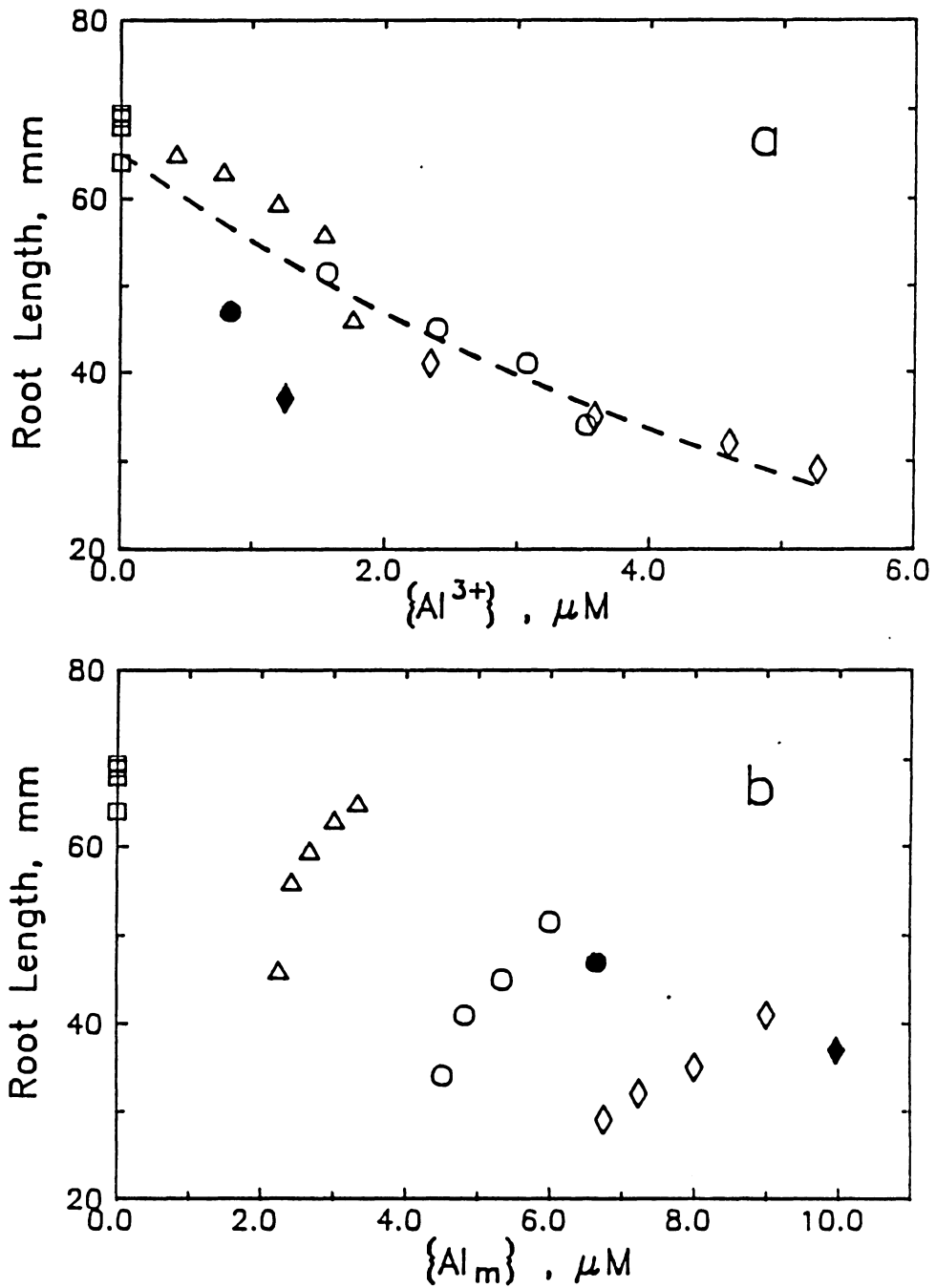


Figure 8. Data from Fig. 7 plotted as root length vs. activities of  $\text{Al}^{3+}$  (a) or sum of mononuclear species (b); Best-fit line for (a) describes the function  $y = 65.2 \exp(-0.17x)$ . Symbols correspond to levels of  $\text{Al}_i$  in Fig. 7.



## Improved Al Experiments

For the following experiments, several series of 0.5 mM Al stock solutions with basicities (molar ratio of OH<sup>-</sup> to Al initially added,  $n_0$ , where a negative value indicates added H<sup>+</sup>) ranging from -4 to 2.9 were prepared which, through trial-and-error, could be diluted to yield test solutions with predictable pH levels and distributions of mono- and polynuclear Al. The stock solutions were always between 14 and 30 d old at the initiation of each experiment to minimize aging effects (Smith, 1971; Turner, 1976; Chapter II), and were prepared from a single primary stock (0.01 M AlCl<sub>3</sub> • 6H<sub>2</sub>O). Final dilutions were prepared one day before transfer of seedlings to promote stabilization of the distribution of Al species. When a certain pH was desired, growth solutions were often supplemented with HCl, but never with base. All growth solutions were assayed for [Al<sub>m</sub>] with ferron at the beginning and end of each experiment. In addition, solution pH was periodically measured during the growth period, and readjusted to the initial value using dilute HCl. Speciation and pH data that follow reflect averages of the initial and final values for individual solutions.

First, a set of dilutions was determined that yielded a range of pH values (4.20, 4.45, 4.70, and 4.95) but a constant value of {Al<sub>m</sub>} (10 μM). As pH increased in such a series, {Al<sup>3+</sup>} and [Al<sub>m</sub>] both decreased (Fig. 9a). Thus, the nature of the root growth response to pH yielded immediate clues as to the species responsible for toxicity. Root length of Seneca wheat in the Al solutions increased with pH up to 4.70 but decreased dramatically at pH 4.95 (Fig. 9b). The pH 4.95 solution was the only one containing significant polynuclear Al (Fig. 9a), suggesting that these complexes were indeed toxic. Again, root growth was not closely associated with {Al<sub>m</sub>}. Virtually identical results were obtained with Tyler wheat at {Al<sub>m</sub>} = 8 μM and [CaCl<sub>2</sub>] = 3 mM (data not shown).

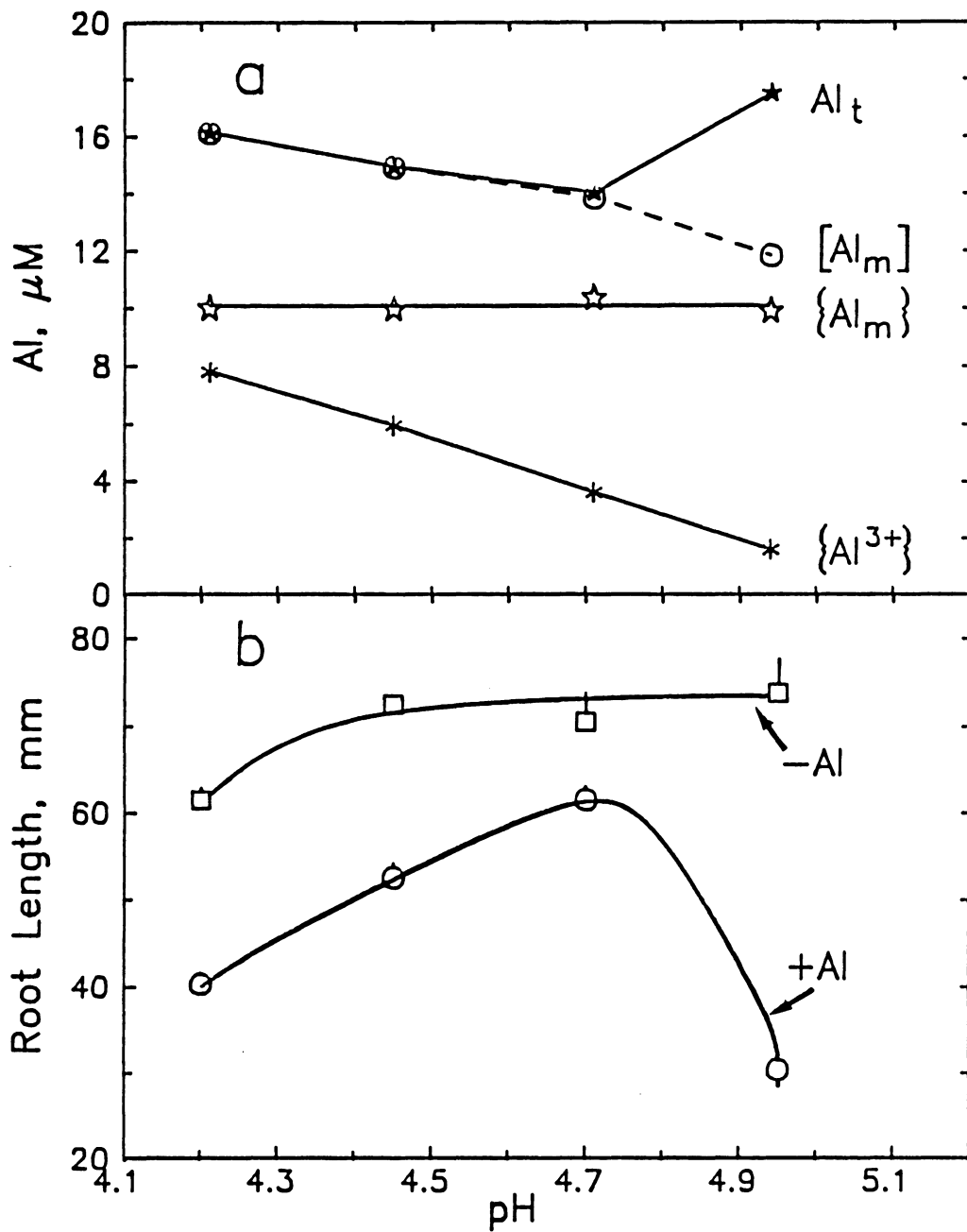


Figure 9. Distribution of Al species (a) and root lengths of Seneca wheat (b) vs. pH at constant sum of mononuclear species activities:  $[\text{CaCl}_2] = 1.0 \text{ mM}$  and points represent means of two identical experiments with two replications each. Lines are hand-fitted and vertical bars signify standard error of the mean where it exceeds symbol size.

Next, experiments were designed wherein pH could be varied, but no polynuclear Al would form. Stock solutions of basicity  $\leq -2$  were diluted and adjusted to the desired pH levels with HCl. At  $Al_t = 4 \mu M$  and  $[CaCl_2] = 1.5 mM$ , a pH range of *ca.* 4.2 to 5.0 was obtained, along with significant inhibition of Tyler root growth. Within detection limits, ferron assays indicated that the solutions were 100% mononuclear. Control (0 Al) treatments at each pH were included. Relative root growth increased steadily with increasing pH, reaching 100% at pH 4.95 (Fig. 10). That  $\{Al^{3+}\}$  decreased over this pH range again suggested an association between toxicity and  $\{Al^{3+}\}$ , while the positive correlation between root growth and  $\{Al_m\}$  again indicated that this quantity is unreliable as a predictor of phytotoxicity (Fig. 10).

Last, the effect of polynuclear Al was examined more closely. An initial experiment was conducted with  $Al_t = 15 \mu M$ ,  $[CaCl_2] = 3 mM$ , and  $n_0$  values of -1, 0, 0.5, 1, 1.5, and 2. Relative root growth of Tyler wheat decreased sharply with increasing  $n_0$ , levelling off to *ca.* 15% at  $n_0 = 1.5$  (data not shown). The range in  $n_0$  was then expanded to see if toxicity could be relieved at higher basicities. Control treatments at pH 4.7 and 5.6 bracketed the pH values of the Al solutions and there was no difference in control root lengths. Again, root growth declined markedly with increasing  $n_0$  up to a value of 2.0 (Fig. 11). Toxicity was alleviated, but only partially, when Al was almost completely neutralized ( $n_0 = 2.9$ , Fig. 11). That at least some of the polynuclear species were quite toxic is evident from the  $n_0 = 2.0$  data, where relative root growth was at a minimum. This solution contained  $2.2 \mu M [Al_m]$  and a pH level of 5.2 -- a clearly nontoxic combination when compared with the conditions depicted in Fig. 10. Very similar results were obtained using Seneca wheat at  $Al_t = 15 \mu M$ ,  $[CaCl_2] = 1.0 mM$ , and the same values of  $n_0$  (data not shown).

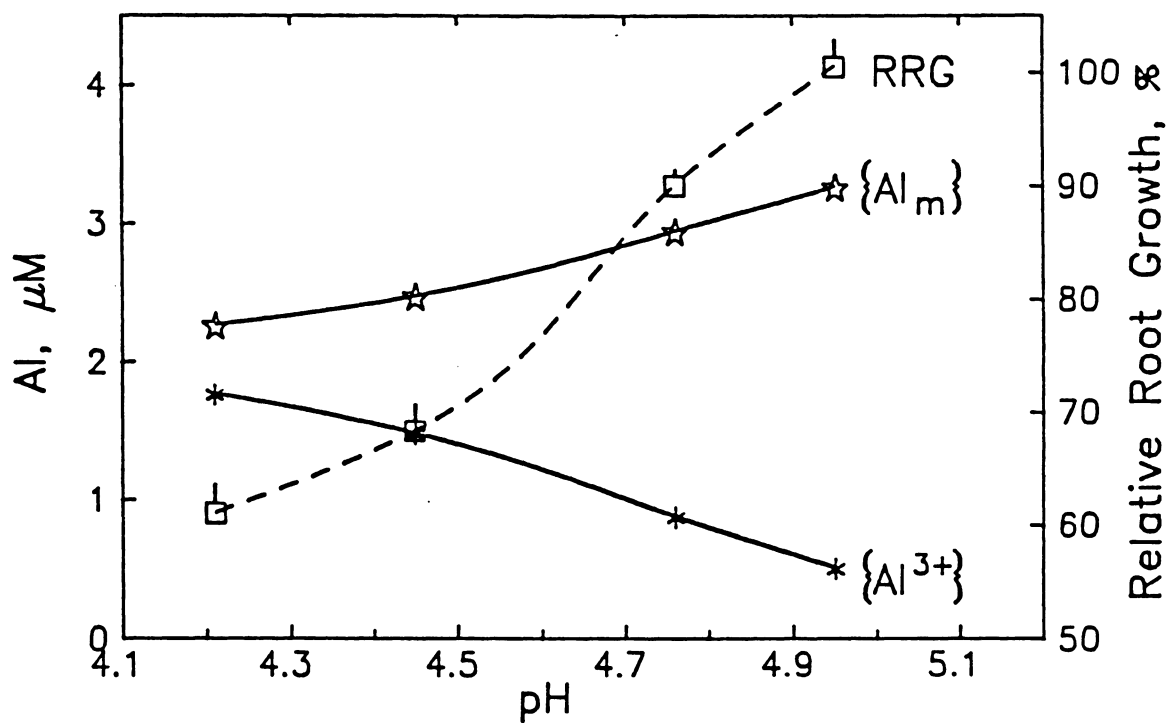


Figure 10. Distribution of Al species and relative root growth (RRG) of Tyler wheat vs. pH in the absence of polynuclear Al:  $Al_t = 4 \mu\text{M}$  and  $[CaCl_2] = 1.5 \text{ mM}$ . Means are for two experiments with two replications each. Vertical bars signify standard error of the mean where it exceeds symbol size, and lines are hand-fitted.

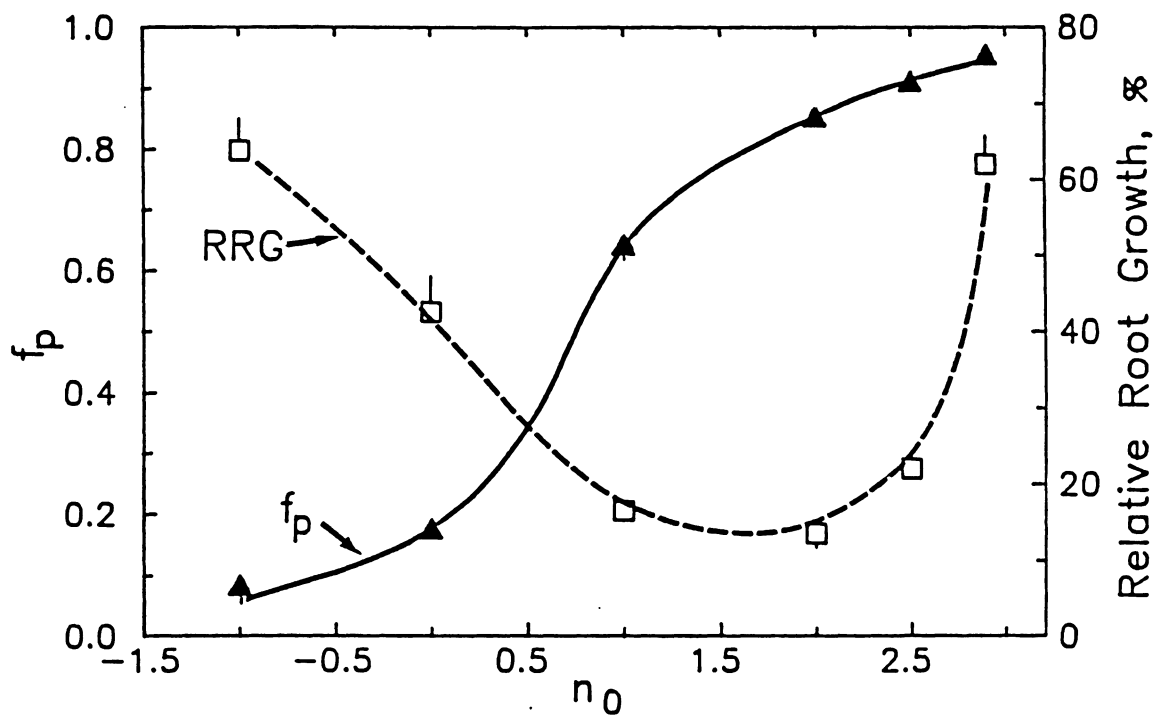


Figure 11. Fraction of polynuclear Al and relative root growth (RRG) of Tyler wheat vs. basicity:  $Al_t = 15 \mu M$  and  $[CaCl_2] = 3 mM$ . Solution pH ranged from 4.7 to 5.6, and control root lengths were constant over this range. Means are for two experiments with two replications each. Vertical bars signify standard error of the mean where it exceeds symbol size, and lines are hand-fitted.

The results from this study shed further light on the initial results presented in Figs. 7 and 8. With increasing pH up to about 4.8, Al toxicity was ameliorated, presumably because  $\text{Al}^{3+}$  is more toxic than the other mononuclear species. At pH 5.0, insufficient HCl was added to prevent formation of toxic polynuclear Al at the higher two levels of  $\text{Al}_4$ , thus explaining the downturn in root growth in Fig. 7 as well as the obvious outlying points (filled symbols) in Fig. 8.

### Lanthanum Experiments

Lanthanum, a trivalent metal that does not hydrolyze over the pH range of interest (Baes and Mesmer, 1976), offered an opportunity to assess the interaction between trivalent cation toxicity and pH without the confounding effect of changes in metal speciation. Clarkson (1965) reported that the trivalent metals Al, La, gallium, and indium induced similar toxicities in onion (*Allium cepa*) roots. Initial results in the present study were encouraging in that La and Al caused similar anatomical symptoms (swelling of the epidermal and outer cortical cells in the zone of elongation), and in that toxicity of both metals could be ameliorated by additions of Ca or Na (Kinraide and Parker, 1987a). However, when grown in solutions with equal activities of  $\text{Al}^{3+}$  or  $\text{La}^{3+}$ , Tyler and Seneca wheat did not exhibit the differential tolerance to La that they did to Al (data not shown). Thus, while there may be physiological similarities between the toxicities of the two metals, it is improbable that the mechanisms are exactly the same. However, the following results using La do serve as an example of a speciation-independent effect of pH on trivalent metal toxicity.

Experiments were conducted where pH was varied from 4.2 to 5.6 in the presence of La and 1.5 mM CaCl<sub>2</sub>. The gradual decline in Tyler root length with increasing pH in the presence of La (Fig. 12) is at this time unexplained. However, the data suggest that there is no tendency for low pH to predispose roots to trivalent metal injury. Similar results were obtained with Seneca wheat at 0, 20, and 40 μM total La (data not shown).

## *Discussion*

Uncertainties in the mononuclear hydrolysis constants for Al confound interpretation of studies such as these. Agreement among published constants appears to be rather poor, especially for the formation of Al(OH)<sub>2</sub><sup>+</sup> and Al(OH)<sub>3</sub><sup>0</sup> (Baes and Mesmer, 1976), and disparate values have been reported for all the constants (Sadiq and Lindsay, 1979). The data indicate a close relationship between the activity of Al<sup>3+</sup> and toxicity to wheat (Figs. 8a and 10) in the absence of polynuclear Al. However, the exact goodness-of-fit for a relationship such as that depicted in Fig. 8a will depend on the choice of constants, and it would be risky to contend that Al<sup>3+</sup> is necessarily the sole toxic species. Nonetheless, there was a consistent trend for alleviation of toxicity with increasing pH (Figs. 7, 9, 10) which, at constant [Al<sub>m</sub>], must yield a decline in {Al<sup>3+</sup>} . In contrast, {Al<sub>m</sub>} must increase with increasing pH (due to the effect of reduced valence on activity coefficients) regardless of choice of constants, and {Al<sub>m</sub>} is clearly not a good predictor of toxicity (Fig. 10). If the hydrolysis product AlOH<sup>2+</sup> were the sole toxic species (Moore, 1974; Alva et al., 1986b), inhibition of root growth should exhibit a maximum at an intermediate pH -- 4.7 using Lindsay's (1979) constants or 4.4 using those given by Alva et al. (1986b). If a significantly different set of constants were in fact correct, it is conceivable

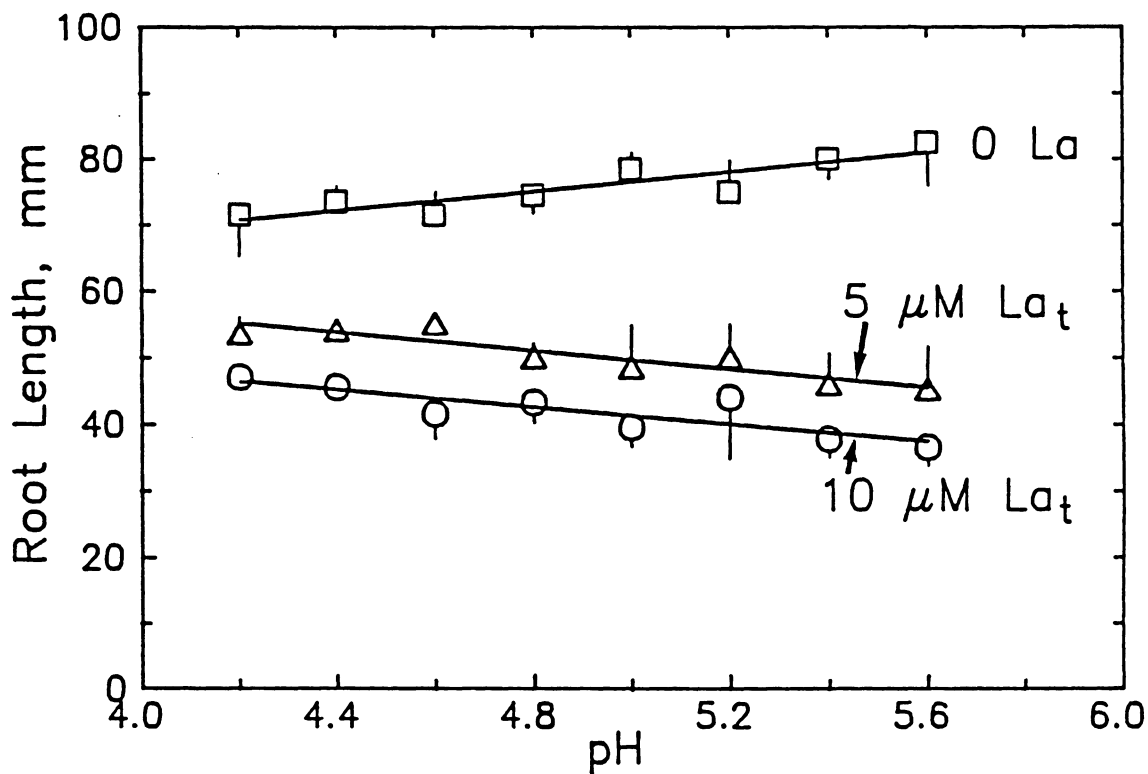


Figure 12. Root length of Tyler wheat as a function of pH in the presence or absence of added La:  $[\text{CaCl}_2] = 1.5 \text{ mM}$ . Lanthanum exists only as the  $\text{La}^{3+}$  ion over this pH range. Means are for two identical unreplicated experiments. Vertical bars signify standard error of the mean where it exceeds symbol size. Slopes of best-fit linear regression lines are all significantly different from zero ( $p < 0.01$ ).



that  $\{AlOH^{2+}\}$  could decline over the entire pH range of Fig. 4 and thereby be indistinguishable from  $Al^{3+}$  in its effect on root growth. Activities of  $Al(OH)_2^+$  will increase over the pH range of Fig. 10 for virtually any set of hydrolysis constants proposed thus far, and this species is unlikely to contribute to toxicity. Consequently, I would concur with the conclusion of other investigators (Adams and Lund, 1966; Brenes and Pearson, 1973; Pavan and Bingham, 1982) that  $Al^{3+}$  is the most toxic of the mononuclear species.

Any doubt regarding the potential toxicity of polynuclear hydroxy-Al can also be removed. While these species have been the subject of innumerable studies, and a host of different formulae proposed (Smith, 1971; Baes and Mesmer, 1976; Hsu, 1977), it is generally agreed that the polymers are metastable intermediates in the kinetically-limited precipitation of solid phase  $Al(OH)_3$  (Smith, 1971; Hsu, 1977). While small differences in the mononuclear fraction are often observed when empirical speciation methods are compared (Bertsch et al, 1986; Hodges, 1987; Chapter II), a survey of published reports reveals remarkable consistency for a given value of  $n_0$ , despite wide variations in  $Al_0$ , solution composition, and method of preparation (Smith, 1971; Bertsch et al., 1986; Jardine and Zelazny, 1987; Chapter II). In the present study, estimates of  $f_p$  by ferron ranged from 0.08 to 0.95 when  $n_0$  was varied from -1 to 2.9 (Fig. 11) -- a wide range in comparison to probable uncertainties in  $f_p$  due to methodology. Although not depicted in Fig. 11, computed activities of all individual mononuclear species, as well as  $\{Al_m\}$ , decreased over the entire range of  $n_0$ . The decline in root growth with increasing  $n_0$  up to 2.0 can only be interpreted as evidence that polynuclear Al can be toxic. The partial amelioration at  $n_0 = 2.9$  (Fig. 11) can be ascribed to formation of larger polymers of low charge or colloidal  $Al(OH)_3$  that was nontoxic. Thus, the findings of Bartlett and Riego (1972) and Wagatsuma and Ezoe (1985) are confirmed by this study. Research on speciation methodology has often focused on quantification of mononuclear Al as a

measure of toxic Al (see, e.g., Hodges, 1987; Jardine and Zelazny, 1987), a practice that must be reevaluated in light of the findings reported here. The large size and polyvalent charge of the polynuclear species, even if known, render computation of activities impractical, and only qualitative comparisons of the relative toxicities of mono- and polynuclear species can be made.

The findings reported here do contrast with several reports including that by Moore (1974), who attributed maximal inhibition of wheat root growth at *ca.* pH 4.5 to a greater toxicity of  $\text{AlOH}^{2+}$  relative to the other species. However, he noted a visible precipitate, presumably gibbsite or amorphous  $\text{Al}(\text{OH})_3$ , in solutions where Al<sub>t</sub> and pH were mutually high. It is probable that at slightly lower pH levels the solutions were also supersaturated with respect to  $\text{Al}(\text{OH})_3$ , but that metastable polymers dominated the system. Thus, it seems likely that Moore's (1974) results reflected toxicity of polynuclear Al, much like that depicted in Fig. 11.

It is more difficult to rationalize the discrepancies between the present study and those of Blamey et al. (1983) and Alva et al. (1986b), who, having accounted for polynuclear Al via a modified aluminon procedure, noted a high correlation between root growth and  $\{\text{Al}_m\}$ . However, five factors can be proposed that might contribute: (i) the presence of ligands (phosphate, citrate) that may have introduced additional uncertainties in speciation, (ii) collinearity among the various mononuclear species, (iii) choice of thermodynamic constants, (iv) real differences in the toxicity of their polynuclear species and the ones in this study, and (v) the possibility that the hydroxy-Al monomers were, in fact, more toxic in their studies using a group of dicotyledonous plants. The problem of collinearity was discussed by Blamey et al. (1983) who noted that *R* values exceeded 0.9 for the regression of relative root length on any one of the mononuclear species, as

well as on  $\{Al_m\}$ . Data presented by Alva et al. (1986b, Table 1) appear to suffer from similar complications -- for example, the simple correlation coefficients between  $\{Al^{3+}\}$  and  $\{AlOH^{2+}\}$  and between  $\{AlOH^{2+}\}$  and  $\{Al_m\}$  were 0.864 and 0.983, respectively. Evaluating which, if any, of the mononuclear species is the primary agent of toxicity is thus highly problematic.

A related problem is that of the choice of constants. Alva et al. (1986b) reanalyzed published data from Blamey et al. (1983) and Alva et al. (1986a), and concluded that  $AlOH^{2+}$  or  $Al(OH)_2^+$  was the primary toxic species. It was not possible to duplicate those calculations, but similar calculations were performed using the data from Table 4 in Blamey et al. (1983) and using two sets of hydrolysis constants -- those given by Lindsay (1979) and those used by Alva et al. (1986b). The ranking of  $R^2$  values for use of the various species activities as independent variables depended on the constants used (Table 5). The similar magnitudes of the various  $R^2$  values can be attributed the high degree of collinearity among the independent variables. Interpretational difficulties are thus likely to persist when  $Al_i$  and pH are both varied, and are compounded by uncertainties in hydrolysis constants. The approach taken in the present study, where pH is varied at a single value of  $Al_i$ ,  $[Al_m]$ , or  $\{Al_m\}$ , minimizes these pitfalls.

That there may be real differences in the toxicity of the polynuclear species cannot be discounted. There is considerable evidence that the nature and reactivity of the polymers can depend on  $Al_i$ , solution age, rate of base addition, presence of ligands such as phosphate and sulfate, and other factors (Smith, 1971; Turner, 1976). Most of the solutions used by Blamey et al. (1983) and Alva et al. (1986a, 1986b) contained phosphate, which may substantially alter the reaction products in partially-neutralized Al solutions (Hsu, 1968). The relationships between the physical and chemical characteristics of

**Table 5.** Coefficients of determination for the regression of root length vs. various species activities using two sets of thermodynamic constants†

Variable	Source of constants	
	Alva et al. (1986b)	Lindsay (1979)
{Al <sup>3+</sup> }	0.946	0.961
{AlOH <sup>2+</sup> }	0.973	0.948
{Al(OH) <sub>2</sub> <sup>+</sup> }	0.920	0.846
{Al <sub>m</sub> } ‡	0.962	0.961

† Regression model of the form  $y = \beta_1 + \beta_2 \exp(-\beta_3 x)$  for all values.

‡ Does not include activity of AlSO<sub>4</sub><sup>+</sup> which is nontoxic (Kinraide and Parker, 1987b).

polynuclear Al and its toxicity to plant roots have not been previously studied, and this question is addressed in Chapter IV.

If only mononuclear Al species are considered, one can now generalize that complexation (with a concomitant reduction in valence) will ameliorate toxicity regardless of whether the ligand is sulfate (Kinraide and Parker, 1987b; Cameron et al., 1986), fluoride (Cameron et al., 1986), an organic acid (Hue et al., 1986), or hydroxyl (this study). In general, charge reductions of ionic species are predicted to enhance diffusional transport across the plasmalemma of cells (Moore, 1974; Haug and Caldwell, 1985; Marschner, 1986, p. 31-32). Although largely speculative, a reasonable interpretation of these findings is that transport of Al into the cell is not necessarily a requisite step in the expression of toxicity and that primary lesions may be extracellular. Similarly, Kinraide and Parker (1987a) reported that the chloride salts of Ca, Mg, Sr, K, and Na ameliorated toxicity above and beyond any effects on the activity of  $Al^{3+}$ , and that divalent cations were 10 to 15 times more effective than monovalents when expressed on an activity basis. These results suggested a highly valence-dependent competition between cations for external, negatively-charged binding sites, although such binding cannot be strictly interpreted as evidence for an external lesion (Kinraide and Parker, 1987a).

That polynuclear hydroxy-Al can also be phytotoxic is consistent with the hypothesis of external lesion. Although polymerization yields a reduction in average positive charge per Al atom (Smith, 1971; Hsu, 1977), the overall polyvalent charge, in conjunction with steric factors, could lead to a high surface affinity for these species. Moreover, the relatively large size (perhaps 100 Al atoms per unit [Smith, 1971]) and high charge of the polymers would argue against ready transport across the plasmalemma. In addition to

Al and La, toxicity experiments were conducted with several other polyvalent cations, including scandium and two polyamines -- spermine ( $\text{H}_3\text{N}(\text{CH}_2)_3\text{H}_2\text{N}(\text{CH}_2)_4\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}_3^+$ ) and spermidine ( $\text{H}_3\text{N}(\text{CH}_2)_3\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}_3^+$ ). All were quite inhibitory to wheat and red clover (*Trifolium pratense* L.) root growth at concentrations in the micromolar range (data not shown). Thus, it would appear that inhibition of root growth by cations of valence  $>2$  is a general phenomenon, in agreement with the findings of Clarkson (1965). Surface binding to membranes or cell walls with subsequent disruption of structure or metabolic function might represent one cause for this inhibition.

These results have practical implications for many Al toxicity studies. When solution culture methods are used, care is needed to design experiments with quantitatively graded levels of toxic Al, particularly where solution composition could lead to the formation of polynuclear species. In the absence of toxic polymers,  $\{\text{Al}^{3+}\}$  appears to provide the most reliable estimate of toxic Al, as long as cation amelioration is also considered (Kinraide and Parker, 1987a). At present, it is not known whether significant quantities of toxic polynuclear Al exist in soil solution. Studies have demonstrated preferential adsorption of polynuclear Al by clay minerals and organic matter, and once adsorbed, these species are generally thought to be difficult to exchange (Zelazny and Jardine, 1988). Thus, an estimate of  $\{\text{Al}^{3+}\}$  in soil solution may be sufficient to assess toxic Al status (Wright et al., 1987). However, Bache and Sharp (1976) proposed that Al extracted by dilute salt solutions, and unreactive with the 8-hydroxyquinoline reagent, represented polynuclear hydroxy-Al that could also exist in soil solution. More data are thus needed to assess the ubiquity of polynuclear Al in soil solution, and its potential role as a phytotoxic agent in acid soils.

## References

- Adams, F., and P. J. Hathcock. 1984. Aluminum toxicity and calcium deficiency in acid subsoil horizons of two Coastal Plains soil series. *Soil Sci. Soc. Am. J.* 48:1305-1309.
- Adams, F., and Z. F. Lund. 1966. Effect of chemical activity of soil solution aluminum on cotton root penetration of acid subsoils. *Soil Sci.* 101:193-198.
- Adams, F., and B. L. Moore. 1983. Chemical factors affecting root growth in subsoil horizons of Coastal Plain soils. *Soil Sci. Soc. Am. J.* 47:99-102.
- Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986a. Effects of phosphorus/aluminum molar ratio and calcium concentration on plant response to aluminum toxicity. *Soil Sci. Soc. Am. J.* 50:133-137.
- Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986b. Relationships between root length of soybean and calculated activities of aluminum monomers in solution. *Soil Sci. Soc. Am. J.* 50:959-962.
- Bache, B. W., and G. S. Sharp. 1976. Soluble polymeric hydroxy-aluminum ions in acid soils. *J. Soil Sci.* 27:167-174.
- Baes, C. F., Jr., and R. E. Mesmer. 1976. *The hydrolysis of cations.* John Wiley and Sons, New York.
- Bartlett, R. J., and D. C. Riego. 1972. Toxicity of aluminum in relation to pH and phosphorus. *Soil Sci.* 114:194-201.
- Bertsch, P. M., W. J. Layton, and R. I. Barnhisel. 1986. Speciation of hydroxy-aluminum solutions by wet chemical and aluminum-27 NMR methods. *Soil Sci. Soc. Am. J.* 50:1449-1454.
- Blamey, F. P. C., D. G. Edwards, and C. J. Asher. 1983. Effects of aluminum, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. *Soil Sci.* 136:197-207.
- Brenes, E., and R. W. Pearson. 1973. Root responses of three gramineae species to soil acidity in an oxisol and an ultisol. *Soil Sci.* 116:295-302.
- Cameron, R. S., G. S. P. Ritchie, and A. D. Robson. 1986. Relative toxicities of inorganic aluminum complexes to barley. *Soil Sci. Soc. Am. J.* 50:1231-1236.
- Clarkson, D. T. 1965. The effect of aluminum and some other trivalent metal cations on cell division in the root apices of *Allium cepa*. *Ann. Bot.* 29:309-315.
- Driscoll, C. T., Jr., J. P. Baker, J. J. Bisogni, Jr., and C. L. Schofield. 1980. Effect of aluminum speciation on fish in dilute acidified waters. *Nature* 284:161-164.

- Foy, C. D. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. *In* F. Adams (ed.) Soil acidity and liming. 2nd ed. Agronomy 12:57-97.
- Haug, A., and C. R. Caldwell. 1985. Aluminum toxicity in plants: The role of the root plasma membrane and calmodulin. p. 359-381. *In* J. B. St. John et al. (ed.) Frontiers of membrane research in agriculture. Rowman and Allanheld, Totowa, NJ.
- Hodges, S. C. 1987. Aluminum speciation: A comparison of five methods. *Soil Sci. Soc. Am. J.* 51:57-64.
- Hsu, P. H. 1968. Interactions between aluminum and phosphate in aqueous solution. *Adv. Chem. Ser.* 73:115-127.
- Hsu, P. H. 1977. Aluminum hydroxides and oxyhydroxides. p. 99-143. *In* J. B. Dixon and S. B. Weed (ed.) Minerals in soil environments. Soil Science Society of America, Madison, WI.
- Hue, N. V., G. R. Craddock, and F. Adams. 1986. Effect of organic acids on aluminum toxicity in subsoils. *Soil Sci. Soc. Am. J.* 50:28-34.
- Jardine, P. M., and L. W. Zelazny. 1987. Influence of inorganic anions on the speciation of mononuclear and polynuclear aluminum by ferron. *Soil Sci. Soc. Am. J.* 51:889-892
- Kinraide, T. B., R. C. Arnold, and V. C. Baligar. 1985. A rapid assay for aluminium phytotoxicity at submicromolar concentrations. *Physiol. Plant.* 65:245-250.
- Kinraide, T. B., and D. R. Parker. 1987a. Cation amelioration of aluminum toxicity in wheat. *Plant Phys.* 83:546-551.
- Kinraide, T. B., and D. R. Parker. 1987b. Nonphytotoxicity of the aluminum sulfate ion,  $AlSO_4^-$ . *Physiol. Plant.* 71:207-212.
- Lindsay, W. L. 1979. Chemical equilibria in soils. John Wiley and Sons, New York.
- Marschner, H. 1986. Mineral nutrition of higher plants. Academic Press, London.
- Moore, D. P. 1974. Physiological effects of pH on roots. p. 135-151. *In* E. W. Carson (ed.) The plant root and its environment. Univ. Press of Virginia, Charlottesville.
- Parker, D. R., L. W. Zelazny, and T. B. Kinraide. 1987. Improvements to the program GEOCHEM. *Soil Sci. Soc. Am. J.* 51:488-491.
- Pavan, M. A., and F. T. Bingham. 1982. Toxicity of aluminum to coffee seedlings grown in nutrient solutions. *Soil Sci. Soc. Am. J.* 46:993-997.
- Pavan, M. A., F. T. Bingham, and P. F. Pratt. 1982. Toxicity of aluminum to coffee in ultisols and oxisols amended with  $CaCO_3$ ,  $MgCO_3$ , and  $CaSO_4 \cdot 2H_2O$ . *Soil Sci. Soc. Am. J.* 46:1201-1207.



- Sadiq, M., and W. L. Lindsay. 1979. Selection of standard free energies of formation for use in soil chemistry. Colorado Exp. Sta. Tech. Bull. 134.
- Smith, R. W. 1971. Relations among equilibrium and nonequilibrium aqueous species of aluminum hydroxy complexes. *Adv. Chem Ser.* 106:250-279.
- Sposito, G., and S. V. Mattigod. 1980. GEOCHEM: A computer program for the calculation of chemical equilibria in soil solution and other natural water systems. Kearney Foundation of Soil Science, Univ. of California, Riverside.
- Turner, R.C. 1976. Effect of aging on properties of polynuclear hydroxyaluminum cations. *Can. J. Chem.* 54: 1528-1534.
- Ulrich, B., R. Mayer, and P. K. Khanna. 1980. Chemical changes due to acid precipitation in a loess-derived soil in central Europe. *Soil Sci.* 130:193-199.
- Wagatsuma, T. and Y. Ezoe. 1985. Effect of pH on ionic species of aluminum in medium and on aluminum toxicity under solution culture. *Soil Sci. Plant Nutr.* 31:547-561.
- Wright, R. J., V. C. Baligar, and S. F. Wright. 1987. Estimation of phytotoxic aluminum in soil solution using three spectrophotometric methods. *Soil Sci.* 144:224-232.
- Zelazny, L. W., and P. M. Jardine. 1988. Surface reactions of aqueous aluminum. *In* G. Sposito (ed.) *Environmental chemistry of aluminum*. CRC Press, Boca Raton, FL (in press).

# Chapter IV

## Phytotoxicity and Characterization of Polynuclear Hydroxy-Aluminum Complexes

### *Abstract*

The objectives of this study were to examine how toxicity of polynuclear Al might vary with experimental conditions or with choice of test plants, and to identify and characterize the toxic polynuclear species. Wheat (*Triticum aestivum* L. cv. 'Tyler' or 'Seneca') or soybean (*Glycine max* (L.) Merr. cv. 'Stafford') seedlings were cultured for 2 d in dilute CaCl<sub>2</sub> solutions containing 15  $\mu$ M total Al at a basicity (molar OH/Al ratio) of 2.0. Increasing rate of base addition, solution age, or levels of added phosphate decreased the fraction of reactive polymers (Al<sub>b</sub>) and increased that of precipitated Al (Al<sub>c</sub>) as determined by the ferron method. Mononuclear Al was consistently  $\leq 3 \mu$ M and did not contribute to toxicity. Inhibition of root growth was well-correlated with [Al<sub>b</sub>] for both

species. That only 3-4  $\mu M$   $[Al_6]$  was required to fully inhibit wheat root growth suggested that detection of toxic polynuclear Al may at times be difficult in chemically complex media. All plants tested were considerably more tolerant of the mononuclear ion  $Al^{3+}$  than of  $Al_6$ , suggesting fundamental physiological differences in the toxicity of the two types of Al. This conclusion was further supported by the finding that the two wheat cultivars exhibited differential tolerance to  $Al^{3+}$ , but not to  $Al_6$ . Several analyses suggested that  $Al_6$  was composed primarily of the so-called  $Al_{13}$  polymer, which may be a consequence of optimal synthesis conditions. Given uncertainties in the ubiquity of this polymeric species, both in the laboratory and in nature, several lines of future investigation are suggested.

## *Introduction*

In Chapter III, the toxicity of polynuclear hydroxy-Al to wheat roots grown in solution was clearly demonstrated. These findings supported isolated reports to the same effect (Bartlett and Riego, 1972; Wagatsuma and Ezoe, 1985; Wagatsuma and Kaneko, 1987) but were in marked contrast to studies that have ascribed Al toxicity only to mononuclear species (Blamey et al., 1983; Alva et al., 1986a, 1986b). The latter view has predominated in the literature, as evidenced by numerous efforts to empirically differentiate mono- and polynuclear Al with the aim of quantifying the biologically active (mononuclear) fraction (see literature review in Chapter II). That differing views exist on the phytotoxicity of various forms of Al may be due to a number of factors (Chapter III), but two seemed particularly amenable to experimental investigation. These were differences in distribution or composition of polynuclear species due to experimental

conditions, and differences in how plant species and/or varieties respond to changes in Al speciation.

When Al solutions are partially neutralized, the fraction of mononuclear Al is relatively uniform for a given OH/Al molar ratio (Chapter II), but the nature of the other species formed is highly dependent on synthesis conditions. Smith (1971) postulated that total Al concentration, temperature, pH, and the anions present could affect the reaction products. He demonstrated that rate of base injection and solution age had profound influences on the distribution of reactive polymers and precipitated hydroxy-Al. Turner (1976) noted that when Al solutions were partially neutralized very slowly (ca. 1 month), the polynuclear complexes only gradually converted to another less reactive polymer. However, when neutralization took ca. 24 h, the same polynuclear species formed initially, but were comparatively rapidly converted to solid phase  $\text{Al}(\text{OH})_3$ . In studying the formation of  $\text{AlO}_4\text{Al}_{12}(\text{OH})_{24}(\text{H}_2\text{O})_{12}^+$  (hereafter termed the 'Al<sub>13</sub>' polymer), Bertsch (1987) identified OH/Al ratio, total Al, base injection rate, stirring rate, and even choice of reaction vessel as influential factors. He proposed a reaction scheme wherein, under optimum conditions, locally high pH at the point of base injection resulted in significant formation of tetrahedrally coordinated hydroxy-Al (aluminate ion), which forms the central core of the Al<sub>13</sub> polymer.

Although there is no *a priori* reason why polynuclear Al should be toxic only to certain plants, this source of variation seemed to warrant investigation. The studies of Blamey et al. (1983) and Alva et al. (1986a, 1986b) utilized dicotyledenous plants throughout; the studies described in Chapter III were conducted only with wheat. Dicots generally have significantly higher root cell wall cation exchange capacities than monocots (Bowling, 1976), a factor that has been implicated in differential Al tolerance (Foy et al.,

1967; Mugwira and Elgawhary, 1979). Given the high affinity of negatively charged surfaces for Al polymers (Zelazny and Jardine, 1988), root CEC represents a possible mediating role in the toxicity of these species.

Although the potential phytotoxicity of polynuclear Al has now been firmly established, there are few indications as to exactly what form of Al is responsible. Moreover, it is uncertain whether toxicity can be quantitatively predicted based on simple chemical analyses. Consequently, the objectives of this study were (i) to examine any variation in the toxicity of polynuclear Al due to experimental conditions, (ii) to compare and contrast how different plant species and cultivars respond to mono- and polynuclear Al, and (iii) to identify and characterize the toxic portion of the polynuclear fraction.

## *Materials and Methods*

### **Seedling Culture**

Wheat seedling culture was performed as described in Chapter III, and the basal medium consisted of 3.0 mM CaCl<sub>2</sub> in all experiments. Soybean seeds were surface-sterilized by soaking in 0.3% NaOCl for 15 min, soaked in aerated 0.5 mM CaCl<sub>2</sub> for 3 h, and then germinated between filter paper moistened with 0.5 mM CaCl<sub>2</sub> for about 38 h. For each pot, five seedlings with radicle lengths of 20 ± 3 mm were selected and transferred to holders consisting of a disk of polypropylene mesh supported by a ring of closed-cell polyethylene foam. Each holder was floated on 1.5 L of aerated test solution in a covered polyethylene pot for 2 d of additional growth at 298 ± 1 K in the dark. For

soybeans, the basal medium consisted of 1.0 mM CaCl<sub>2</sub>. At termination, tap root length was measured for each seedling, and the average for each pot used as the measure of toxicity. Root length was measured from the top of the slight swelling at the transition from hypocotyl to root, just above the emerging lateral roots. Root growth in the absence of Al was vigorous (1.5 to 1.8 mm hr<sup>-1</sup>) despite the omission of nutrients other than Ca from the growth media. Relative root growths were computed as for wheat, but using 20 mm as the initial root length.

### **Solution Preparation and Speciation**

All reagents were of analytical grade or higher purity. Double deionized (DDI) water was used throughout, and all glassware was acid-washed. The pH of all solutions was measured at the beginning and end of each growth experiment with a pH meter equipped with glass and calomel electrodes and calibrated with commercial buffers. In addition, solution pH was periodically checked and readjusted with dilute HCl during the growth period.

For experiments involving polymer toxicity, all growth solutions were prepared from 0.5 mM stock solutions of AlCl<sub>3</sub> which had been partially neutralized to a basicity (molar NaOH/Al ratio initially added,  $n_0$ ) of 2.0. These stock solutions were derived from a primary stock solution (0.01 M AlCl<sub>3</sub> • 6H<sub>2</sub>O) prepared by dissolving the salt in water. Standardized 0.05 M NaOH was slowly injected (generally 0.5 mL min<sup>-1</sup>) using an autoburette with the delivery tip submerged and the solutions vigorously stirred to minimize locally high concentrations of base (Smith and Hem, 1972; Bertsch, 1987). Total Al (Al<sub>t</sub>) was determined in these stocks by inductively-coupled plasma emission

spectroscopy (ICPES) using standards prepared from pure Al wire. Total Al in the growth solutions was nominally 15  $\mu\text{M}$  throughout, but was computed exactly for each solution based on the ICPES analysis of the corresponding stock solution.

The modified version of the spectrophotometric ferron procedure described in Chapter II was used to estimate the fractions of mono- and polynuclear Al in the growth solutions. One mL of sample and 0.8 mL of the mixed ferron reagent were used. Absorbance increases were monitored for one hour such that three fractions could be distinguished:  $\text{Al}_1$ ,  $\text{Al}_b$ , and  $\text{Al}_c$  in the parlance of Smith and Hem (1972), corresponding to mononuclear Al, reactive polymers, and unreactive polymers or solid phase  $\text{Al}(\text{OH})_3$ , respectively. The Al reacted at 30 min was taken to be  $[\text{Al}_c + \text{Al}_b]$  (brackets indicate concentrations throughout), and the first 24 min used to fit the binary-species kinetics function to obtain  $[\text{Al}_c]$ . The quantity  $[\text{Al}_b]$  was computed as the difference between  $\text{Al}_1$  and  $[\text{Al}_c + \text{Al}_b]$ .

For wheat experiments, two liters of each test solution were prepared. Three 500-mL aliquots (replications) were placed in beakers for measuring root growth, and the remainder retained for analysis. The ferron assay was performed in duplicate on this sample, but not on individual beakers. For soybean experiments, three 1.5-L solutions (replications) were prepared individually, and a 10-mL subsample taken from each. A single run of the ferron assay was then performed on each subsample. All replicate ferron assays agreed within  $\pm 5\%$  of  $\text{Al}_1$ ; only means are reported here. All test solutions were prepared and allowed to age overnight prior to transfer of seedlings and analysis by ferron.

Experiments were also conducted at pH 4.3 to measure plant response to  $\text{Al}^{3+}$ , which appears to be the most significant toxic monomeric species (Chapter III). Growth sol-

utions were prepared from 1.0 mM stock solutions of  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  that were also  $\geq 1$  mM in HCl. These solutions were checked with the ferron assay and were found to contain only mononuclear Al. Concentrations of mononuclear species were computed with the program GEOCHEM using the Al hydrolysis constants reported by May et al. (1979), and the constants for Ca-Cl complexes given in Table 4.

### Characterization of $\text{Al}_b$

An ultrafiltration (UF) method based on principles outlined by Staub et al. (1984) and Parthasarathy and Buffle (1985) was used to estimate the size of the  $\text{Al}_b$  polymers. The apparatus consisted of a Spectra-Por stirred cell (Spectrum Medical Industries, Inc., Los Angeles, CA) fitted with 43 mm diameter membrane filter discs which were pre-washed with a minimum of 150 mL DDI water. Fifty-mL samples were filtered under 300 kPa pressure (supplied via  $\text{N}_2$  gas) with vigorous stirring through Spectrum Type C filters with nominal molecular weight cutoffs (NMWCO) ranging from  $1 \times 10^3$  to  $1 \times 10^5$  g. The filtrates were collected in 1-mL increments using a fraction collector. Based on preliminary experiments with acidified (mononuclear) Al solutions, the first 3 mL were discarded and the subsequent 4 mL retained and combined for analysis of total Al by ICPEs. The fraction of Al retained by each filter was computed based on the difference in  $\text{Al}_t$  in the filtered and unfiltered solutions.

The bound OH/Al ratio of the  $\text{Al}_b$  fraction,  $n_b$ , was estimated for a number of solutions according to the relationship:

$$[\text{OH}_{\text{TB}}] = [\text{OH}_f] + [\text{OH}_s] = n_a[\text{Al}_a] + n_b[\text{Al}_b] + n_c[\text{Al}_c] \quad [\text{IV.1}]$$



where:  $[\text{OH}_{\text{TB}}]$  = concentration of total OH bound to Al  
 $[\text{OH}_i]$  = concentration of OH initially added  
 $[\text{OH}_s]$  = additional bound OH due to spontaneous hydrolysis  
 $n_a, n_b, n_c$  = the bound OH/Al ratio in the respective Al fractions

The quantities  $[\text{Al}_a]$ ,  $[\text{Al}_b]$ , and  $[\text{Al}_c]$  were all obtained from the ferron assay. The quantity  $[\text{OH}_i]$  was estimated based on  $[\text{H}^+]$ ; the latter was computed based on the measured pH and the single-ion activity coefficient for  $\text{H}^+$  calculated using the Davies equation (Stumm and Morgan, 1981). The estimate of  $[\text{OH}_i]$  was refined by consideration of  $\text{CO}_2$  equilibria using methods and constants outlined by Stumm and Morgan (1981). This entailed modification of the proton condition to include  $[\text{HCO}_3^-]$  based on measured pH and an assumed  $p\text{CO}_2$  of  $1 \times 10^{-3.5}$  atm. This is equivalent to a slight reduction in the mineral acidity to achieve the observed pH, and thus slightly reduces  $[\text{OH}_i]$ . It was assumed that  $n_c$  was 3.0 (Smith, 1971), and calculated  $n_c$  with GEOCHEM using measured pH and  $[\text{Al}_c]$  as inputs. Equation IV.1 was then rearranged and solved for  $n_b$ .

The  $^{27}\text{Al}$  NMR methods described by Bertsch et al. (1986a,b) were used to check for the presence of the  $\text{Al}_{13}$  polymer in two representative stock solutions ( $\text{Al}_t = 0.5 \text{ mM}$ ,  $n_0 = 2.0$ ) neutralized at 0.05 and 12.0  $\text{mL min}^{-1}$ . Spectra were obtained at 78.2 MHz using a Varian XL-300 spectrometer with a super conducting magnet in the Fourier transform mode. The cycle time was 1.6 s, the digital resolution was 0.61 Hz, the pulse width was 7.0  $\mu\text{s}$ , and the sweep width was 10 kHz. An acidified 0.037 M Al standard was run for spectral calibration; the peak corresponding to the hydrated  $\text{Al}^{3+}$  ion was taken to be 0 ppm. For the partially neutralized stock solutions, the peak at 62.5 ppm downfield corresponds to the tetrahedrally-coordinated Al in the center of the  $\text{Al}_{13}$  polymer. The integrated intensity of this peak was compared with the 'Al monomer'

peak just downfield from 0 ppm to obtain a qualitative estimate of the amounts of  $Al_3$  polymer in the stock solutions (Bertsch et al., 1986a,b).

## *Results and Discussion*

### **Wheat Experiments**

Initial pH values ( $pH_i$ ) are reported here, but final values generally differed by 0.1 unit or less. In all experiments, pH exceeded 5.0 and  $[Al_c]$  did not exceed  $3 \mu M$ , so toxicity can be ascribed solely to polynuclear Al (Chapter III). A single control treatment at pH 5.1 was included in each experiment, there being no response to increasing pH above ca. 4.5 (Chapter III). Average final root lengths of both Seneca and Tyler wheat grown in control solutions ranged from 65 to 72 mm. Ferron assays were conducted at initiation only; assays of selected solutions at termination indicated that decreases in  $[Al_b]$  and increases in  $[Al_c]$  were significant during the growth period. However, in the absence of information on the temporal response of roots to Al speciation, root growth was related to initial speciation only.

In Chapter III, growth solutions were derived from partially-neutralized stocks that had been prepared with base addition rates of  $0.50 \text{ mL min}^{-1}$ . Here, stocks were prepared using rates of 0.05, 0.5, 3.0, and  $12.0 \text{ mL min}^{-1}$ , such that it took 196, 19.6, 3.3, or 0.8 min to achieve a basicity of 2.0. For the fastest rate the delivery tip was suspended above the stirred solution, but was submerged for the other three rates. The stock solutions were 6 d old (Tyler) or 8 d old (Seneca) when the growth solutions were prepared.

Increasing rate of addition decreased  $[Al_b]$  and increased  $[Al_c]$ , while  $[Al_e]$  was relatively constant (Table 6), consistent with the findings of Smith and Hem (1972). Relative root growths of both Tyler and Seneca wheat were  $< 20\%$  for all rates of base addition (Table 6). The  $0.5 \text{ mL min}^{-1}$  rate was used for subsequent experiments.

Experiments were then conducted where the Al stock solutions had been aged from 1 to 180 d. Again,  $[Al_b]$  decreased and  $[Al_c]$  increased (Table 7) in a manner consistent with other reports (Smith and Hem, 1972; Turner, 1976; Jardine and Zelazny, 1986). Root growth of both cultivars improved markedly in the solutions prepared from the oldest stocks, and some amelioration was evident for stocks that were  $\geq 52$  d old (Table 7).

Last, small quantities of phosphate (as  $KH_2PO_4$ ) were added to the growth solutions to achieve P/Al molar ratios of 0.1, 0.2 and 0.3. The Al and P stocks were added separately to 800-mL aliquots of water before mixing to avoid temporarily high concentrations and possible precipitation. The Al stocks used were 12 d old (Tyler) or 15 day old (Seneca) when growth solutions were prepared, and these were aged 2 d instead of the usual 1 d before transfer of seedlings. Two control treatments were included -- 0 and  $4.5 \mu M$  P -- and average final root length differed by  $< 1$  mm for these solutions.

Increasing concentrations of P decreased  $[Al_b]$ , increased  $[Al_c]$ , while  $[Al_e]$  was again fairly constant (Table 8). Root growth of both cultivars was slightly improved at P/Al = 0.2, and was dramatically increased at P/Al = 0.3 (Table 8). For Tyler the anomalously high root growth at the highest P/Al ratio was significantly greater than both of the controls, suggesting some stimulation of growth when both P and Al are present, but when only  $[Al_b]$  is rather low. Root growth of Seneca was also high at P/Al = 0.3, but not significantly greater than control growth.

Table 6. Influence of rate of base addition to Al stock solutions on speciation and root growth of Tyler and Seneca wheat. All growth solutions contained  $15 \mu\text{M Al}_i$  at  $n_0 = 2.0$ , and  $3.0 \text{ mM CaCl}_2$ . For each variety, results are from a single experiment with three replications.

Rate of Addition	Relative Root Growth	Ferron Assay				$k_b$	$\text{pH}_i$
		$[\text{Al}_a]$	$[\text{Al}_b]$	$[\text{Al}_c]$			
$\text{mL min}^{-1}$	%	----- $\mu\text{M}$ -----			$\text{min}^{-1}$		
Tyler							
0.05	$13 \pm 1 \dagger$	2.5	9.1	3.2	0.17	5.21	
0.50	$16 \pm 2$	2.5	7.2	5.2	0.17	5.16	
3.0	$17 \pm 1$	2.2	6.3	6.2	0.17	5.11	
12.0	$18 \pm 0$	2.2	5.5	6.9	0.18	5.10	
Seneca							
0.05	$15 \pm 0$	1.7	9.8	3.5	0.16	5.25	
0.50	$11 \pm 1$	2.1	8.2	4.7	0.18	5.21	
3.0	$15 \pm 1$	2.7	6.9	5.5	0.18	5.21	
12.0	$13 \pm 1$	2.9	6.2	6.6	0.17	5.18	

$\dagger \pm$  standard error of the mean.

**Table 7.** Influence of stock solution age on Al speciation and root growth of Tyler and Seneca wheat. All growth solutions contained  $15 \mu\text{M Al}_i$  at  $n_0 = 2.0$ , and  $3.0 \text{ mM CaCl}_2$ . For each variety, results are from a single experiment with three replications.

Stock Age	Relative Root Growth	Ferron Assay				
		$[\text{Al}_a]$	$[\text{Al}_b]$	$[\text{Al}_c]$	$k_b$	$\text{pH}_i$
days	%	----- $\mu\text{M}$ -----			$\text{min}^{-1}$	
Tyler						
1	$13 \pm 1 \dagger$	1.0	9.0	4.9	0.16	5.25
25	$15 \pm 2$	2.4	4.7	7.2	0.17	5.19
65	$32 \pm 0$	2.2	2.2	9.3	0.16	5.12
153	$85 \pm 6$	3.0	0.5	11.2	-- ‡	5.03
Seneca						
12	$16 \pm 2 \dagger$	1.3	8.4	5.3	0.18	5.20
52	$28 \pm 2$	2.0	2.6	10.2	0.19	5.11
92	$62 \pm 10$	2.3	1.4	10.9	0.16	5.08
180	$88 \pm 2$	2.4	0.5	11.5	-- ‡	5.05

†  $\pm$  standard error of the mean.

‡ Could not fit an accurate value due to low level of  $[\text{Al}_b]$ .

**Table 8.** Influence of added phosphate on Al speciation and root growth of Tyler and Seneca wheat. All growth solutions contained 15  $\mu\text{M}$   $\text{Al}_i$  at  $n_0 = 2.0$ , and 3.0 mM  $\text{CaCl}_2$ . For each variety, results are from a single experiment with three replications.

P Added	Relative Root Growth	Ferron Assay				
		$[\text{Al}_i]$	$[\text{Al}_i]$	$[\text{Al}_i]$	$k_b$	pH <sub>i</sub>
$\mu\text{M}$	%	----- $\mu\text{M}$ -----			$\text{min}^{-1}$	
		Tyler				
0	13 ± 2 †	1.5	7.8	5.8	0.18	5.19
1.5	14 ± 1	2.1	5.1	7.9	0.17	5.25
3.0	26 ± 3	2.6	2.5	10.0	0.15	5.25
4.5	110 ± 2	2.3	0.8	12.0	-- ‡	5.20
		Seneca				
0	18 ± 1	1.8	6.7	6.5	0.19	5.19
1.5	18 ± 1	2.5	4.5	8.0	0.17	5.24
3.0	43 ± 5	1.7	2.0	11.3	0.14	5.22
4.5	103 ± 3	1.8	0.9	12.3	-- ‡	5.21

† ± standard error of the mean.

‡ Could not fit an accurate value due to low level of  $[\text{Al}_i]$ .

It remains to be seen whether the anomalously high root growths observed for the P/Al = 0.3 treatment (Table 8) are consistent phenomena, not consequences of experimental variation. White (1976) postulated (but did not conclusively demonstrate) that, at certain combinations of pH (or basicity), and concentrations of P and Al, polynuclear hydroxyphosphate-Al complexes of low or neutral charge could form that would be readily taken up by plants. If the Al could be internally detoxified, the P could become metabolically available, and thus explain occasional reports of growth stimulation by low levels of Al (White, 1976; Foy, 1984). The author is reluctant to support this model based on a single experiment, but it may provide an explanation for these results.

Increasing rate of base addition did not yield as pronounced effects as did increasing age of the stock solution, but both decreased  $[Al_6]$ . Phosphate was also effective in this regard, an observation that is generally consistent with other reports (Hsu, 1968; Blamey et al., 1983). Taken together, the data from these experiments suggest a close association between  $[Al_6]$  and toxicity, although many of the treatments resulted in minimum growth (Fig. 13). The two outliers in Fig. 13 correspond to the highest P/Al ratio treatments discussed above. That wheat appears to be remarkably sensitive to the  $Al_6$  polymers suggests that detection of polymer toxicity may at times be difficult, and may thus explain the infrequency of reports on this effect.

Computations of activities for  $Al_6$  are precluded by the large size, polyvalent charge, and unknown structure of the polymers, and it is thus difficult to compare relative toxicities of  $Al_6$  and  $Al^{3+}$ . However, relative root growth approached a minimum at about  $3 \mu M$   $[Al_6]$  (Fig. 13). Experiments were conducted at pH 4.3, with  $Al_6$  ranging from 0 to  $48 \mu M$ , and the same level of  $CaCl_2$ . All Al was mononuclear according to the ferron assay. Root growth was much less affected by  $Al^{3+}$  (Fig. 14) than by  $Al_6$  (Fig. 13) when both

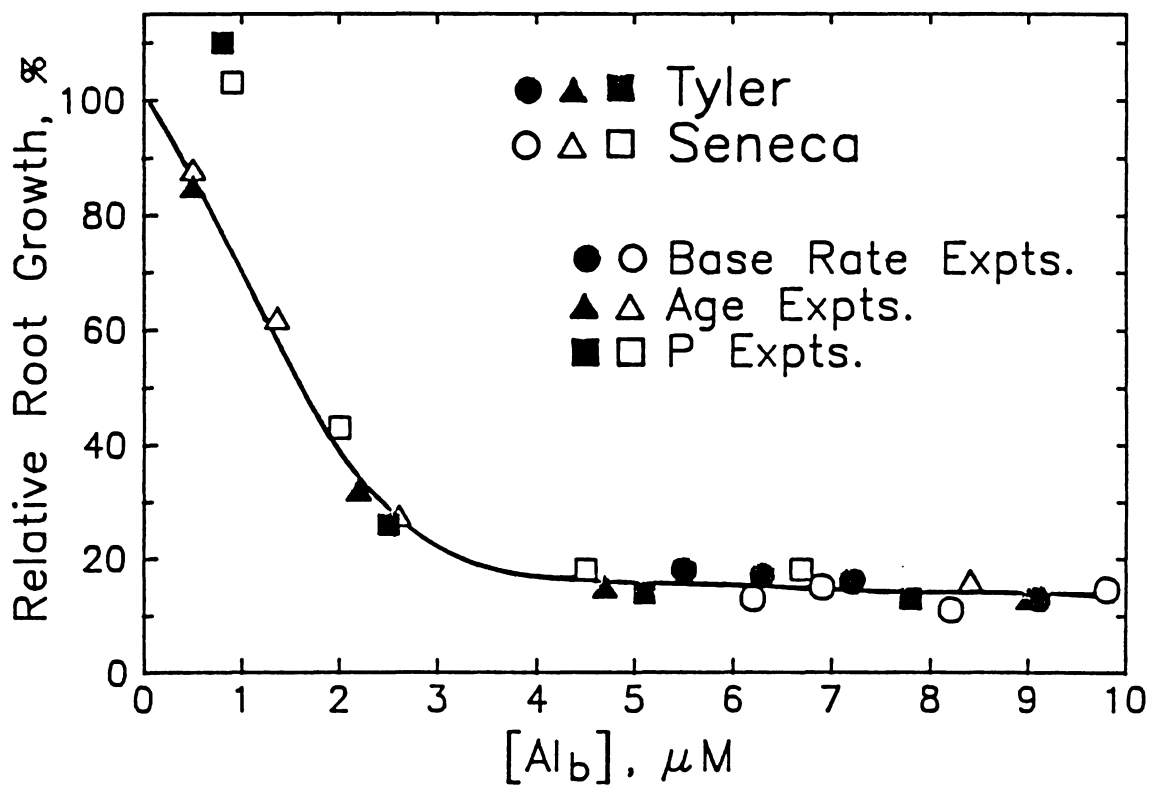


Figure 13. Relative root growth of Tyler and Seneca wheat vs. concentration of  $Al_b$ : Data are taken from tables 6-8 and line is hand-fitted.



were expressed as concentrations. Moreover, although the two cultivars exhibited marked differential tolerance to  $Al^{3+}$  (Fig. 14), their response to  $Al_6$  was identical (Fig. 13). These findings indicate that there are physiological differences in the mechanism of mono- and polynuclear Al toxicity, or perhaps in the means whereby tolerance to the two forms is achieved.

## Soybean Experiments

Experiments similar to those described in Chapter III were conducted to ensure that mononuclear Al could be ruled out as a toxicant in studies of polymer toxicity. Solutions were prepared from acidified Al stocks such that  $Al_1$  was  $5 \mu M$ ,  $[CaCl_2]$  was  $1.0 \text{ mM}$ , and pH ranged from 4.20 to 4.85. The solutions were entirely mononuclear according to the ferron assays. Root growth was not significantly different from control growth at any pH (data not shown), and it is thus improbable that mononuclear Al contributed to inhibition in the experiments described below, where again  $[Al_1]$  was  $\leq 3 \mu M$  throughout.

For soybeans, test solutions were prepared from an assortment of stock solutions of varying ages and base injection rates; phosphate was not included as a variable. As with wheat, a single control at pH 5.1 was included, there being no response to increasing pH above 4.6 in control solutions (data not shown). Trends in distributions of  $Al_1$ ,  $Al_6$ , and  $Al_7$  were quite similar to those observed in the wheat experiments, and solution pH was  $\geq 4.9$  throughout (data not shown). Pooling data from three experiments, a relationship between root growth and  $[Al_6]$  was again observed (Fig. 15). Precision did not seem as high as with wheat, and root growth did not approach a minimum at as low values of

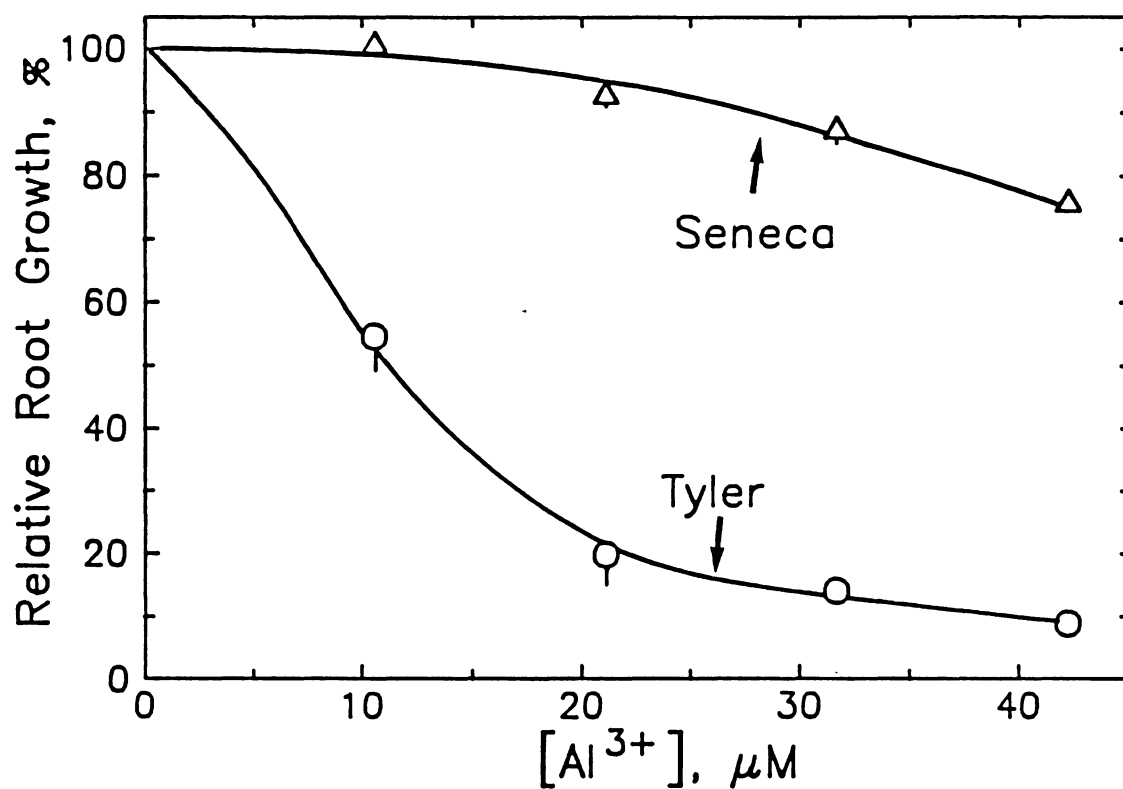


Figure 14. Relative root growth of Tyler and Seneca wheat vs. concentration of Al<sup>3+</sup> at pH 4.3: Means are for a single experiment with two replications. Vertical bars indicate standard error of the mean, and lines are hand-fitted.

[Al<sub>p</sub>], but the responses were qualitatively identical. Soybeans were also much less sensitive to Al<sup>3+</sup> (at pH 4.3) than to Al<sub>p</sub> (Fig. 15), further supporting the notion that these two forms of Al affect root growth in physiologically different ways.

It would thus seem that there is no fundamental difference in the response of monocots and dicots to polynuclear Al -- both seem quite sensitive. Moreover, toxic polymers persisted even when solutions were aged for several months or contained moderate levels of P. Thus, the apparent nontoxicity of polynuclear Al observed by others (Blamey et al., 1983; Alva et al., 1986a,b) is not readily explained by these results. However, comparisons are confounded by a lack of unambiguous definitions. For example, the nonmononuclear fraction has been divided into anywhere from two to four subfractions using various techniques (Smith, 1971; Turner, 1976; Jardine and Zelazny, 1986; Bertsch et al., 1986). The ferron method has been employed here to differentiate three fractions of Al. Blamey et al. (1983) and Alva et al. (1986a,b) utilized a modified aluminon method wherein the absorbance at 30 min was taken as mononuclear Al, and the 'total' Al (wherein the sample is acidified and heated prior to assay) was used to differentiate soluble (monomeric plus polymeric) and solid phase Al. It is difficult to say how those results compare with the ferron assays reported here, or even if reactive (and phytotoxic) polymers were present in their solutions.

### **Characterization of Al<sub>p</sub>**

Having established the relationship between the reactive polynuclear species as measured by ferron (Al<sub>p</sub>) and toxicity, it seemed desirable to elucidate the size, charge, and probable structure of these species. Values of the fitted first-order rate coefficient for the

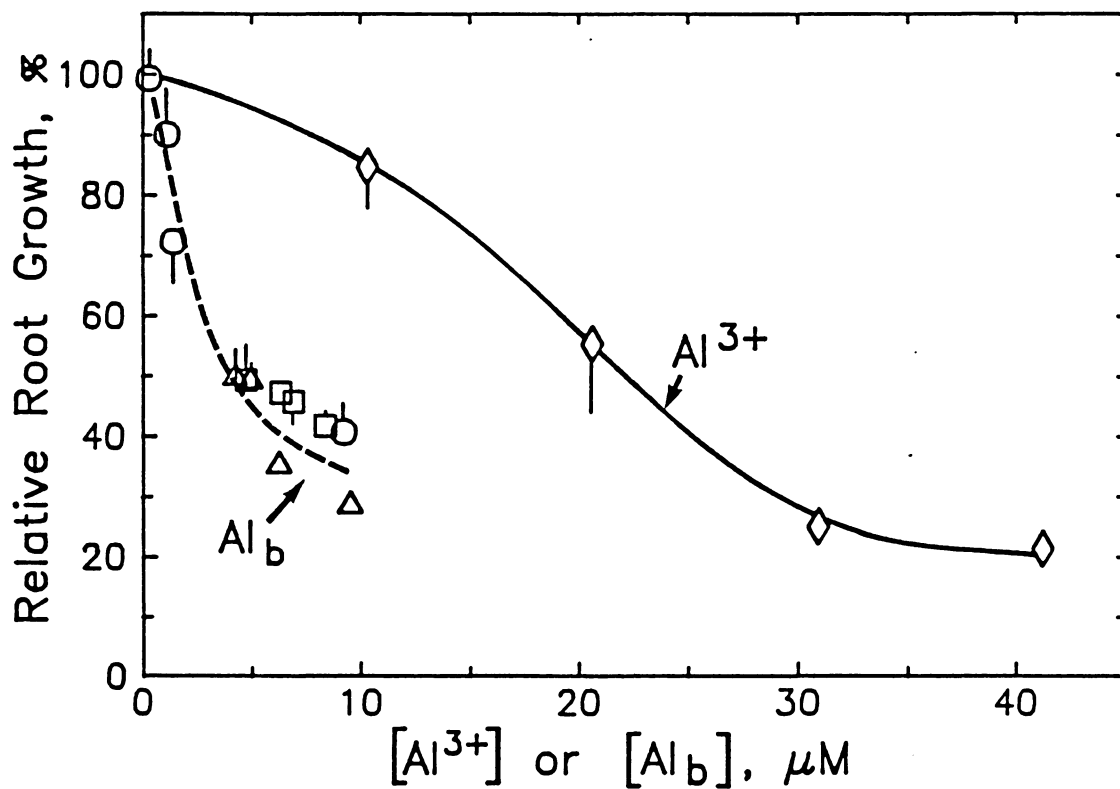


Figure 15. Relative root growth of Stafford soybeans vs. concentration of  $Al_b$  or concentration of  $Al^{3+}$  at pH 4.3.:  $[CaCl_2] = 1.0$  mM throughout. Each symbol type corresponds to a single experiment with three replications. Vertical bars indicate standard error of the mean, and lines are hand-fitted.

$Al_3$ -ferron reaction,  $k_b$ , were very consistent over all base addition rates, stock additions, and P levels (Tables 1-3 plus data for soybeans not shown). In contrast to other reports, no 'breaks' in the first-order plots of the  $Al_3$ -ferron reaction were observed (Bertsch, et al., 1986b), nor was there any appreciable reaction between ferron and  $Al_3$  after 30 min (Jardine and Zelazny, 1986). It seemed therefore reasonable that, with the method of preparation employed in this study,  $Al_3$  is composed of a single polymer, or a suite of similarly-sized polymers, that are equally reactive with the ferron reagent. It was therefore assumed that an analysis of any solution derived from a stock prepared by these methods would be representative of all the solutions used in plant growth experiments, at least in terms of the nature of  $Al_3$ .

It was noted that, due to analytical limitations and small adsorption losses on the membranes, the UF method was not very workable at the  $15 \mu M Al_3$  level employed in the toxicity experiments. Consequently, an experiment was performed to test the independence of Al speciation on dilution. A single  $0.5 mM$  stock was prepared using the  $0.05 mL \text{ min}^{-1}$  rate of base addition. After 1 d aging, this stock was diluted to 15, 50, and  $100 \mu M Al_3$  in  $3 mM CaCl_2$ . Ferron assays performed immediately after dilution, and again after 2 d aging, indicated that the distributions of  $Al_3$ ,  $Al_2$ , and  $Al_1$  were very similar for all levels of  $Al_3$  at both sampling times (data not shown). The volumes of sample and reagent had been adjusted to yield a molar ferron/ $Al_3$  ratio of exactly 50 for each sample. The fitted values of  $k_b$  increased with increases in the absolute concentration of ferron in the reaction mixture, and such changes are expected in that  $k_b$  represents a conditional rate coefficient that incorporates the concentration of ferron (Stumm and Morgan, 1981, p. 93). It was thus concluded that the nature and extent of  $Al_3$  was unaffected by  $Al_3$  over the range examined.

Duplicate UF runs were performed on freshly prepared 100  $\mu M$  solutions (3.0 mM in  $\text{CaCl}_2$ ) derived from a single stock solution (neutralized at 0.05 mL  $\text{min}^{-1}$ ) after the stock had been aged 1 or 26 d. The inclusion of  $\text{CaCl}_2$  in the solutions was found to be helpful in minimizing adsorption losses of Al on the membrane filters. Ferron assays were performed at the same time, and the fractions of  $\text{Al}_b$ ,  $\text{Al}_c$ , and  $\text{Al}_e$  are indicated by the vertical scale on the left of Fig. 16. The fraction of Al retained by filters with NMWCO's  $\leq 5 \times 10^3$  g corresponds closely with the sum of the  $\text{Al}_b$  and  $\text{Al}_c$  fractions, and the fraction of  $\text{Al}_e$  is roughly approximated by the 'tail' at high NMWCO's (Fig. 16). The sharp inflection between NMWCO's of  $5 \times 10^3$  and  $2 \times 10^4$  g corresponds to the breakthrough of the  $\text{Al}_b$  fraction (Fig 16). Two other UF analyses were performed on similar solutions prepared from a 54-d-old stock that had been neutralized at the 0.5 mL  $\text{min}^{-1}$  injection rate, or a 15-d-old stock that had been neutralized at the 12.0 mL  $\text{min}^{-1}$  rate. Although  $\text{Al}_b$  comprised only 33% and 49% of  $\text{Al}_e$ , respectively, the breakthrough of  $\text{Al}_b$  occurred at the same pore size (data not shown). Thus, there is no indication that the  $\text{Al}_b$  polymers are any larger after aging or with fast rates of base injection, consistent with the findings of Parthasarathy and Buffle (1985). The manufacturer's estimated estimated pore size for the  $1 \times 10^4$  NMWCO filter is 25 Å. That more than 50% of  $\text{Al}_b$  passes this filter suggests that this represents the upper size limit of these polymers (Staub et al., 1984), and they may well be in the 10 – 20 Å range reported by Parthasarathy and Buffle (1985).

Calculation of  $n_b$  values also suggested that  $\text{Al}_b$  is composed of relatively small polymeric units. Only solutions where  $\text{Al}_b$  constituted at least 40% of the total Al were considered in order to minimize errors due to assumptions about  $n_b$  and  $n_c$ . The average value of  $n_b$  for 20 solutions with  $\text{Al}_e$  ranging from 15 to 100  $\mu M$  was 2.38 with a standard error of 0.14. Computations such as those outlined by Smith and Hem (1972), which relate

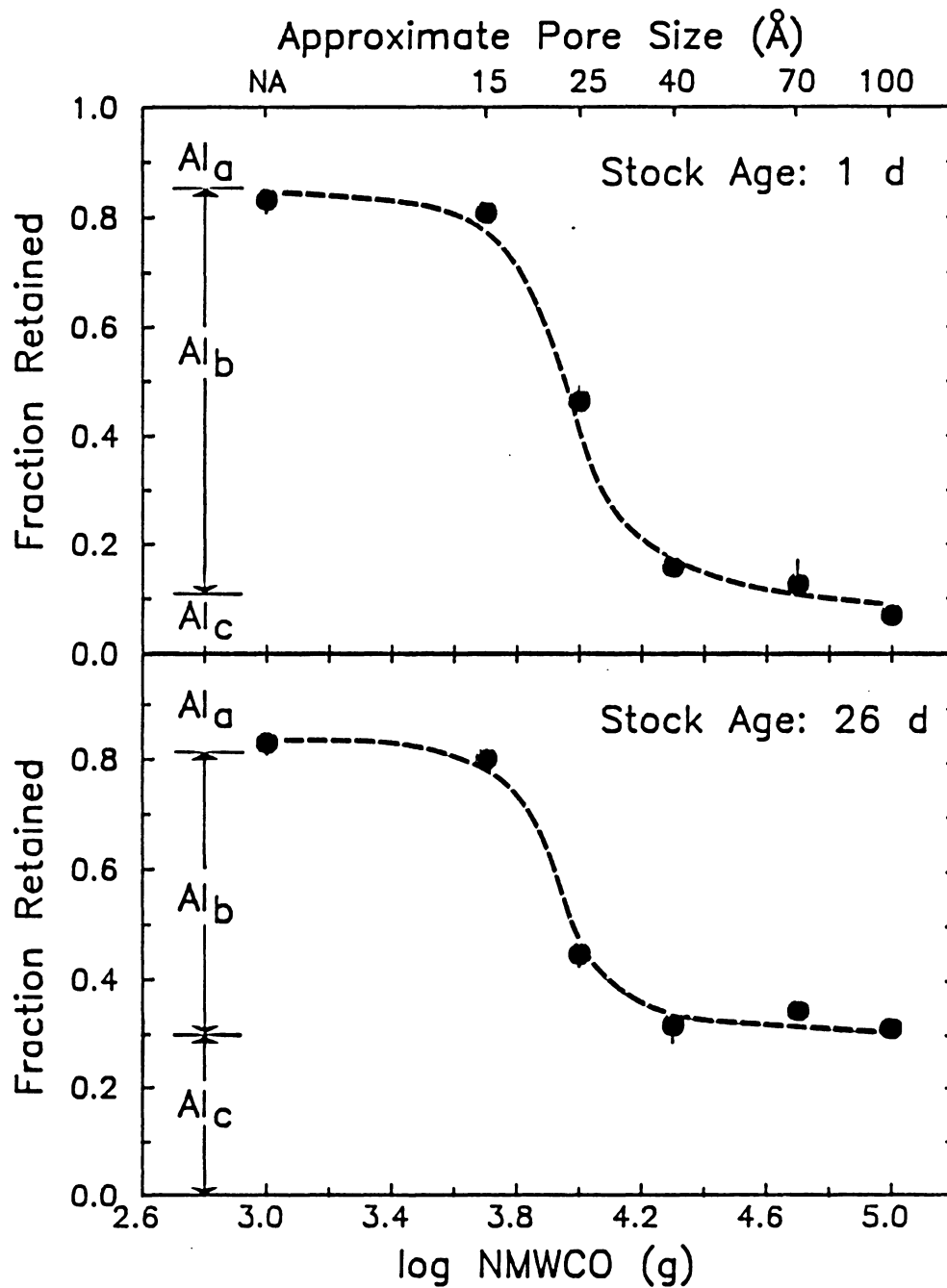


Figure 16. Fraction of Al<sub>i</sub> retained vs. nominal molecular weight cutoff of several membrane filters: Solutions were prepared from a preneutralized stock solution after 1 or 26 d aging. Vertical bars indicate standard error where 1 SE > symbol size.

the number of Al atoms per unit to  $n_b$ , suggested that the polymers contain perhaps 13 to 24 Al atoms assuming a linked hexagonal-ring structure. However, the  $Al_{13}$  polymer ( $n_b = 2.46$ ) could also be accommodated based on these analyses.

Two stock solutions, neutralized at the 0.05 and 12 mL min<sup>-1</sup> base injection rate, were analyzed by <sup>27</sup>Al-NMR between 48 and 96 h after preparation. Counting times were 17 and 9.5 h for the slowest and fastest base injection rate, respectively. At approximately the same time, ferron assays were performed on freshly-prepared 5x dilutions (100 μM Al<sub>i</sub>) of both stocks. According to the ferron assay, Al<sub>i</sub> comprised 71 and 54% of Al<sub>i</sub> for the slowest and fastest base injection rate, respectively. The integrated intensity of the  $Al_{13}$  peak was ≥9 times that of the Al monomer peak for both solutions; the amount of Al<sub>i</sub> was between 15 and 18% of Al<sub>i</sub> according to the ferron assays. Although only qualitative estimates of the quantities of  $Al_{13}$  polymer, these results indicate that, in the partially neutralized stock solutions, the Al<sub>i</sub> fraction was largely (if not entirely) the  $Al_{13}$  polymer. Parthasarathy and Buffle (1985) reported that this polymer is about 15 Å in diameter, consistent with the UF-based size estimate.

Detection limits of the <sup>27</sup>Al-NMR method preclude direct examination of the more dilute growth solutions. It can be assumed, however, that the  $Al_{13}$  polymers persist for some time after dilution (Parthasarathy and Buffle, 1985), and are therefore the primary toxic species in the experiments described earlier. The formation of this polymer is, however, dependent on synthesis conditions (Bertsch, 1987), and it is thus difficult to predict its occurrence. Moreover, it is equally difficult to say whether the small, linked hexagonal-ring polymers envisioned by some (Smith, 1972; Hsu 1977) ever form in partially neutralized solutions, and if so, whether these species are also phytotoxic. More recent investigations, which have employed <sup>27</sup>Al-NMR, suggest that the polymeric



species that reacts rapidly with ferron could be  $Al_3$ , exclusively (Bertsch et al., 1986b; Bertsch, 1987; Denney and Hsu, 1986; Parthasarathy and Buffle, 1985). Other components of partially neutralized solutions may include very small polymers (e.g., dimers or trimers) and a much less reactive (and thus perhaps larger) polymer (Bertsch et al., 1986b). Further study is needed to better define the relationships between synthesis conditions, total Al, basicity, etc. and the reaction products formed, as well as the role of various polynuclear species in Al phytotoxicity.

## *Summary and Conclusions*

Despite variations in the distribution of  $Al_6$  and  $Al_7$  due to experimental conditions, toxic polymers, represented by the  $Al_6$  fraction, were found to be persistent in all test solutions except those prepared from very old partially neutralized stocks or those containing rather high levels of P. Thus, the reports of others (Blamey et al., 1983; Alva et al., 1986a,b) who have not observed polymer toxicity in some similar solutions cannot be readily explained. It should be noted, however, that no attempt was made to duplicate their experimental conditions exactly, and that the formation of toxic polymers in this study may have been a consequence of optimal synthesis conditions. The rather low levels of  $Al_6$  required to substantially inhibit root growth suggest that detection of polymer toxicity may at times be problematic, especially in chemically complex media.

Both wheat and soybeans were considerably more sensitive to equal concentrations of  $Al_6$  than mononuclear  $Al^{3+}$ , and two wheat cultivars exhibited differential tolerance only to the latter. These findings suggest fundamental physiological differences in how the

two types of Al adversely affect root growth, an area of future study that may warrant attention. In addition, results from screening of genetic material for differential Al tolerance should be viewed with caution unless steps were taken to preclude toxic polymer formation.

The characterization of the Al<sub>n</sub> polymers reported here was not comprehensive, but did suggest that they are relatively small, possess substantial positive charge, and are dominantly the so-called Al<sub>13</sub> polymer. It remains to be seen whether any other similar species (i.e. the hexagonal ring units envisioned by some) can also be formed and yield similar chemical and toxicological effects. If not, the toxic Al<sub>13</sub> polymers observed here may be unique to certain combinations of total Al, basicity, and synthesis conditions (Bertsch, 1987). However, relatively few studies have employed <sup>27</sup>Al-NMR, the only definitive means of identifying Al<sub>13</sub>, and it is thus quite difficult to assess the ubiquity of this species.

Similarly, it remains to be seen whether Al polymer toxicity is of any general significance in acid soils. It is noteworthy, however, that there are reports of lime-induced growth depressions that could not be readily explained by micronutrient deficiencies or other factors (Pearson, 1975; Farina et al., 1980). Since conventional wisdom dictates that liming will precipitate Al as innocuous solid phases, the possibility of toxicity due to polynuclear species may have been overlooked. It is conceivable, however, that Al<sub>13</sub> (or some other toxic polymer) could form in the vicinity of a dissolving limestone particle (Bertsch, 1987). In addition to these results with both a monocot and a dicot, polymer toxicity has been demonstrated with fish (Baker and Schofield, 1982) and with the symbiotic bacterium *Rhizobium trifolii* (Wood and Cooper, 1984), suggesting that the toxicity may be rather general. Future studies of Al toxicity should carefully consider

formation of polynuclear species and their possible adverse effect on growth and survival.

## *References*

- Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986a. Effects of phosphorus/aluminum molar ratio and calcium concentration on plant response to aluminum toxicity. *Soil Sci. Soc. Am. J.* 50:133-137.
- Alva, A. K., D. G. Edwards, C. J. Asher, and F. P. C. Blamey. 1986b. Relationships between root length of soybean and calculated activities of aluminum monomers in solution. *Soil Sci. Soc. Am. J.* 50:959-962.
- Baker, J. P., and C. Schofield. 1982. Aluminum toxicity to fish in acidic waters. *Water Air Soil Pollut.* 18:289-309.
- Bartlett, R. J., and D. C. Riego. 1972. Toxicity of aluminum in relation to pH and phosphorus. *Soil Sci.* 114:194-201.
- Bertsch, P. M. 1987. Conditions for  $Al_{13}$  polymer formation in partially neutralized aluminum solutions. *Soil Sci. Soc. Am. J.* 51:825-828.
- Bertsch, P. M., W. J. Layton, and R. I. Barnhisel. 1986a. Speciation of hydroxy-aluminum solutions by wet chemical and aluminum-27 NMR methods. *Soil Sci. Soc. Am. J.* 50:1449-1454.
- Bertsch, P. M., G. W. Thomas, and R. I. Barnhisel. 1986b. Characterization of hydroxy-aluminum solutions by aluminum-27 nuclear magnetic resonance spectroscopy. *Soil Sci. Soc. Am. J.* 50:825-830.
- Blamey, F. P. C., D. G. Edwards, and C. J. Asher. 1983. Effects of aluminum, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. *Soil Sci.* 136:197-207.
- Bowling, D. J. F. 1976. Uptake of ions by plant roots. Halsted Press, John Wiley & Sons, New York.
- Denney, D. Z., and P. H. Hsu. 1986.  $^{27}Al$  nuclear magnetic resonance and ferron kinetic studies of partially neutralized  $AlCl_3$  solutions. *Clays Clay Min.* 34:604-607.
- Farina, M. P. W., M. E. Sumner, C. O. Plank, and W. S. Letzsch. 1980. Aluminum toxicity in corn at near neutral soil pH levels. *J. Plant Nutr.* 2:683-697.

- Foy, C. D., A. L. Fleming, G. R. Burns, and W. H. Armiger. 1967. Characterization of differential aluminum tolerance among varieties of wheat and barley. *Soil Sci. Soc. Am. Proc.* 31:513-520.
- Hsu, P. H. 1968. Interactions between aluminum and phosphate in aqueous solution. *Adv. Chem. Ser.* 73:115-127.
- Hsu, P. H. 1977. Aluminum hydroxides and oxyhydroxides. p. 99-143. *In* J. B. Dixon and S. B. Weed (ed.) *Minerals in soil environments*. Soil Science Society of America, Madison, WI.
- Jardine, P. M., and L. W. Zelazny. 1986. Mononuclear and polynuclear aluminum speciation through differential kinetic reactions with ferron. *Soil Sci. Soc. Am. J.* 50:895-900.
- May, H. M., P. A. Helmke, and M. L. Jackson. 1979. Gibbsite solubility and thermodynamic properties of hydroxy-aluminum ions in aqueous solution at 25° C. *Geochim. Cosmo. Acta.* 43:861-868.
- Parthasarathy, N. and J. Buffle. 1985. Study of polymeric aluminum (III) hydroxide solutions for application in waste water treatment. Properties of the polymer and optimal conditions of preparation. *Water Res.* 19:25-36.
- Pearson, R. W. 1975. Soil acidity and liming in the humid tropics. *Cornell Int. Agric. Bull.* 30, Cornell Univ., Ithaca, N.Y.
- Smith, R. W. 1971. Relations among equilibrium and nonequilibrium aqueous species of aluminum hydroxy complexes. *Adv. Chem Ser.* 106:250-279.
- Smith, R. W., and J. D. Hem. 1972. Effect of aging on aluminum hydroxide complexes in dilute aqueous solutions. U.S. Geol. Surv. Water-Supply Paper 1827-D.
- Staub, C., J. Buffle, and W. Haerdi. 1984. Measurement of complexation properties of metal ions in natural conditions by ultrafiltration: Influence of various factors on the retention of metals and ligands by neutral and negatively charged membranes. *Anal. Chem.* 56:2843-2849.
- Stumm, W., and J. J. Morgan. 1981. *Aquatic chemistry*. 2nd ed. John Wiley & Sons, New York.
- Tsai, P.P., and P.H. Hsu. 1984. Studies of aged OH-Al solutions using kinetics of Al-ferron reactions and sulfate precipitation. *Soil Sci. Soc. Am. J.* 48:59-65.
- Turner, R.C. 1976. Effect of aging on properties of polynuclear hydroxyaluminum cations. *Can. J. Chem.* 54: 1528-1534.
- Wagatsuma, T., and Y. Ezoe. 1985. Effect of pH on ionic species of aluminum in medium and on aluminum toxicity under solution culture. *Soil Sci. Plant Nutr.* 31:547-561.

- Wagatsuma, T., and M. Kaneko. 1987. High toxicity of hydroxy-aluminum polymer ions to plant roots. *Soil Sci. Plant Nutr.* 33:57-67.
- White, R. E. 1976. Studies on mineral adsorption by plants. III. The interaction of aluminum, phosphate, and pH on the growth of *Medicago sativa*. *Plant Soil* 46:195-208.
- Wood, M., and J. E. Cooper. 1984. Aluminum toxicity and multiplication of *Rhizobium trifolii* in a defined growth medium. *Soil Biol. Biochem.* 16:571-576.
- Zelazny, L. W., and P. M. Jardine. 1988. Surface reactions of aqueous aluminum. In G. Sposito (ed.) *Environmental chemistry of aluminum*. CRC Press, Boca Raton, FL (in press).

# Chapter V

## Summary and Conclusions

Although it has long been recognized that toxicity of aluminum (Al) to plants is a major growth-limiting factor in acid soils, relationships between the aqueous chemistry of Al and phytotoxicity are still not fully understood. At the initiation of these studies, the effect of hydroxyl ions was particularly problematic in that, although raising solution pH generally relieves Al toxicity, there were no consistent indications as to why this occurs. The effect has been ascribed to differential toxicity of the mononuclear hydrolysis products, but conflicting reports have appeared as to which are the more toxic species. Alternatively, the formation of nontoxic polynuclear hydroxy-Al complexes or solid phase  $\text{Al}(\text{OH})_3$  has been invoked to explain the effect, although there was a small body of evidence that the polynuclear species are also toxic. A third, heretofore untested, hypothesis was that low pH may predispose plants to Al injury, and that amelioration with increasing pH is a physiological response, independent of changes in Al speciation.

In order to attack this problem, an accurate and reliable method was needed for speciating aqueous Al. Polynuclear hydroxy-Al complexes are generally viewed as metastable species, and are thus more amenable to empirical than to computational approaches to speciation. The objectives of the first study were to evaluate and compare the ferron (8-hydroxy-7-iodo-5-quinoline-sulfonic acid), aluminon (aurintricarboxylic acid, triammonium salt), and 8-hydroxyquinoline methods for their ability to differentiate mono- and polynuclear Al in solutions typical of those used in subsequent phytotoxicity studies. Test solutions were 20  $\mu\text{M}$  in  $\text{AlCl}_3$ , 0.4  $\text{mM}$  in  $\text{CaCl}_2$  or 40  $\text{mM}$  in  $\text{NaCl}$ , and had basicities (molar  $\text{OH}/\text{Al}$  ratios) ranging from -1 to 2.25.

Reactions of ferron and aluminon with mixtures of mono- and polynuclear Al-hydroxide species were kinetically modeled as two simultaneous reactions, yielding estimates of the mononuclear fraction. The reaction of 8-hydroxyquinoline with mononuclear Al was essentially instantaneous, and the use of an arbitrary cutoff time unavoidable. Although each method provided a consistent, reproducible estimate of the mononuclear fraction, differences between methods occurred. At low basicities, the estimates of  $f_m$  were in the order 8-hydroxyquinoline > ferron > aluminon, while at higher basicities the general order is aluminon  $\geq$  8-hydroxyquinoline > ferron. In comparison to the other methods, aluminon appeared to be somewhat insensitive to changes in speciation.

Significant quantities of polynuclear Al were detected even in solutions without added base or with slight acidification. Increasing ionic strength of test solutions increased estimates of  $f_m$  by all methods. Aging solutions up to 32 days resulted in decreased estimates of  $f_m$  using aluminon, but estimates using ferron and 8-hydroxyquinoline were only minimally affected. At  $\text{Al}_i = 20 \mu\text{M}$ , all three methods yielded results of adequate precision for most applications. The order of precision is 8-hydroxyquinoline > ferron

> aluminon. Ferron was only one-half as sensitive as the other two methods, and may be of limited use at Al concentrations much below  $10 \mu\text{M}$ , although careful adjustment of the molar ferron/Al ratio can improve sensitivity somewhat.

Ferron may be the preferred method, at least in chemically simple media, due to its simplicity, precision, and moderately rapid kinetics. Additional information regarding the nature and reactivity of polynuclear and solid phase Al can be readily obtained using a kinetic approach. The best kinetic model is one where monomers obey a pseudo second-order rate law, reactive polymers a pseudo first-order law, and the data are corrected for unreactive polynuclear species and/or solid phases.

Solution culture techniques were then used to examine the relationships between Al speciation and phytotoxicity in dilute, chemically well-defined systems wherein  $\text{OH}^-$  was the only Al-complexing ligand. Two-day old wheat (*Triticum aestivum* L. cv. 'Tyler' or 'Seneca') seedlings were transferred to 500-mL test solutions for 2 days of additional growth at 298 K in the dark, and final root lengths used as the measure of Al toxicity. Quantities of polynuclear hydroxy-Al (considered to be all nonmononuclear Al for this study) were estimated with the ferron procedure, and activities of mononuclear species were calculated using the computer program GEOCHEM.

In the absence of polynuclear hydroxy-Al, raising solution pH consistently ameliorated Al toxicity suggesting that, although  $\text{Al}^{3+}$  is not necessarily the sole toxic species, its activity is the best single indicator of Al stress. The results did not confirm other reports that the sum of the mononuclear species activities is a reliable predictor of Al phytotoxicity. Polynuclear hydroxy-Al was also demonstrably toxic, supporting isolated reports of the same finding, and indicated that the common practice of excluding the polynuclear fraction from estimates of toxic Al should be reevaluated. Experiments with



lanthanum, a trivalent metal that does not hydrolyze over the pH range of interest (4.2 to 5.6), suggested that there is no tendency for low pH to predispose plant roots to metal toxicity. Variations in Al toxicity with increasing pH can therefore be ascribed primarily to changes in Al speciation.

Inasmuch as the demonstrated phytotoxicity of polynuclear hydroxy-Al contrasted with several earlier reports, a third study was undertaken to examine how toxicity of these complexes might vary with experimental conditions or with choice of test plants. A further objective was to identify and characterize the toxic polynuclear species. Wheat or soybean (*Glycine max* (L.) Merr. cv. 'Stafford') seedlings were cultured for 2 d in dilute  $\text{CaCl}_2$  solutions containing  $15 \mu\text{M}$  total Al at a basicity (molar OH/Al ratio) of 2.0. Test solutions were derived from  $0.5 \text{ mM}$  stock solutions that had been preneutralized at varying base injection rates ( $0.05$  to  $12 \text{ mL min}^{-1}$ ) and aged for 1 to 180 d. Phosphate additions to the growth solutions ( $0$  to  $4.5 \mu\text{M}$  as  $\text{KH}_2\text{PO}_4$ ) were also included as a source of variation.

Increasing stock solution age, rate of base addition, or levels of added phosphate decreased the fraction of reactive polymers ( $\text{Al}_b$ ) and increased that of precipitated Al ( $\text{Al}_c$ ) as determined by the ferron method. Mononuclear Al was consistently  $\leq 3 \mu\text{M}$  and did not contribute to toxicity. Inhibition of root growth was well correlated with  $[\text{Al}_b]$  for both species. Despite wide variation in the distribution of  $\text{Al}_b$  and  $\text{Al}_c$  due to experimental conditions, the toxic polymers were persistent in all test solutions except those prepared from very old stocks or those containing rather high levels of P. Thus, the reports of others, who have not observed this phenomenon in some similar solutions, cannot be readily explained. However, the rather low levels of  $\text{Al}_b$  required to substan-

tially inhibit root growth suggest that detection of polymer toxicity may at times be problematic, especially in chemically complex media.

All plants tested were far more tolerant of the mononuclear ion  $\text{Al}^{3+}$  (at pH 4.3) than of  $\text{Al}_6$ , suggesting fundamental physiological differences in the toxicity of the two types of Al. This was further supported by the finding that the two wheat cultivars Tyler and Seneca exhibited marked differential tolerance to  $\text{Al}^{3+}$ , but not to  $\text{Al}_6$ . These findings suggest that future research on the physiological basis of the two types of Al toxicity may be warranted.

Analyses using ferron kinetics, ultrafiltration, calculated stoichiometry of the solutions, and  $^{27}\text{Al}$  – NMR indicated that  $\text{Al}_6$  is composed of relatively small polymers with significant residual positive charge that are principally the so-called  $\text{Al}_{13}$  polymer. Although formation of this polymer may depend on unique combinations of total Al, basicity, and synthesis conditions, it is usually observed by researchers who employ  $^{27}\text{Al}$  – NMR and may thus be more ubiquitous than generally believed. Proponents of a small polymer with a hexagonal ring structure have generally not utilized  $^{27}\text{Al}$  – NMR, and the importance of such species is presently very difficult to assess.

The studies described in this report have significantly improved our understanding of the relationships between Al phytotoxicity and chemistry in systems where hydroxyl is the dominant Al-complexing ligand. At the same time, several areas of future research have been identified. Although the ferron method has been shown to adequately separate the three major classes of Al in relatively 'clean' solutions, it suffers from interferences by common soil constituents and lacks sensitivity at very low ( $< 10 \mu\text{M}$ ) levels of total Al. Consequently, there is still a need for a method that can overcome these limitations yet provide the same kind of unambiguous speciation data obtained with ferron. Further

resolution of the toxicity or nontoxicity of the mononuclear Al species may be possible, especially if a very reliable set of hydrolysis constants can be obtained. The overall relationships between Al speciation and toxicity may lend credence to a hypothesis wherein the physiological basis of toxicity is primarily an extracellular event. Further study is sorely needed to verify or discount this theory.

It remains to be seen whether Al polymer toxicity is of any general significance in acid soils. There are, however, reports of lime-induced growth depressions that could not be explained by micronutrient deficiencies or other factors. Since conventional wisdom dictates that liming will precipitate Al as innocuous solid phases, the possibility of toxicity due to polynuclear species may have been largely overlooked in the soils literature. A detailed study of soil solution chemistry in situations where formation of these complexes seems most probable would be a useful contribution to our overall understanding of acid soil infertility. In addition, the possible formation and phytotoxicity of polynuclear species other than  $Al_3$  merits investigation.

In addition to the results reported here with both a monocot and a dicot, polymer toxicity has been demonstrated with fish and with the symbiotic bacterium *Rhizobium trifolii*, suggesting that the toxicity may be rather general. The profoundly different response of wheat and soybeans to  $Al^{3+}$  vs.  $Al_3$  suggests that past research efforts may not always be readily compared, especially when pH approached or exceeded ca. 5.0. Future studies of Al toxicity should carefully consider formation of polynuclear species, and their possible adverse effect on growth and survival.

# Appendix A

## Additional Data for Chapter II

### Calcium Interference with Aluminon

An experiment was conducted to evaluate the effect of Ca on the aluminon assay over a  $\text{CaCl}_2$  concentration range relevant to toxicity studies. The test solutions contained  $20 \mu\text{M}$  acidified (monomeric)  $\text{AlCl}_3$ . One mL of sample and 1.4 mL reagent were mixed and, after 2 h at 298 K, absorbance was measured at 546.2 nm. No effect on absorbance was observed for Ca levels up to 6.4 mM (Table 9).

### Kinetics of Al Monomer Reactions with Ferron and Aluminon

Typical linearized second-order plots for the reaction of acidified, monomeric Al solutions with ferron and aluminon are presented in Figs. 17 and 18. The procedure was as described in Chapter II. The linear nature of the plots suggested that both reactions are

**Table 9. Effect of calcium on absorbance of Al monomer-aluminum mixtures**

[CaCl <sub>2</sub> ] mM	Absorbance
0	0.296 ± 0.009
0.4	0.281 ± 0.006
0.8	0.283 ± 0.008
1.6	0.285 ± 0.008
3.2	0.281 ± 0.004
6.4	0.281 ± 0.007

well-approximated as pseudo second-order with respect to Al. In contrast, first-order plots showed marked curvilinearity (data not shown).

## **Kinetics of Al Monomer-8-Hydroxyquinoline Reactions**

The standard volumes of sample ( $20 \mu\text{M}$  Al, acidified) and reagents were used with the beaker-stir-bar version of the 8-hydroxyquinoline method. The sample was rapidly injected into the stirred reagent mixture using an automatic pipet, and the reaction stopped after varying times by rapid injection of the butyl acetate using a repipet. Absorbance of the organic phase was measured at 388.4 nm and showed no consistent increasing trend as a function of time (Table 10). It was thus concluded that the reaction was, for all practical purposes, instantaneous.

## **Additional Method Comparison Data**

In addition to the data presented in Fig. 3, all three methods were used to analyse the  $0.4 \text{ mM}$   $\text{CaCl}_2$  solutions after 30 d aging and the  $40 \text{ mM}$   $\text{NaCl}$  solutions after 3 d aging. The results (Figs. 19 and 20) were quite similar to those depicted in Fig. 3.

## **Comparison of Ferron and 'Instantaneous' 8-Hydroxyquinoline Methods**

A set of solutions containing  $20 \mu\text{M}$  Al at  $n_0 = 0, 0.75, 1.50,$  or  $2.25$  and aged 7 d were assayed with ferron and the beaker-stir-bar version of the 8-hydroxyquinoline method described on p. 15 and 25, As described in Chapter II, this resulted in better agreement

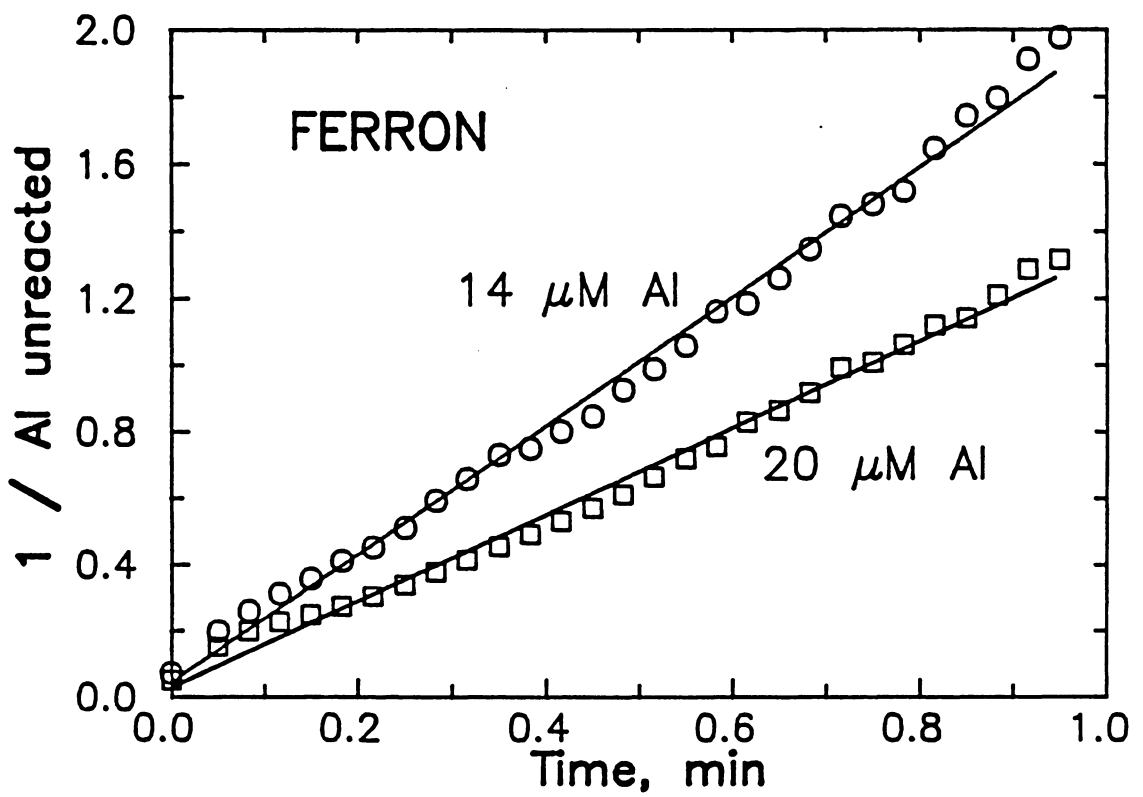


Figure 17. Linearized second-order plot for the reaction of ferron and mononuclear Al

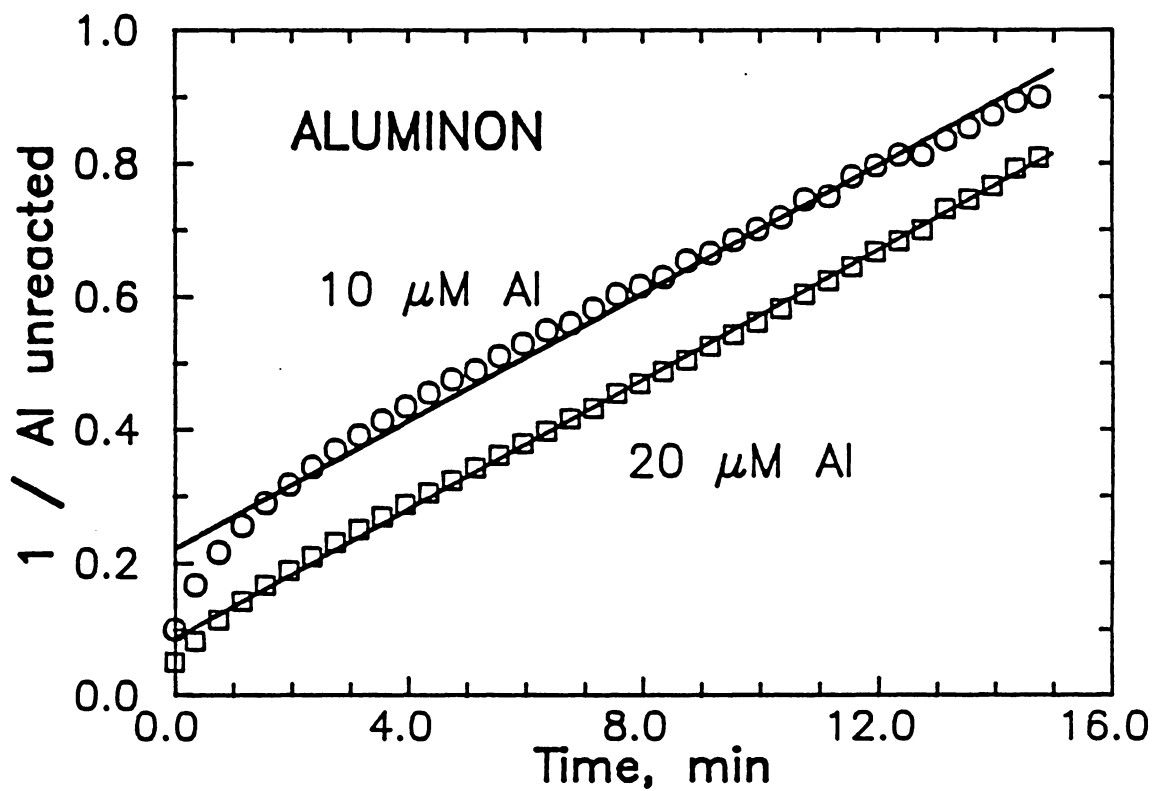


Figure 18. Linearized second-order plot for the reaction of aluminon and mononuclear Al



**Table 10. Effect of reaction time on absorbance of Al monomer-8-hydroxyquinoline mixtures**

Time	Absorbance
sec	
5	0.301
10	0.262
15	0.240
20	0.223
30	0.240
60	0.286

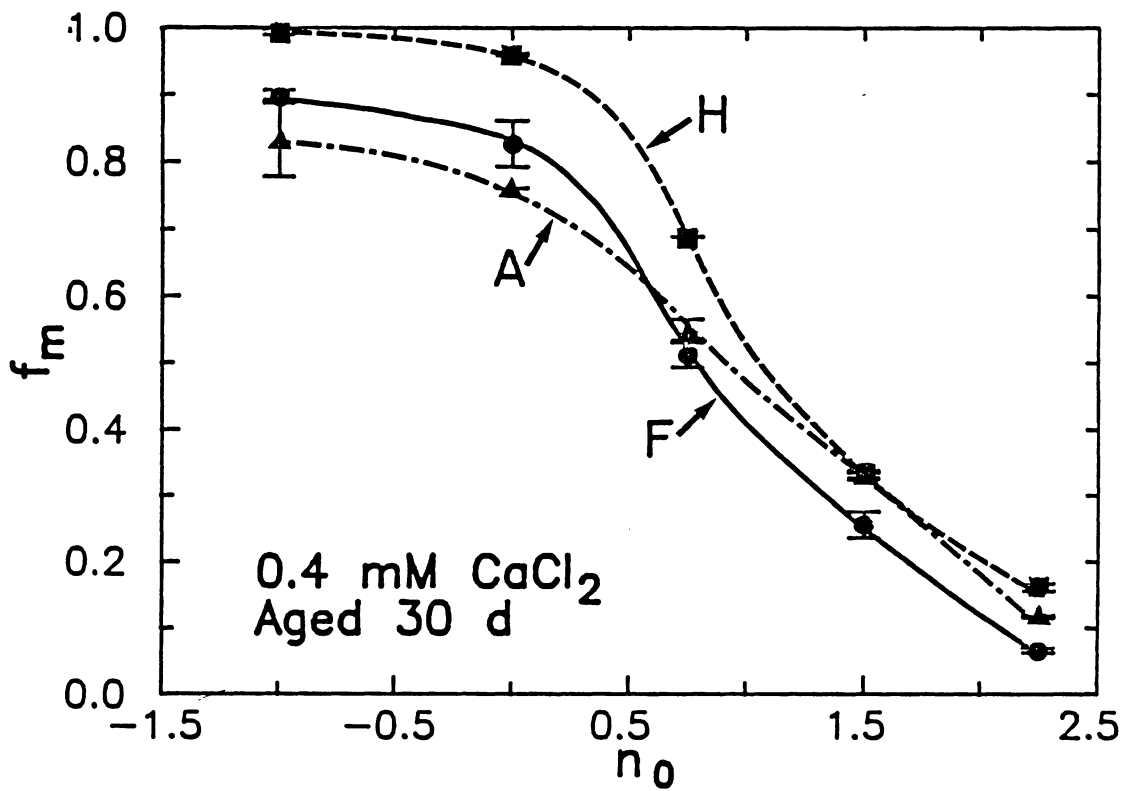


Figure 19. Fraction of mononuclear Al vs. basicity by the three methods for the 0.4 mM  $\text{CaCl}_2$  solutions after 30 d aging: F = ferron, A = aluminon, and H = 8-hydroxyquinoline. Vertical bars indicate standard errors of duplicate determinations where  $1 \text{ SE} > \text{symbol size}$ .

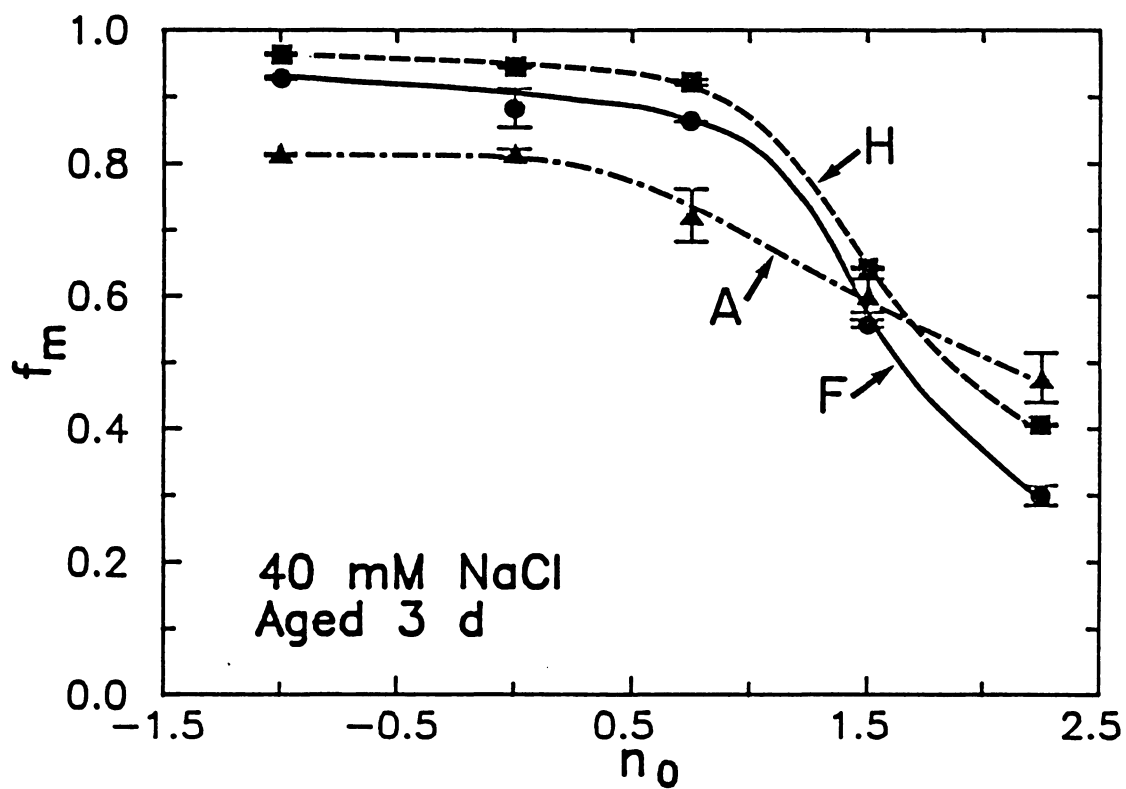


Figure 20. Fraction of mononuclear Al vs. basicity by the three methods for the 40 mM NaCl solutions after 3 d aging: F = ferron, A = aluminon, and H = 8-hydroxyquinoline. Vertical bars indicate standard errors of duplicate determinations where  $1 \text{ SE} > \text{symbol size}$ .

between the two methods at basicities  $\geq 1.5$ , but did not remove discrepancies at lower basicities (Table 11).

## Kinetics of Ferron-Polymer Reactions

The solutions depicted in Figs. 5 and 6 and Table 3 were analyzed to determine the reaction order of the ferron-polymer reaction. The first 90 s of data were omitted to eliminate any contribution of monomeric Al, and the analysis carried out to 24 min. Linearized first-order (Fig. 21) and second-order (Fig. 22) plots were constructed. The greater linearity observed in Fig. 21 suggested that this reaction was best approximated as a first-order reaction with respect to Al.

## Speciation of Solutions Described in Table 3 After 35 Days Aging

The solutions depicted in Figs. 5 and 6 and Table 3 were analyzed with ferron again after 35 d aging, and the data fit to the same five reaction schemes used in Table 3. Estimates of  $f_m$  were slightly lower than at 3 d aging (Tables 3 and 12). Again, the best fit to the first 24 min of data was obtained by correcting for  $Al_c$ . As before, when only the first 5 min of the reaction was considered, estimates of  $f_m$  were comparable to those using the 'best' model, regardless of whether  $n'$  was 1 or 2.

Table 11. Fraction of mononuclear Al by ferron and the 'instantaneous' 8-hydroxyquinoline methods

$n_0$	$f_m$	
	Ferron	8-hydroxy.
0.00	$0.854 \pm 0.004$	$0.973 \pm 0.071$
0.75	$0.585 \pm 0.054$	$0.700 \pm 0.028$
1.50	$0.356 \pm 0.032$	$0.322 \pm 0.016$
2.25	$0.091 \pm 0.010$	$0.077 \pm 0.006$

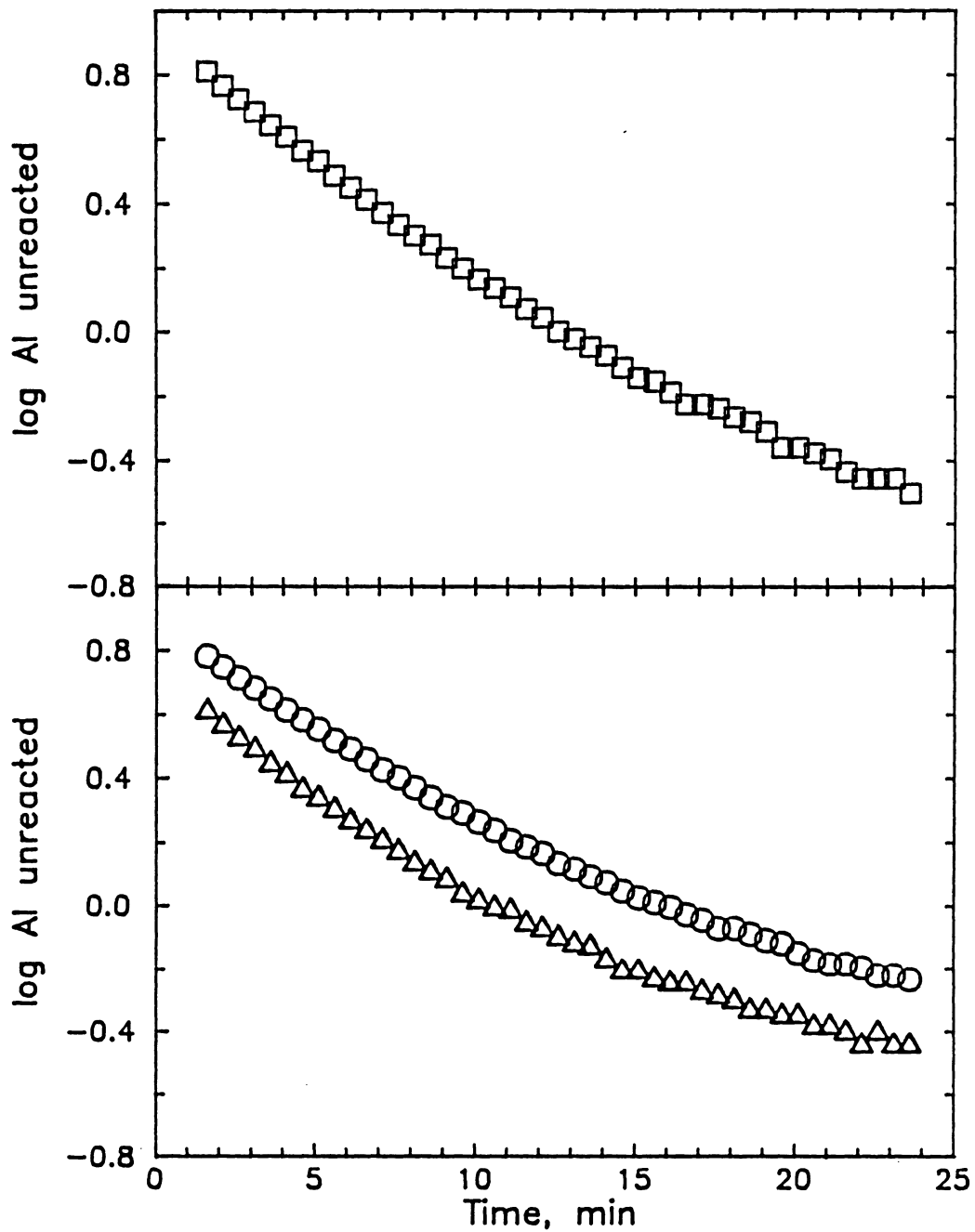


Figure 21. Linearized first-order plots for the reaction of ferron and polynuclear Al: Solutions contained  $20 \mu\text{M}$   $\text{Al}_t$  and  $0.4 \text{ mM}$   $\text{CaCl}_2$  and were aged 3 d prior to analysis. Values of  $n_0$  were 0.75 (triangles), 1.50 (squares), and 2.25 (circles).

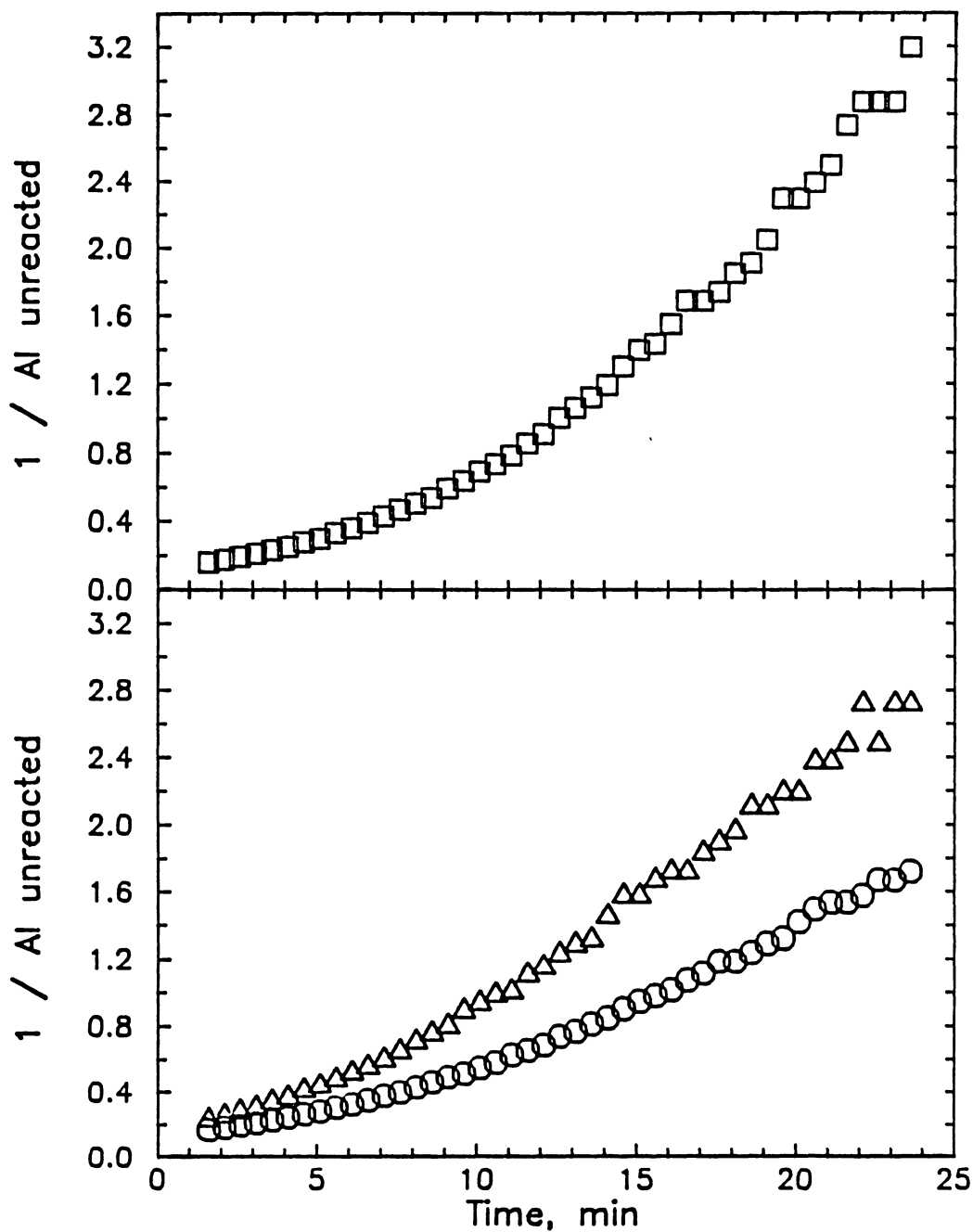


Figure 22. Linearized second-order plots for the reaction of ferron and polynuclear Al: Solutions contained  $20 \mu\text{M}$   $\text{Al}_t$  and  $0.4 \text{ mM}$   $\text{CaCl}_2$  and were aged 3 d prior to analysis. Values of  $n_0$  were 0.75 (triangles), 1.50 (squares), and 2.25 (circles).

Table 12. Computed fraction of mononuclear Al and coefficients of determination for the solutions from Table 3 after 35 d aging. The alternative kinetic models are all based on the same data representing single determinations at each value of  $n_q$ .

Model	Rxn. orders		Duration min	Corrected for Al <sub>c</sub> ?	$n_q = 0.75$		$n_q = 1.50$		$n_q = 2.25$	
	$n$	$n'$			$f_m$	$R^2$	$f_m$	$R^2$	$f_m$	$R^2$
1	2	1	24	no	0.642	0.952	0.349	0.916	0.097	0.949
2	2	2	24	no	0.603	0.978	0.343	0.923	0.097	0.951
3	2	1	24	yes	0.578	0.991	0.276	0.980	0.068	0.988
4	2	1	5	no	0.580	0.975	0.292	0.935	0.076	0.959
5	2	2	5	no	0.576	0.973	0.292	0.935	0.076	0.959



## **Appendix B**

### **Additional Data for Chapter III**

#### **Experiments 14 and 15 -- Tyler Root Growth at Constant Sum of Mononuclear Species Activities**

An experiment similar to that depicted in Fig. 9 was conducted using Tyler wheat and  $\{Al_m\}$  held at  $8 \mu M$ . The distribution of Al species and root growth (Fig 23) were similar to that observed with Seneca, again suggesting that (a) some of the polynuclear Al is toxic, and (b) that  $\{Al_m\}$  is not well-correlated with toxicity.

#### **Experiment 16 -- Tyler Root Growth as a Function of Basicity**

A single experiment where  $n_0$  ranged from -1 to 2.0 was conducted prior to the experiments depicted in Fig. 11. Relative root growth of Tyler wheat declined steadily with increasing  $n_0$  (and  $f_p$ ) and levelled off at ca.  $n_0 = 1.5$  (Fig 24).

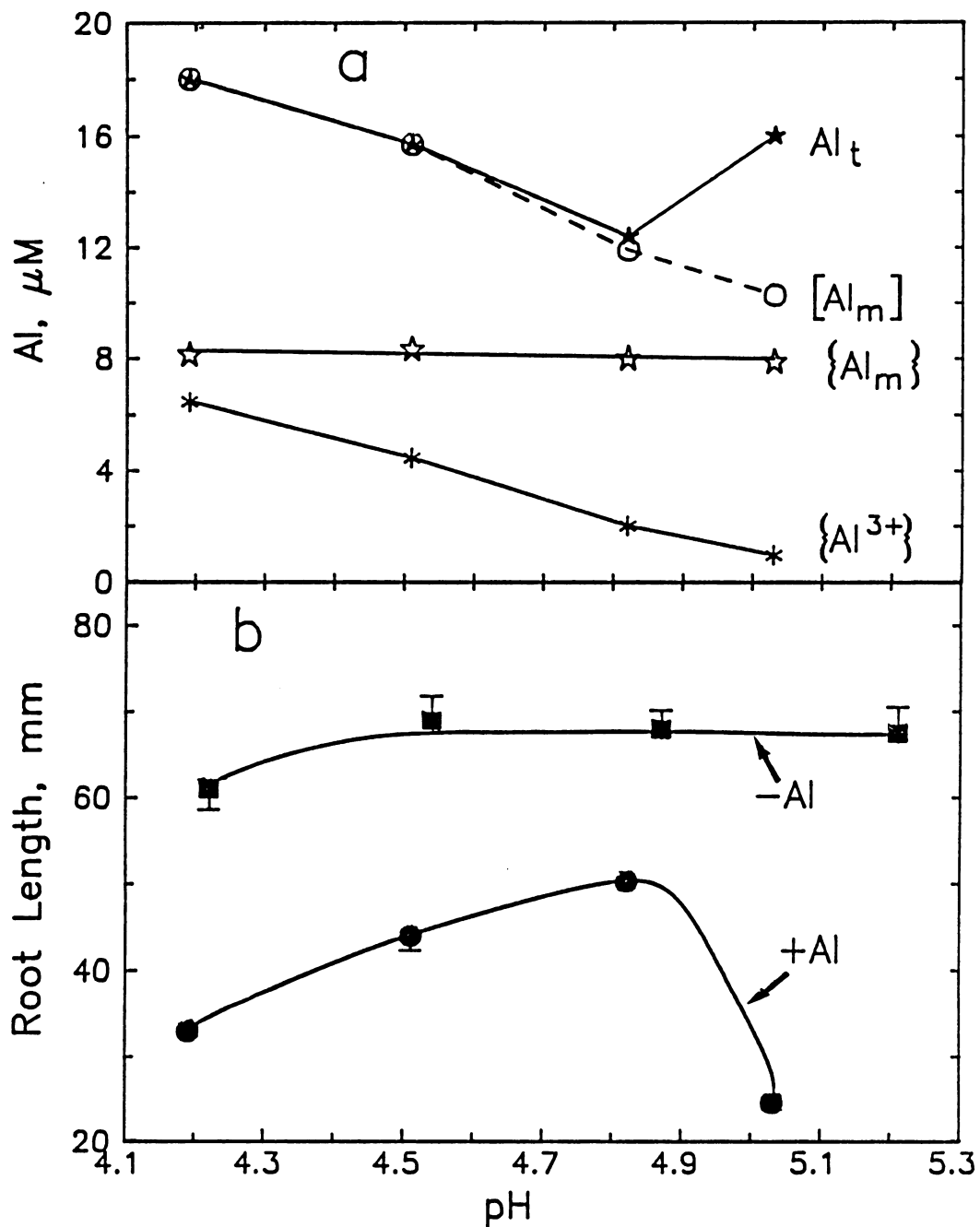
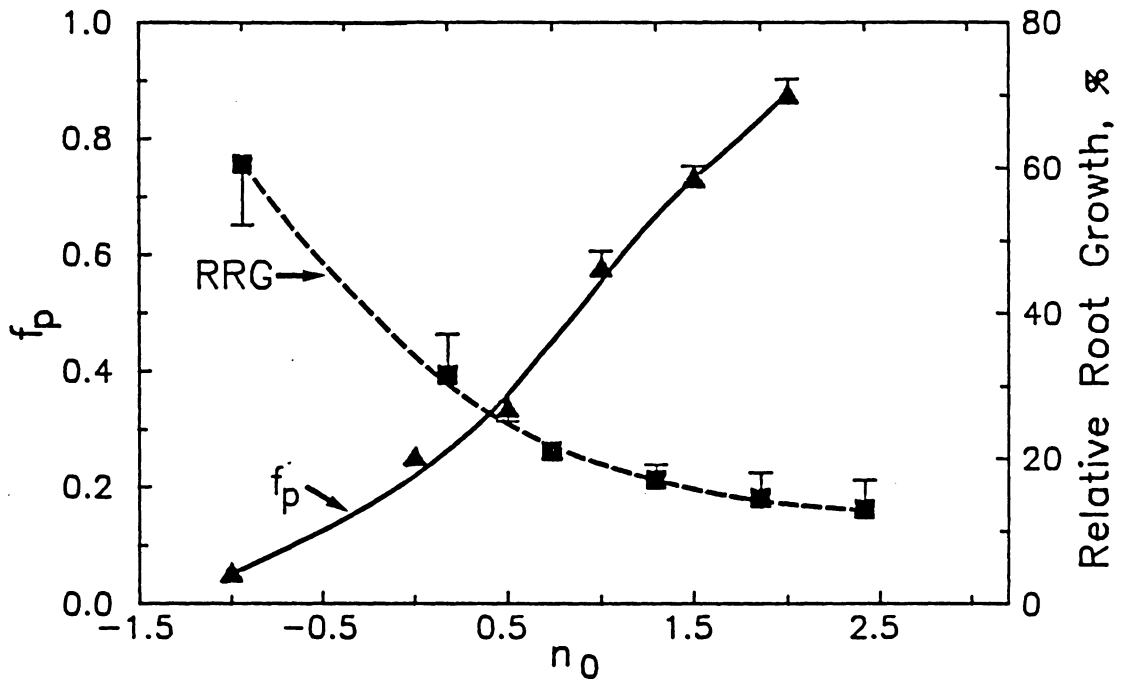


Figure 23. Distribution of Al species (a) and root lengths of Tyler wheat (b) vs. pH at constant sum of mononuclear species activities:  $[\text{CaCl}_2] = 3.0 \text{ mM}$  and points represent means of two identical experiments with two replications each. Lines are hand-fitted and vertical bars signify standard error of the mean where it exceeds symbol size.



**Figure 24.** Fraction of polynuclear Al and relative root growth (RRG) of Tyler wheat vs. basicity:  $Al_t = 15$  and  $\mu M$  and  $[CaCl_2] = 3.0$  mM. Solution pH ranged from 4.7 to 5.2, and control root lengths were constant over this range. Means are for a single experiment with two replications. Vertical bars signify standard error of the mean where it exceeds symbol size, and lines are hand-fitted.

## **Experiments 31 and 32 -- Seneca Root Growth as a Function of Basicity**

Experiments identical to those depicted in Fig 11 (except for Ca level) were conducted with Seneca wheat. The results (Fig. 25) were very similar to those previously obtained with Tyler. The slight increase in root growth as  $n_0$  increased from -1 to 0 can be ascribed to alleviation of toxicity due to  $Al^{3+}$ . At  $n_0 > 0$ , the response is dictated by the toxic polymers (Fig. 25).

## **Tabulated Species Activities for Figures 11 and 25**

The average (4 observations for each cultivar) pH and computed mononuclear species activities are given in Table 13. In general, all species activities, including the sum, decline with increasing  $n_0$ . That root growth declines over most of this range indicates that polymeric species are responsible for most of the inhibition seen in Figs. 11 and 25.

## **Experiments 18 and 33 -- Response of Tyler and Seneca to Al and La at pH 4.3**

Two experiments were conducted to assess the differential tolerance of Tyler and Seneca wheat to Al and La. Both used two Ca levels -- 0.4 and 3.0 mM  $CaCl_2$ . Tyler was grown at  $Al^{3+}$  or  $La^{3+}$  activities of 0 to 6  $\mu M$  and a single replication per treatment. Seneca was grown at  $Al^{3+}$  or  $La^{3+}$  activities of 0 to 16  $\mu M$  and two replications per treatment.

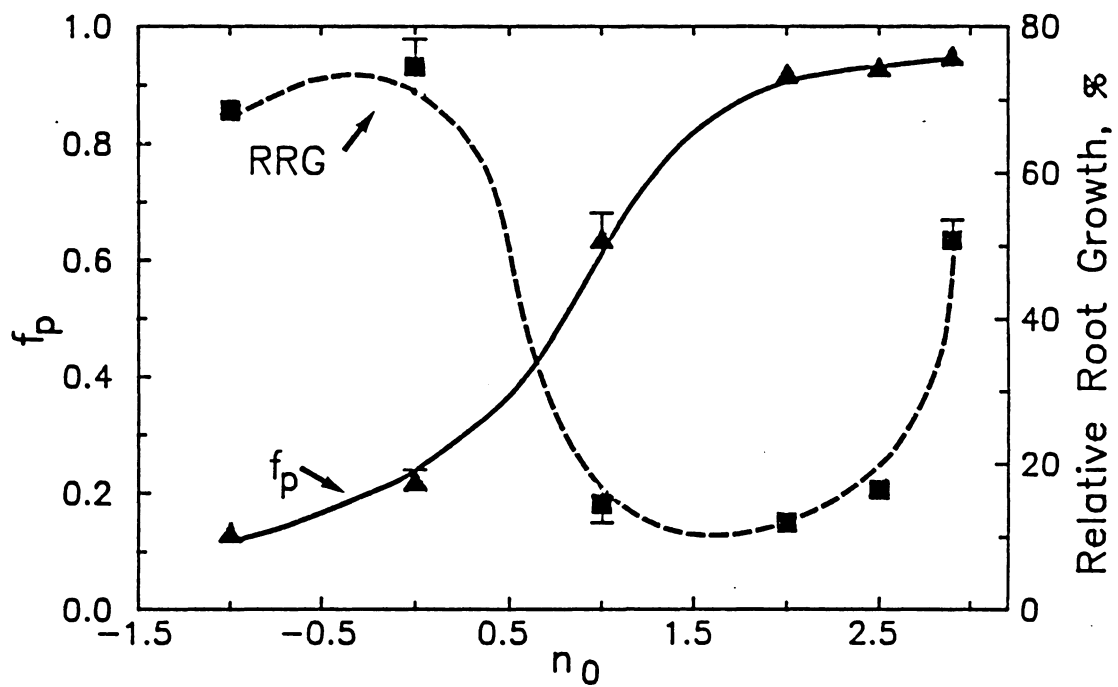


Figure 25. Fraction of polynuclear Al and relative root growth (RRG) of Seneca wheat vs. basicity:  $Al_t = 15$  and  $\mu M$  and  $[CaCl_2] = 1.0$  mM. Solution pH ranged from 4.7 to 5.6, and control root lengths were constant over this range. Means are for two experiments with two replications each. Vertical bars signify standard error of the mean where it exceeds symbol size, and lines are hand-fitted.

Table 13. Computed species activities and pH for the experiments depicted in Figs. 11 and 25

$n_0$	pH	$\{Al^{3+}\}$	$\{AlOH^{2+}\}$	$\{Al(OH)_2^+\}$	$\{Al(OH)_3^0\}$	$\{Al_m\}$
		----- $\mu M$ -----				
Tyler						
-1.0	4.78	2.49	1.44	4.51	0.56	9.00
0	5.01	1.15	1.13	6.00	1.26	9.54
1.0	5.07	0.40	0.45	2.77	0.67	4.29
2.0	5.24	0.08	0.14	1.23	0.47	1.92
2.5	5.40	0.02	0.06	0.73	0.38	1.19
2.9	5.60	< 0.01	0.02	0.33	0.27	0.62
Seneca						
-1.0	4.72	3.27	1.62	4.49	0.48	9.86
0	4.89	1.85	1.36	5.55	0.88	9.64
1.0	5.03	0.53	0.54	3.00	0.66	4.73
2.0	5.12	0.08	0.10	0.71	0.19	1.08
2.5	5.30	0.03	0.06	0.62	0.25	0.96
2.9	5.51	< 0.01	0.02	0.41	0.27	0.71

The results are summarized in Fig. 26 and indicate that the two varieties exhibit almost no differential tolerance to La. In contrast, they separate considerably in the presence of Al, especially at the higher Ca level (Fig. 26).

### **Experiments 249 and 250 -- Response of Seneca to La and pH**

Experiments very similar to those depicted in Fig. 12 were conducted using Seneca wheat. Total La was 0, 20, or 40  $\mu M$  and  $[CaCl_2]$  was 1.5 mM. Again there was a slight, but significant, decline in root length with increasing pH in the presence of La (Fig. 27).

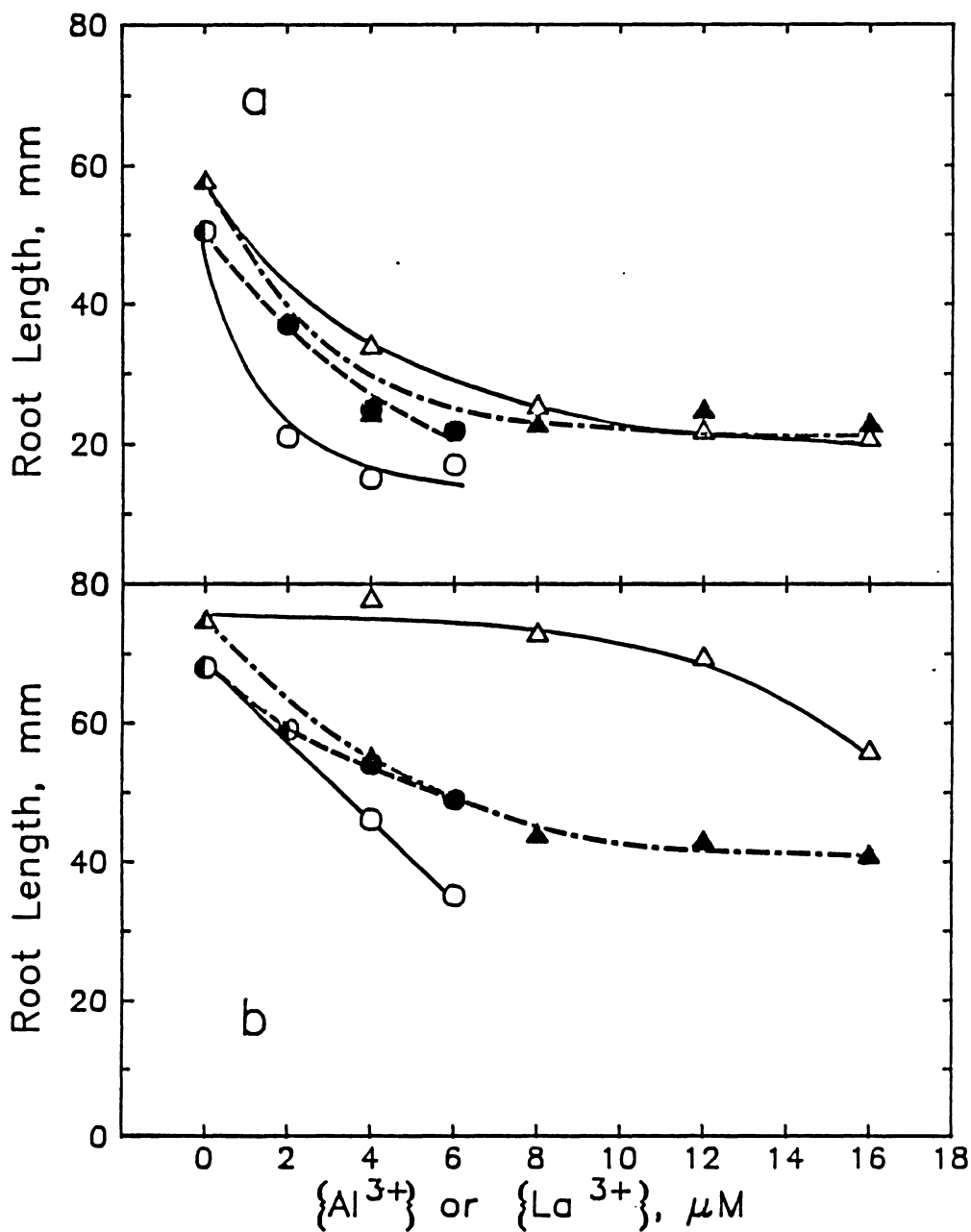


Figure 26. Root length of Tyler and Seneca wheat vs.  $Al^{3+}$  or  $La^{3+}$  activity at pH 4.3:  $[CaCl_2]$  was either 0.4 (a) or 3.0 (b) mM. Circles indicate Tyler and triangles Seneca. Closed symbols are for La, while open symbols indicate Al.



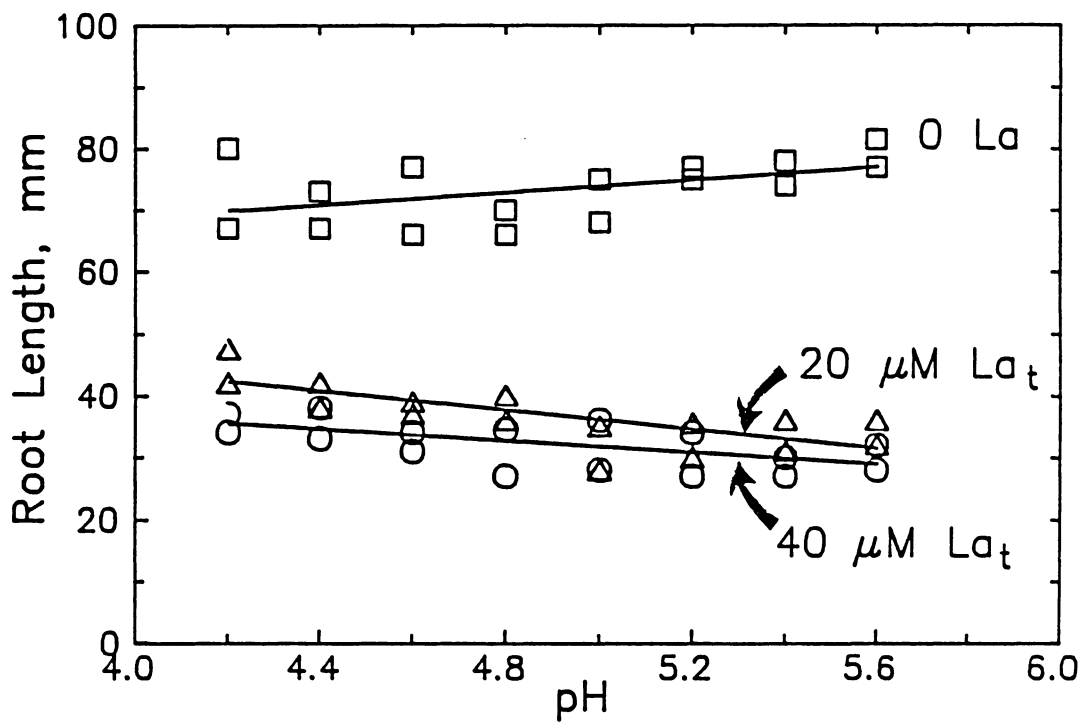


Figure 27. Root length of Seneca wheat as a function of pH in the presence or absence of added La: Lanthanum exists only as the  $\text{La}^{3+}$  ion over this pH range. Points are from two identical unreplicated experiments. Slopes of best-fit linear regression lines are all significantly different from zero ( $p < 0.01$ ).

## Appendix C

### Additional Data for Chapter IV

#### **Expt. 63 - Soybean Response to Mononuclear Al at Variable pH**

An experiment was conducted in 1.0 mM CaCl<sub>2</sub> solutions at pH levels ranging from 4.2 to 4.85. Solutions contained either 0 or 5 μM Al<sub>i</sub>; the latter were derived from stocks with  $n_0 \leq -2$ . No polynuclear Al could be detected in any solutions with ferron. There were two replications per treatment. No significant inhibition of Stafford soybean root growth due to Al occurred at any pH (Table 14). This finding was used as the justification for ascribing all inhibition in partially-neutralized Al solutions to polynuclear Al.

#### **Expts. 71, 72, 75 - Soybean Response to Polynuclear Al**

The tabulated speciation and growth data corresponding to the experiments depicted in Fig. 14 are presented in Table 15.

## Effect of Dilution on Ferron Speciation of Partially-Neutralized Solutions

A 1-d old stock that had been neutralized to  $n_0 = 2.0$  at  $0.05 \text{ mL min}^{-1}$  was diluted to yield solutions containing 15, 50, or  $100 \mu\text{M Al}_i$ . These solutions were then assayed with ferron immediately after dilution, and again after 2 d further aging. The volumes of reagents and sample were adjusted such that the molar ferron/ $\text{Al}_i$  ratio was exactly  $\frac{1}{2}0$  in all cases. For  $\text{Al}_i = 50$  or  $100$ , the Al reacted at 18 min was taken to be  $[\text{Al}_a + \text{Al}_b]$ , and the binary-species kinetics function fit to the first 12 min of data. At  $\text{Al}_i = 15$ , the procedure described in Chapter II was used. All assays were performed in duplicate. The distribution of Al species was not drastically affected by dilution for either 0 or 2 d aging (Table 16). The fitted values of  $k_b$  increased with increasing  $\text{Al}_i$  (Table 16). This is due to the corresponding increases in the absolute concentration of ferron in the reaction mixtures, and the fact that  $k_b$  is a conditional rate coefficient that includes the concentration term for ferron. The observed values of  $k_b$  were consistent with an approximate reaction order of  $3/2$  with respect to ferron, although little can be inferred about stoichiometry from such an observation.

## Additional Ultrafiltration Data

Two additional UF runs were made on  $100 \mu\text{M Al}_i$  solutions that were prepared from a 54-d-old stock that had been neutralized at  $0.5 \text{ mL min}^{-1}$ , or from a 15-d-old stock neutralized at  $12.0 \text{ mL min}^{-1}$ . Again, the UF results closely corresponded to the ferron analysis, and the breakthrough of  $\text{Al}_i$  occurred near a NMWCO of  $1 \times 10^4 \text{ g}$  (Figs. 28 and 29).

## Tabulated Values for Computations of $n_b$

The tabulated data for the computation of  $n_b$  are presented in Table 17. All solutions contained 3.0 mM CaCl<sub>2</sub>. The first 14 solutions in Table 17 contained 15  $\mu$ M Al<sub>i</sub>. Solution 672 contained 50  $\mu$ M Al<sub>i</sub>, and the remaining solutions 100  $\mu$ M Al<sub>i</sub>.

Table 14. Final root lengths of Stafford soybeans in solutions containing only mononuclear Al

Al <sub>i</sub>	pH	Root Length
$\mu M$		mm
0	4.18	95 ± 4 †
	4.43	112 ± 2
	4.66	128 ± 7
	4.85	120 ± 3
5	4.22	96 ± 3
	4.46	109 ± 4
	4.68	126 ± 8
	4.85	123 ± 11

† ± standard error of the mean

Table 15. Influence of stock solution age and rate of base injection on Al speciation and root growth of Stafford soybeans. All growth solutions contained  $15 \mu\text{M Al}_i$  at  $n_0 = 2.0$ , and  $1.0 \text{ mM CaCl}_2$ . Each entry represents the mean of three replications.

Stock Age	Rate of Addition	Relative Root Growth	Ferron Assay				$k_d$	pH <sub>i</sub>
			[Al <sub>i</sub> ]	[Al <sub>L</sub> ]	[Al <sub>L</sub> ]	[Al <sub>L</sub> ]		
days	mL min <sup>-1</sup>	%	----- $\mu\text{M}$ -----			min <sup>-1</sup>		
Experiment 71								
9	0.05	41 ± 5 †	1.5	9.2	4.5	0.21	5.13	
79	0.5	72 ± 7	1.9	1.4	11.5	--‡	4.99	
130	0.05	90 ± 8	1.6	1.1	12.7	--‡	4.96	
145	0.05	99 ± 5	2.8	0.3	9.2	--‡	4.96	
Experiment 72								
7	0.5	42 ± 2	1.3	8.4	5.5	0.19	5.12	
6	3.0	46 ± 4	0.9	6.9	7.0	0.20	5.04	
6	12.0	47 ± 2	0.7	6.3	8.0	0.19	5.03	
30	0.5	49 ± 6	0.8	4.7	8.5	0.19	5.03	
Experiment 75								
1	0.05	29 ± 2	2.6	9.5	2.7	0.18	5.15	
24	0.5	36 ± 1	2.2	6.2	6.5	0.18	5.00	
23	3.0	50 ± 2	2.0	4.9	7.8	0.18	4.99	
23	12.0	50 ± 4	2.4	4.2	8.4	0.20	4.97	

† ± standard error of the mean.

‡ Could not fit an accurate value due to low level of [Al<sub>L</sub>].

Table 16. Ferron-based speciation of partially neutralized Al solutions as a function of dilution

Stock Age	Al <sub>t</sub>	Fraction of			k <sub>b</sub>	pH
		Al <sub>s</sub>	Al <sub>b</sub>	Al <sub>e</sub>		
days	$\mu M$				min <sup>-1</sup>	
0	15	0.11	0.64	0.25	0.12	5.30
	50	0.17	0.70	0.13	0.34	5.05
	100	0.15	0.71	0.14	0.44	4.93
2	15	0.14	0.60	0.26	0.13	5.27
	50	0.16	0.70	0.14	0.34	5.04
	100	0.15	0.72	0.13	0.48	4.88

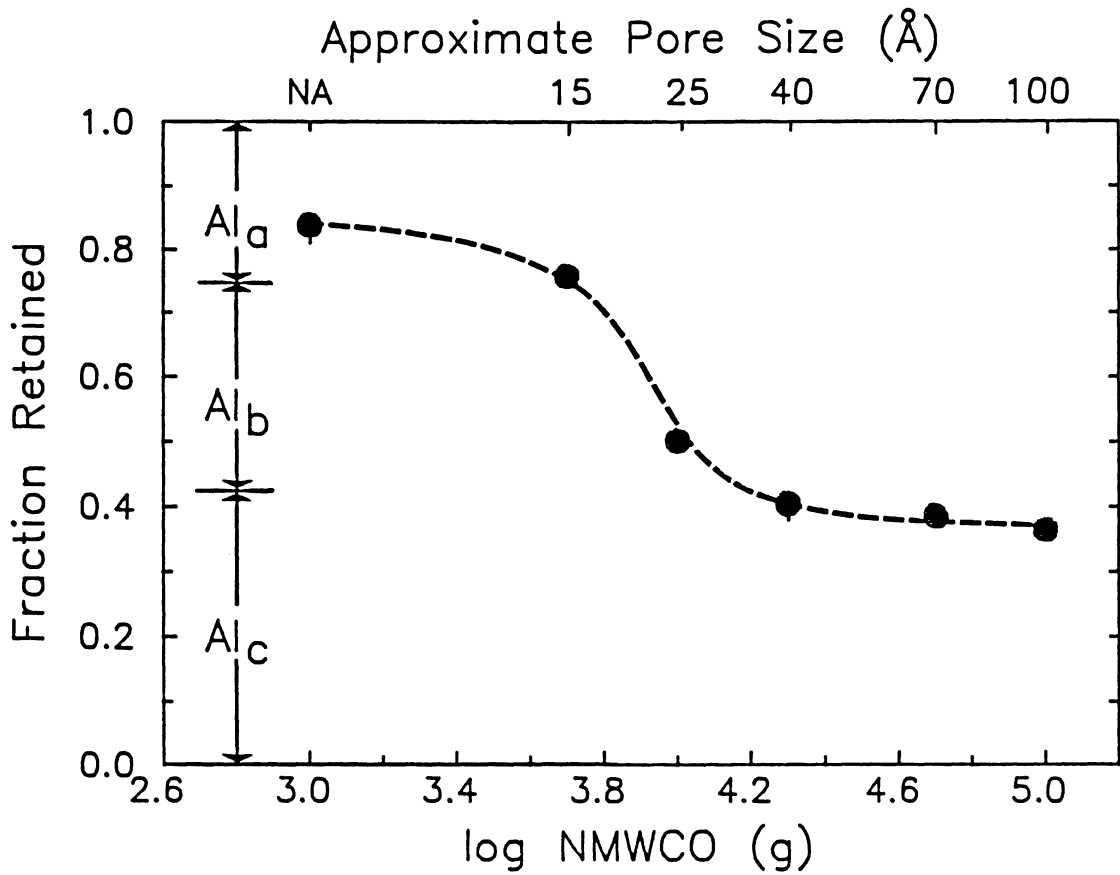


Figure 28. Fraction of Al<sub>i</sub> retained vs. nominal molecular weight cutoff of several membrane filters: The solution was prepared from a preneutralized stock solution after 54 d aging. Vertical bars indicate standard error where 1 SE > symbol size.



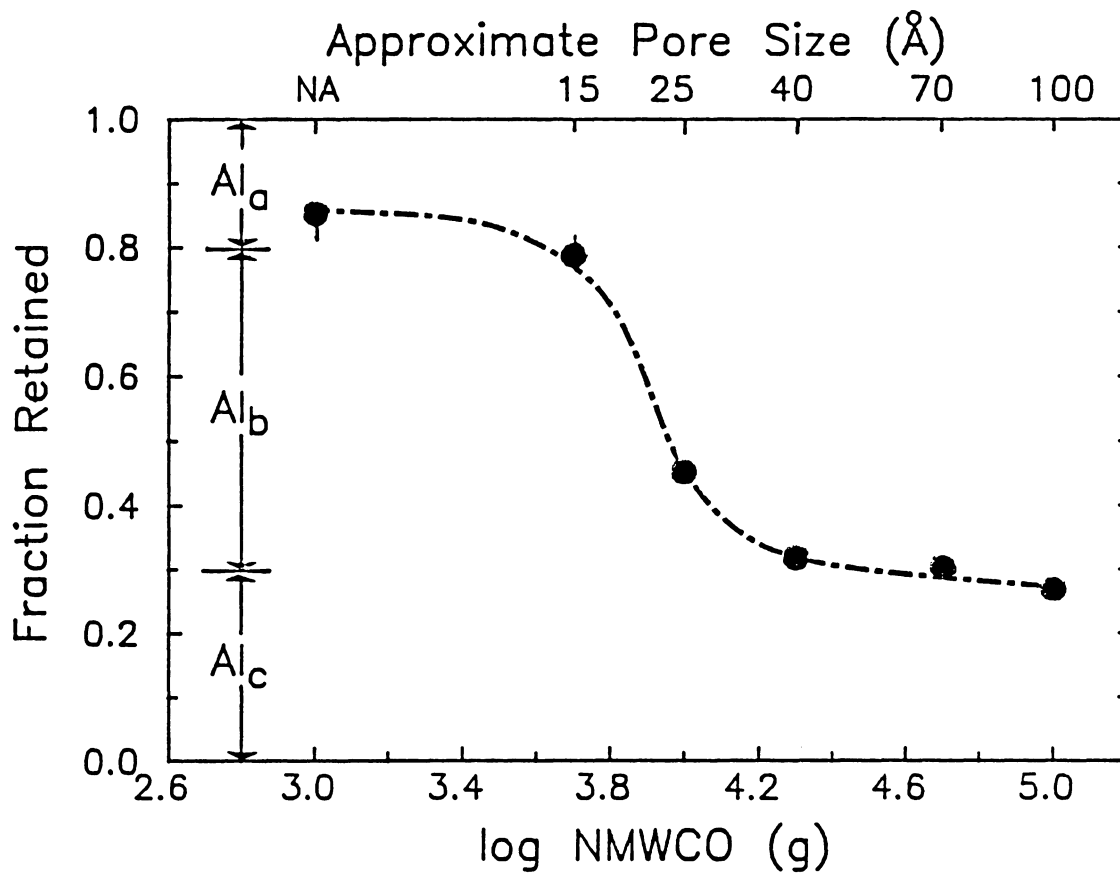


Figure 29. Fraction of  $Al_d$  retained vs. nominal molecular weight cutoff of several membrane filters: The solution was prepared from a 15-d-old stock solution neutralized at  $12.0 \text{ mL min}^{-1}$ . Vertical bars indicate standard error where  $1 \text{ SE} > \text{symbol size}$ .

Table 17. Data for computations of the bound OH/Al ratio in the Al<sub>b</sub> polymers

Solution	pH	Al <sub>a</sub>	Al <sub>b</sub>	Al <sub>c</sub>	n <sub>a</sub>	n <sub>b</sub>
		-----μM -----				
412	5.25	1.0	9.0	4.9	1.06	2.14
422	5.21	2.5	9.1	3.2	1.00	2.57
423	5.16	2.5	7.2	5.2	0.92	2.60
424	5.11	2.2	6.3	6.2	0.84	2.67
442	5.19	1.5	7.8	5.8	0.97	2.26
462	5.25	1.7	9.8	3.5	1.06	2.34
463	5.21	2.1	8.2	4.7	1.00	2.40
464	5.21	2.7	6.9	5.5	1.00	2.45
465	5.18	2.9	6.1	6.2	0.95	2.54
472	5.20	1.3	8.4	5.3	0.99	2.25
482	5.19	1.8	6.7	6.5	0.97	2.24
542	5.16	2.4	6.8	5.8	0.92	2.53
673a	5.30	1.6	9.6	3.8	1.10	2.22
673b	5.27	2.2	8.9	3.9	1.07	2.34
672a	5.05	8.4	34.8	6.8	0.75	2.37
672b	5.04	7.8	35.1	7.1	0.74	2.35
671a	4.93	15.0	71.0	14.0	0.59	2.28
671b	4.88	15.0	72.0	13.0	0.53	2.32
701	4.95	13.7	74.3	11.0	0.61	2.27
761	4.72	17.7	51.7	29.6	0.37	2.39

**The vita has been removed from  
the scanned document**