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THE UNIVERSITY OF ALBERTA

ALUMINUM TOLERANCE OF *Betula papyrifera* MARSH. ON NATURALLY ACIDIC SOIL

by

WARREN G. GIBERSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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IN

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Date 88-04-21

Abstract

The native plant community established on a site of low soil pH (4.1 to 4.4) and high $CaCl_2$ -extractable Al (25.5 ppm) near Buffalo Head Prairie, Alberta was described. Betula papyrifera Marsh. was selected, as the dominant species, for assessment of Al tolerance limits in comparison with members of the same species originating from a site near Devon, Alberta on soil of pH 5.1 to 6.2 and $CaCl_2$ -extractable Al = 0.0 ppm. The Al tolerance mechanism of these native plants, and its intra-specific variation, was investigated after formation of the hypothesis that Betula papyrifera originating from sites of high plant-available Al might exhibit greater Al tolerance.

In root elongation experiments, Buffalo Head Prairie plants showed significantly greater growth in 100 and 150 ppm Al treatments than did Devon plants. Root morphology was unaffected in either population after 14 days' growth in the presence of 25 ppm Al, however 100 ppm treatments induced classical coralloid root morphology in both. The frequency of mitotic figures in root tip smears of both populations decreased after 24 h in 100 ppm Al.

Foliar Al levels were found to be in the order of tissue Al concentrations of crop plants. Diameter class was noted

to be a variable in stem Al concentrations. Al concentrations in leaves, stems and catkins were significantly higher in Devon plants for nearly all diameter classes. Energy dispersive analysis of X-rays revealed that roots grown in the presence of 100 ppm Al for 14 days had levels of Al in their cortical and vascular tissues well above those in controls. In experiments with excised roots, Devon plants were shown to adsorb twice the amount of Al (7.0 ppm) as Buffalo Head Prairie plants, as calculated by difference after distilled water and $LaCl_3$ washes.

Betula papyrifera is tolerant of high levels of soluble Al through a mechanism not involving complete avoidance, nor Al accumulation and sequestration. Differential tolerance within the species was observed.

Acknowledgements

I wish to express my sincere appreciation and deep gratitude to Dr. J.M. Mayo, Dr. W. Moser, and Dr. K.O. Higginbotham for their guidance and patience during this work. Credit must also go to my parents and my wife, Vicki, for their support. Thanks also to Dr. Peter Nosko and to Mr. Robert Ellis for their valued help in the field.

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CHAPTER I

INTRODUCTION

Anthropogenic acidification of the environment has attained a high profile over the past several decades, an example being the concern over acid rain (Likens et al., 1979). Coupled with acid deposition is the phenomenon of soil acidification leading to decreased productivity of agricultural land and natural vegetation. Smelter and industrial plant-derived pollutant stresses including sulfur dioxide, sulfuric acid, and heavy metal accumulation administer severe biological effects in industrial areas (Freedman and Hutchinson, 1980) by creating conditions of low soil pH and high toxic metal concentrations. Concern over soil acidification has focused the attention of this study on naturally acidic sites, their native vegetation, and the ability of native plant species to tolerate strong subsoil acidity and its potential for associated high levels of soluble aluminum.

Recent interest in research on aluminum toxicity has been prompted by an increasing number of reports of forest decline in eastern North America and Europe (Ulrich, 1980; Scott et al., 1984; van Breemen, 1985). Ulrich (1980) postulated that this decline is attributable to increasing levels of soluble aluminum secondary to soil acidification by acid deposits. Particular concern over forest decline

has focused on tree species (Steiner et al., 1978; Schier, 1985; van Praag et al., 1985; Cummings et al., 1985, 1986; Hutchinson et al., 1986; Thornston et al., 1986).

Soil acidity generally reduces rooting depth, increases susceptibility to drought, and decreases nutrient use (Hewitt, 1952). Hydrogen ion concentration is determined by a number of factors including soil-parent material, type of litter, and soil microbial populations (Lodhi, 1982). Decreased pH may result in replacement of cations in the humic-clay exchange complexes of the soil by H^+ and Al^{3+} such that nutrients are leached to lower horizons (Likens et al., 1979; Lodhi, 1982).

Early Studies of Aluminum Toxicity

The toxic response to Al of plants grown on acid soils has long been investigated due to its importance as a growth-limiting factor for plants of agricultural importance. Hartwell and Pember (1918) were first to discover that Al was of much greater significance than hydrogen ion concentration as a toxic factor affecting barley growth on acid soils. Deleterious effects are particularly evident in soils below pH 5.0, but can occur at pH levels as high as 5.5 (McCart and Kamprath, 1965; Foy, 1974). Severe toxicity may result as Al solubility increases such that more than half the cation exchange

sites in the soil are occupied by Al (Evans and Kamprath, 970). At these low levels, Al is present almost entirely as the free trivalent ion, precipitating out as hydroxides as the pH rises to appear again in solution as aluminate under alkaline conditions (Magistad, 1925). Al solubility in soils is also determined by the type of clay mineral, concentration of other cations, total salt concentrations, and organic matter content (Foy, 1974).

The existence of Al in extracts of acid soils was first observed by Vietch (1904). Early plant physiological work (Ruprecht, 1915; Hartwell and Pember, 1918) illustrated the toxic effects of Al on crop plants. Both inhibitory and stimulative effects have subsequently been cited, and the related physiology extensively reviewed (Foy, 1974; Foy et al., 1978). Al is generally regarded as a nonessential element, yet contradictory reports have been cited (Stoklasa, 1911; McClean and Gilbert, 1927; MacLeod and Jackson, 1965; Hackett, 1962, 1967; Dios and Broyer, 1962). For example, Paterson (1965) reported that 0.25 to 0.50 ppm Al stimulated growth of young corn plants in Hoagland's solution. Bertrand and deWolf (1968) concluded that Al is actually required by corn, leading to its designation as a dynamic microelement.

Symptoms, Physiology, and Biochemistry

Plant symptoms specific to Al toxicity are usually manifested in disrupted root cell division (Fleming and Foy, 1968; Clarkson, 1965, 1969; Reid et al., 1971), inhibition of calcium uptake and utilization (Paterson, 1965; Lance and Pearson, 1969; Clarkson and Sanderson, 1971), and inhibition of phosphorus uptake and utilization (Foy and Brown, 1963, 1964; Chaisson, 1964). Clarkson and Sanderson (1971) found Al neutralized or reversed the negative charge on the pores of the free space, thus reducing their ability to bind Ca. Both Al and P accumulate in a variety of plant roots, and it has been hypothesized that internal precipitation of aluminum phosphate accounts for reduction of P transport to shoots (Wright, 1948; Wright and Donahue, 1953). Foliar symptoms may be present with P deficiency leading to formation of stunted dark green leaves, purpling of stems, leaves and veins, and yellowing of leaf tips. Symptoms may also resemble Ca deficiency with its characteristic curling of young leaves and collapse of plant apex and petioles (Foy et al., 1978). Inhibition of root cell division gives roots a characteristically coralloid morphology with the absence of fine branching (Fleming and Foy, 1968; Clarkson, 1969; Reid et al., 1971).

Al is known to increase the viscosity of protoplasm

and to decrease overall permeability to salts, dyes, and water (Stoklasa, 1911; McClean and Gilbert, 1927; Hoefler, 1958; Aimi and Murakami, 1964). Such findings are attributed to crosslinking of adjacent protein molecules (Clarkson and Sanderson, 1969) and crosslinking of cell wall pectins in the middle lamella (Rorison, 1968). At the cellular level, Al has been implicated in causing inhibition of root cell division and subsequent elongation (Rios and Pearson 1964; Clarkson, 1965). The latter worker showed that cessation of root elongation in onion exposed to 10^{-3} to 10^{-5} M Al was closely correlated with the disappearance of mitotic figures, and Clarkson and Sanderson (1969) hypothesized that Al blocked the mitotic cycle during interphase. Fleming and Foy (1968) observed bionucleate cells in the meristematic regions of Al-treated wheat root tips. Al has been observed to accumulate in the nuclei of some injured plants (McClean and Gilbert, 1927; Aimi and Murakami, 1964). From here, it may reduce DNA synthesis (Clarkson, 1969) through crosslinking of polymers which could increase the rigidity of the double helix (Clarkson and Sanderson, 1969). Sampson et al., (1965) found Al treated plants synthesized a metabolically unstable DNA of unusual base composition, while Woolhouse (1969), and Klimashevskii and Berezovskii (1973) found Al inhibited acid phosphatase and ATP-ase activity. Zltao et al. (1987) report that Al^{3+} and Ca^{2+} alter membrane

permeability, listing architectural changes in membrane lipids.

Differential Tolerance

Wide ranges of tolerance to Al have been reported for different species and varieties within species. Again most efforts have focused on crop plants. Cranberry is a highly tolerant species requiring addition of 150 ppm Al to nutrient solutions (pH 3.5) for reduction of shoot growth (Medappa and Dana, 1968). Alfalfa and cotton are particularly sensitive, with injury occurring at levels of 0.5 ppm Al (Rios and Pearson, 1964; MacLeod and Jackson, 1965). Studies of the genus Agrostis showed A. stolonifera to be injured at 5.4 ppm Al and A. canina at 10.8 ppm, while A. tenuis was unable to grow at a concentration of 43.2 ppm Al to which A. setacea was tolerant (Clarkson, 1966). McCormick and Steiner (1978) observed the relative Al tolerance of 11 tree species, finding a Populus hybrid clone and Elaeagnus umbellata to be sensitive to 10-40 ppm Al, while Alnus glutinosa, Betula spp., Pinus spp., and Quercus spp. were tolerant to concentrations as high as 160 ppm.

Differences in tolerance among varieties are cited for snapbeans (Foy et al., 1967); Naidoo, 1976), rice (Ota, 1968), wheat and barley (Foy et al., 1965a, 1965b,

and 1967; Maclean and Chaisson, 1966), alfalfa (Ouellette and Dessureaux, 1958), potatoes (Lee, 1971), peanuts, (Adams and Pearson, 1967), and perennial ryegrass (Vose and Randall, 1962). Steiner et al., (1980) found that provenances of paper birch throughout North America differed significantly in root elongation after exposure to 120 ppm Al.

While the exact nature of Al toxicity has not been elucidated at the biochemical and physiological levels, and may not involve a single metabolic process, several properties attributed to differential tolerance have been summarized (Foy, 1974). Greater Al tolerance in association with the ability to continue root elongation and resist morphological damage to root tips was postulated for 'Atlas 66' wheat in comparison to the 'Monon' variety (Fleming and Foy, 1968), and in sugarbeet cultures by Keser et al., (1977). Differential plant-induced pH changes in the root zone may also determine tolerance capabilities. By raising the pH in the vicinity of the root, tolerant plants may decrease Al solubility and subsequent toxicity (Foy et al., 1965a, 1967; Subramoney and Sankaranarayanan, 1964; Otsuka, 1968). Whether pH adjustments are a primary cause of differential tolerance or simply the result of differential growth under Al stress is yet unclear (Foy et al., 1978).

Aluminum uptake and transport have also been postulated as processes involved in differential tolerance

mechanisms. Steiner et al., (1980) found foliar Al concentrations to be slightly higher in Al -sensitive provenances of paper birch than in tolerant provenances indicating that foliar elemental concentrations are only remotely involved in the actual tolerance of this species.

Ability to absorb and utilize other nutrients such as Ca, P, Mg, K, and Si in the presence of Al has also been postulated as a general tolerance mechanism leading to differential tolerance. Ouellette and Dessureaux (1958) found that Al-tolerant alfalfa clones retained higher concentrations of Ca and Al in their roots than did sensitive clones. Al-sensitive wheat and barley varieties were shown by Foy et al., (1967) to be more susceptible to Ca deficiencies than tolerant varieties after addition of 3 ppm Al, with Ca retention in tops of 25% versus 97%.

Hartwell and Pember (1918) first suggested that observed P deficiency symptoms in barley shoots might be due to internal precipitation of P by Al. Work in support of this theory was cited by Wright (1943), Wright and Donahue (1953), Rasmussen (1968), McCormick and Borden (1972, 1974), Naidoo (1976). Waisel et al., (1970) have presented contradictory results. Differential Al tolerance credited to P utilization has been reported for buckwheat and barley (Foy and Brown, 1964), Deschampsia flexuosa (Hackett, 1967), Agrostis spp. (Clarkson, 1966a), barley (Maclean and Chiasson, 1966), and wheat (Foy et al, 1965,

1967). Ouellette and Dessureaux (1958) found that aluminum phosphate precipitation was not at the heart of Al sensitivity in alfalfa clones.

With regard to capacity for P utilization in the presence of Al, and subsequent differential tolerance, chelation mechanisms have been evoked (Drake and Steckel, 1955). Aluminum was found to affect both ^{32}P and K utilization in Red Spruce seedlings (Cumming et al., 1985, 1986). Naturally occurring organic acids have proven effective in prevention of Fe and Al precipitation (Struthers and Sieling, 1950). Acid tolerance and citric acid content of roots have been positively correlated in some plants (Chamura and Koike, 1960), and Clarkson (1966) found that cationic Al, but not chelated Al, inhibited cell division in Agrostis stolonifera.

Ecological Considerations

Plant response to Al has received little attention in an ecological setting (Clarkson, 1969). Studies of Al tolerance in some native Al-accumulating species include Hu et al., (1957), Mooman et al., (1957), Hess (1963) and Medappa and Dana (1968). Resistance in these plants appears to be related to prevention of Al from reaching critical metabolic sites (Turner, 1969).

Clarkson (1966) undertook a significant investigation

of Al tolerance in 4 species of Agrostis common to soils of different pH and exchangeable Al. A. setacea originating on the most acid soil, a sandy podsol (pH 4.1), showed greatest tolerance. It was noted that since A. canina and A. tenuis have a wide distribution on acid and neutral soils, one might expect natural selection to produce populations of differing Al tolerance, however seed was collected for each species from only one site so that this parameter could not be assessed. Further to this, Snaydon and Bradshaw (1961) have shown edaphic ecotypes to be present within species of populations on different soil types. After discovering Betula papyrifera to be tolerant of high Al concentrations (120 ppm) (McCormick and Steiner, 1978; Steiner et al., 1980) examined differential Al response of paper birch provenances from locations in the U.S. and Canada. Al treatments elicited variable response as determined by root elongation and uptake of Al, Ca, P, and other elements such that tolerant and intolerant provenances could be defined. It was tentatively concluded that provenances from central and eastern portions of the species' distribution display greatest Al tolerance. Deleterious effects of Al are known for other tree species including Sugar Maple, Red Spruce and Balsam Fir (Schier, 1985; Thornston et al., 1986).

Objectives and Experimental Design

It is well established that Al toxicity is limiting to plant growth on acid soils, thus persistence of vegetation on naturally acidic sites implies inherent tolerance mechanisms.

The objectives of this study were to:

1. describe a plant community established on acid soil (high soluble Al) and examine some ecological interactions.
2. select a dominant species (Betula papyrifera, Marsh.) and define its Al tolerance limits in comparison to plants of the same species originating from a site of more neutral soil pH (low soluble Al).
3. investigate the general tolerance mechanisms of these native plants and their intraspecific variability.

With respect to heavy metal tolerance mechanisms, Antonovics, Bradshaw, and Turner (1971) have postulated differential uptake of ions, and removal of ions from metabolism through vacuolar deposition or transformation into an innocuous form. Similarly, native plants could i) avoid absorption and transport of Al, or ii) resist toxicity by sequestering the ion intracellularly. These postulates, in conjunction with the above objectives, were tested as outlined in Fig.1.

I. Assessment of Al Tolerance Limits

Parameters Assessed

A. Root elongation
B. Abundance of mitotic figures
C. Root morphology

Differential Tolerance	No edaphic ecotypes nor plasticity within genotype
Betula from high soluble-Al site is tolerant	Betula from high and low soluble Al-site is tolerant

II. Assessment of General Tolerance Mechanisms

Parameters Assessed

A. Al concentration in aerial plant parts
B. Root affinity for Al ion
C. Cellular localization of Al

Avoidance of Al toxicity by exclusion	Resistance to Al toxicity

Fig.1. Overview of the experimental design.

CHAPTER II

DESCRIPTION OF THE STUDY SITES

Location

In selecting an undisturbed plant community tolerant to the injurious effects of high plant-available Al, the study of Hoyt and Nyborg (1971) was reviewed. This work deals with the addition of lime to a field near Buffalo Head Prairie, Alberta, in which soil acidity and soluble Al inhibited growth of crop plants. Upon surveying the area, a unique plant community was apparent adjacent to the test plots set out in the earlier study. This site was examined intensively in terms of edaphic features, and vascular plant species and their ecological interactions, as described later in this chapter. A reference site was later selected at the Devonian Botanical Garden near Edmonton, Alberta.

The Buffalo Head Prairie study site is situated 5 km NE of the townsite, west of the Buffalo Head Hills at $58^{0}3'N$ and $116^{o}16'W$ (Fig. 2). Much of this area is now farmed, leaving isolated woodlots as seen in Plate 1. The topography is that of a level lacustrine plain at the base of the Buffalo Head Hills.

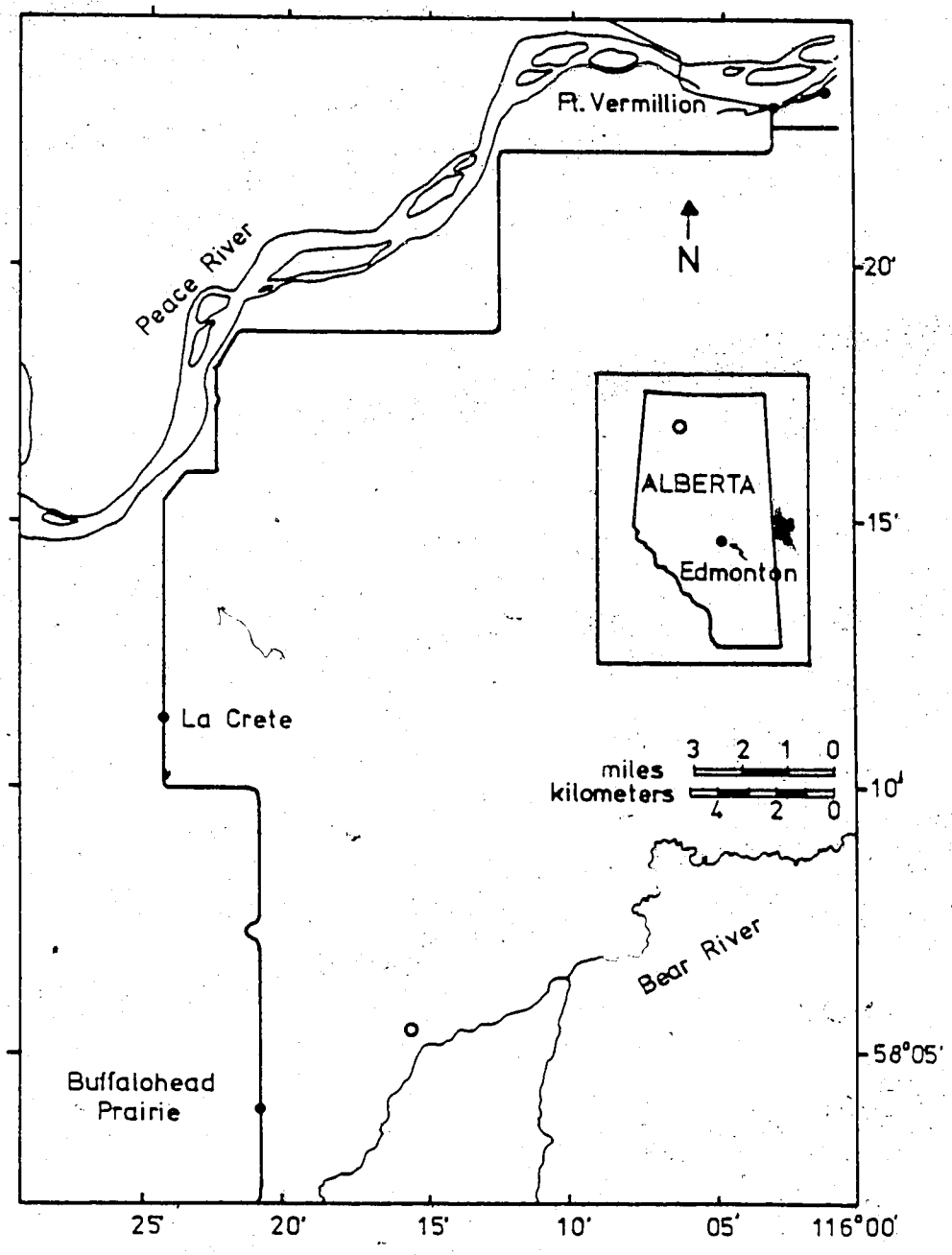


Figure 2. Location of the Buffalo Head Prairie study site with low soil pH and high soluble Al.

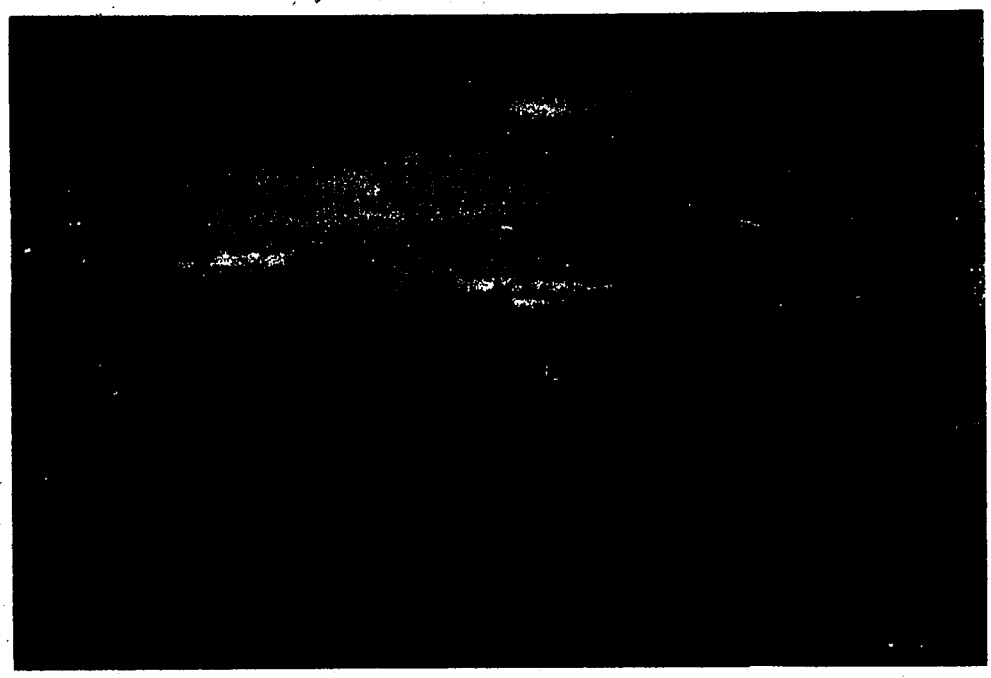


Plate 1. The study site at Buffalo Head Prairie, Alberta.

The reference site at the University of Alberta Devonian Botanic Garden was selected after Betula papyrifera was found to be the dominant vascular plant species at the Buffalo Head Prairie site. The location at the Botanic Garden was particularly well-suited for providing plant material for comparative physiological studies of limits of Al tolerance, and tolerance mechanisms, due to its near neutral soil pH and dominant cover of Betula papyrifera. The Devonian Botanic Garden is situated 23 km SW of Edmonton off Highway 60. The reference stand was located in the Betuletum adjacent to the Forest Ecological Reserve in the SW corner of the Garden, on a 15° slope of N-NW exposure.

Climate

Continuous climatic data have been compiled for both study sites by the Atmospheric Environment Service, Environment Canada (1980) as summarized (Fig. 3) in standard climatic diagrams (Walter and Lieth, 1967). Cold, dry winters and warm, short summers are prevalent at the Buffalo Head Prairie site. Devon has a dry autumn period.

The annual mean precipitation at Buffalo Head Prairie over the period from 1951 to 1980 was 407.6 mm compared with 528.8 mm at Stony Plain, the nearest station to the Devon site for which data are available (20 km NW of

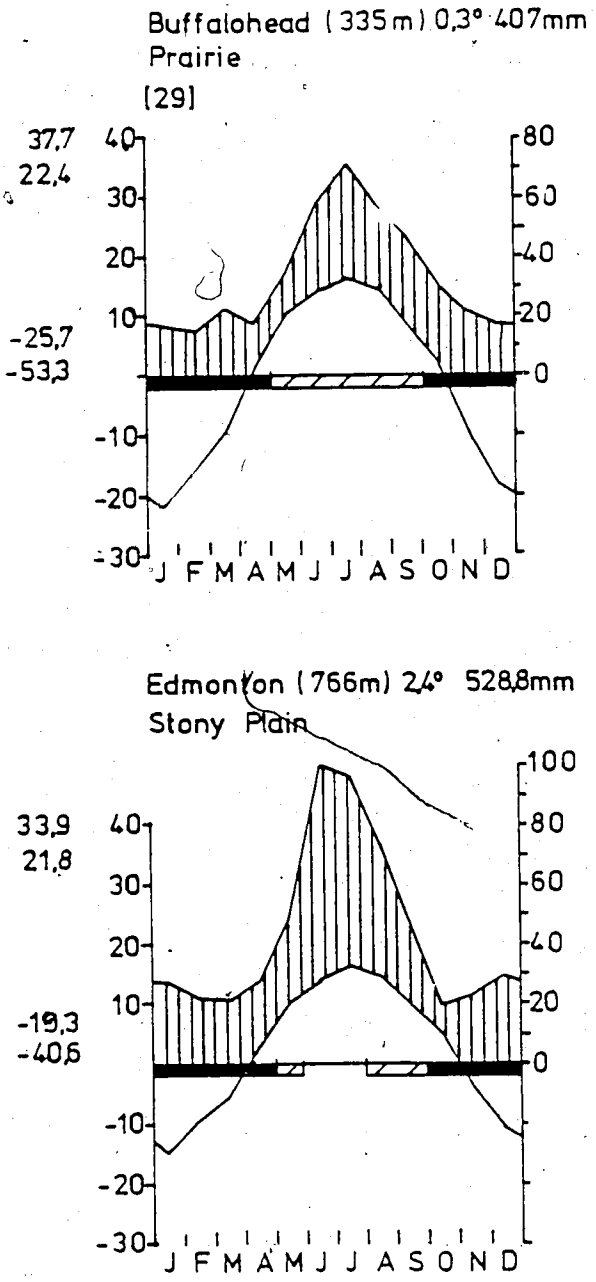


Figure 3. Standard climatic diagrams (Walter and Lieth, 1967) for Buffalo Head Prairie and Stony Plain weather stations based on data from Atmospheric Environment Service, Environment Canada (1980).

Devonian Botanic Garden). The mean annual temperature for the period was -0.3 ^{0}C at Buffalo Head Prairie and 2.4 ^{0}C near the reference site. In contrast with the reference site, all months had absolute minimum temperatures below zero in Buffalo Head Prairie. While the climatic diagrams show similarities, the Buffalo Head Prairie study site is subject to lower mean annual temperatures and annual mean precipitation.

Soils

The Buffalo Head Prairie site which was chosen for the study due to its strong acidity and subsequently elevated levels of soluble Al is included in the Savage Series in the Rego Gleysol subgroup. The parent material of this soil is gray, may be strongly to extremely acidic, and is fine-textured material of lacustrine deposition. It is generally agreed that this material was washed from the Buffalo Head Hills to be deposited on the level plains below. For this topographic reason, soil drainage is poor. Laminae of organic material are characteristic throughout the profile and on the soil surface and are comprised of decayed rushes, grasses and reeds.

Soil samples from the Devon site were collected with the objective of developing a pH and $CaCl_2$ -extractable Al

profile for comparative purposes. More neutral pH and exceedingly low levels of $CaCl_2$ -extractable Al justify selection of the reference site.

Methods

Soils were studied at both the Buffalo Head Prairie study site and the Devon reference site, particular attention being given to pH and plant-available Al. Soils were described in the field and samples of each horizon were analyzed for physical and chemical properties. At Devon, samples were collected at 10 cm intervals throughout the homogeneous profile for more accurate description.

Colour was determined in diffuse sunlight. All subsequent analysis was done on the 2 mm fraction. Particle size analysis was completed according to the hydrometer method of Bouyoucos (1951). Organic matter, N (NO_3 only), P, K, S, SO_4 concentrations, conductivity, $CaCl_2$ -extractable, Mn, and NH_4OAC extractable Ca, Mg, and Na were determined by the Alberta Soil and Feed Testing Laboratory. The pH was determined on a soil-water paste (1:1) by glass electrode. Plant-available Al was determined by 0.01 M $CaCl_2$ extraction according to Hoyt and Nyborg (1971).

Results and Discussion

The soil profile at the Buffalo Head Prairie site was found to be representative of the Rego Gleysol classification, of moderately fine textured material with an extremely acid soil reaction in all horizons (Table 1). Values of pH = 4.1 to 4.9 may be explained by mobilization of elemental sulphur from the Buffalo Head Hills followed by its oxidation. Another explanation could be that shales beneath the level plain might themselves render elemental S (Nyborg, pers. comm.).

In this regard, sulphate was determined to be 20 ppm in two horizons. The low soil reaction determined here agrees closely with the discovery of Hoyt and Nyborg that Savage surface soils (0-15 cm) sampled from cultivated and virgin land in the Peace River region of Alberta varied from pH 4.02 to 4.18, well below the level of pH 5.5 above which Al toxicity does not generally occur (McCart and Kamprath, 1965). Low soil pH in the Buffalo Head Prairie profile was found to result in $CaCl_2$ -extractable Al (= plant available Al; Hoyt and Nyborg, 1971) concentrations ranging from 10.7 to 25.5 ppm. Again this is well above the critical level for Al toxicity in most crop plants (Foy, 1974).

Table 1. Soil characteristics of the study site, Buffalo Head Prairie, Alberta.

HORIZON	DEPTH(cm)	% of <2 mm			pH	COND. mmhos	ORGANIC MATTER %	N	P (NO_3 only)	K (ppm)	EXTRACTABLE CATION (ppm)						COLOUR (DRY)
		SAND	SILT	CLAY							Al*	Mn*	Ca+	Mg+	Na+	SO_4	
Oh	3- 0				4.9	-	-	-	-	-	-	-	-	-	-	-	
	0- 2	27	10	63	4.1	0.2	16.2	0	4	200	10.7	5.1	1356	233	160	11	5YR 3/6
Cg_1	2-27	23	17	60	4.2	0.6	7.8	0	3	131	25.5	19.0	3240	866	99	15	10YR 3/2
	27-30	23	19	58	4.3	1.2	17.9	0	100	136	17.4	19.2	2571	677	164	20	7.5YR 3/3
Cg_2	30-50	21	24	55	4.3	0.7	3.9	0	3	129	16.4	21.0	3188	1145	131	15	10YR 3/3
	50-65	17	13	70	4.4	1.9	5.4	0	5	198	16.3	25.0	3452	1082	272	20	10YR 2/2
Cg_3	65+	10	36	54	4.1	1.2	2.2	0	2	126	13.4	17.1	2094	703	196	20	10YR 4/6

* $CaCl_2$ extraction

\+ NH_4OAc extraction

The reference site at Devon was chosen as a Betula papyrifera dominated community on soil of nearer neutral pH (Table 2). The pH in the soil profile to a depth of 1 m ranged from 5.1 to 6.2 with corresponding $CaCl_2$ -extractable Al concentrations of 0.0 ppm. This is a sandy soil with low percent organic matter and good drainage. Conductivity is lower than that found in the Buffalo Head Prairie profile due to better drainage. Sulphate levels extend only to 5 ppm in accordance with the higher pH. It is pertinent to this study that Betula papyrifera exhibits such a wide breadth of physiological adaptation to edaphic factors.

Other studies comparing Al concentration and soil pH with associated tree species are largely lacking. Hutchinson et al. (1986) studied five boreal conifers on 35 conifer dominated boreal sites (Podzolic soils) in northern Ontario. Organic and mineral soil horizons were compared. Mean pH was 3.7 (range 3.2 - 4.6) in organic horizons and 4.3 (range 3.5 - 6.2) in mineral horizons. Extractable Al levels in organic horizons were 50 mg/l (range 15 - 257) and 12 mg/l (range 2 - 40) in mineral horizons.

Table 2. Soil characteristics of the reference site, Devon, Alberta.

SAMPLE	DEPTH(cm)	% of <2 mm			pH	COND. mmhos	ORGANIC MATTER %	N	P (NO_3 only)	K (ppm)	EXTRACTABLE CATION (ppm)						COLOUR (DRY)
		SAND	SILT	CLAY							Al*	Mn*	Ca+	Mg+	Na+	SO_4	
LFH	3- 0				5.9	-	-	-	-	-	-	-	-	-	-	-	
1	0-10	83	8	9	5.1	0.1	1.4	0	10	269	0.0	15.0	642	41	25	5	10YR 4/3
2	10-20	83	7	10	5.2	0.1	1.2	0	36	225	0.0	7.3	545	40	35	3	10YR 5/4
3	20-30	85	6	9	5.5	0.1	0.8	0	46	238	0.0	3.1	604	54	36	-	10YR 6/3
4	30-40	86	5	9	5.9	0.1	0.6	1	21	140	0.0	1.8	627	79	18	2	10YR 6/3
5	40-50	85	4	11	5.6	0.1	0.5	1	24	186	0.0	1.8	776	96	23	2	10YR 6/3
6	50-60	82	4	14	6.0	0.1	0.5	1	23	167	0.0	1.0	1154	147	21	-	10YR 6/3
7	60-70	80	4	16	6.2	0.2	0.9	1	22	280	0.0	0.8	1277	163	41	2	10YR 5/4
8	70-80	80	4	16	6.0	0.1	0.9	1	21	244	0.0	0.6	1344	176	51	2	10YR 5/4
9	80-90	85	4	11	6.0	0.1	0.8	1	7	266	0.0	0.6	1172	165	69	-	10YR 5/4
10	90-100	88	3	9	6.0	0.1	0.6	1	5	269	0.0	0.6	924	131	51	-	10YR 5/4

* $CaCl_2$ extraction

\+ NH_4OAc extraction

Community Description

A description of the native plant community adapted to the high Al site at Buffalo Head Prairie was undertaken. Such an ecological investigation was importand because of the lack of information on native Al-tolerant plants and their phytosociology (Clarkson, 1966; McCormick and Steiner, 1978, Steiner et al., 1980). For comparative purposes, a similar study was done at the Devon site.

Methods

Community analysis at Buffalo Head Prairie was accomplished by examining the vegetation within two 5 x 5 m quadrats placed randomly on each of ten N-S transects spaced evenly along the E-W fence demarcating the southern boundary of the study site (Plate 2). The transect originated at the east corner of the site, and extended 350 m to the west.

Plate 2.

the vegetation

Ocular estimates of cover class were made for each species within the quadrat, whether rooted or overhanging, using the following range of values:

R = rare

t = 1%

1 = 1-5%

2 = 6-15%

3 = 16-25%

4 = 26-50%

5 = 51-75%

6 = 76-95%

7 = 96-100%

Subsequent calculations were based on midpoints of cover classes. Also documented were number of individual Betula papyrifera trees (2.5 cm DBH), number of stems, and DBH of stems. Increment cores from a range of size classes were taken for analysis. Voucher speciments were collected for all vascular plant species occurring on the study site.

These parameters were also assessed at the Devon site, however, due to a smaller area, one 5 x 5 m quadrat was located using a random numbers table, on each of ten N-S transects spaced evenly along a 150 m E-W transect, for equitable sampling intensity (Plate 3).

Plate 3. Five

Results and Discussion

Analysis of the plant community composition at the Buffalo Head Prairie site revealed a community strongly dominated by Betula papyrifera and Calamagrostis canadensis (bluejoint). This trend is easily observed in the dominance diversity curve (Whittaker, 1965) of combined strata (Fig. 4), leading to its designation as a Betula/Calamagrostis association.

The novelty of this community structure in Alberta is supported by the lack of references to such an association in the literature. Birch forests are generally not common in Western Canada (Halliday and Brown, 1943), although the Swan Hills exhibited this type of vegetation with a Calamagrostis understory before logging during World War II (La Roi, pers. comm.). In his description of forest communities in northwestern Alberta, Moss (1953) referred to Betula papyrifera as a "less prominent" forest tree species. Here birch was described in relation to tamarack vegetation established on a Drepanocladus-Carex-Betula bog under relatively wet conditions. Birch species present included Betula papyrifera and B. glandulosa, with Calamagrostis spp. scattered throughout the mosaic. Similarly, the Buffalo Head Prairie study site was wet, especially in its eastern extremity, more pronounced in the spring, when the water table was noted to be within 20 cm

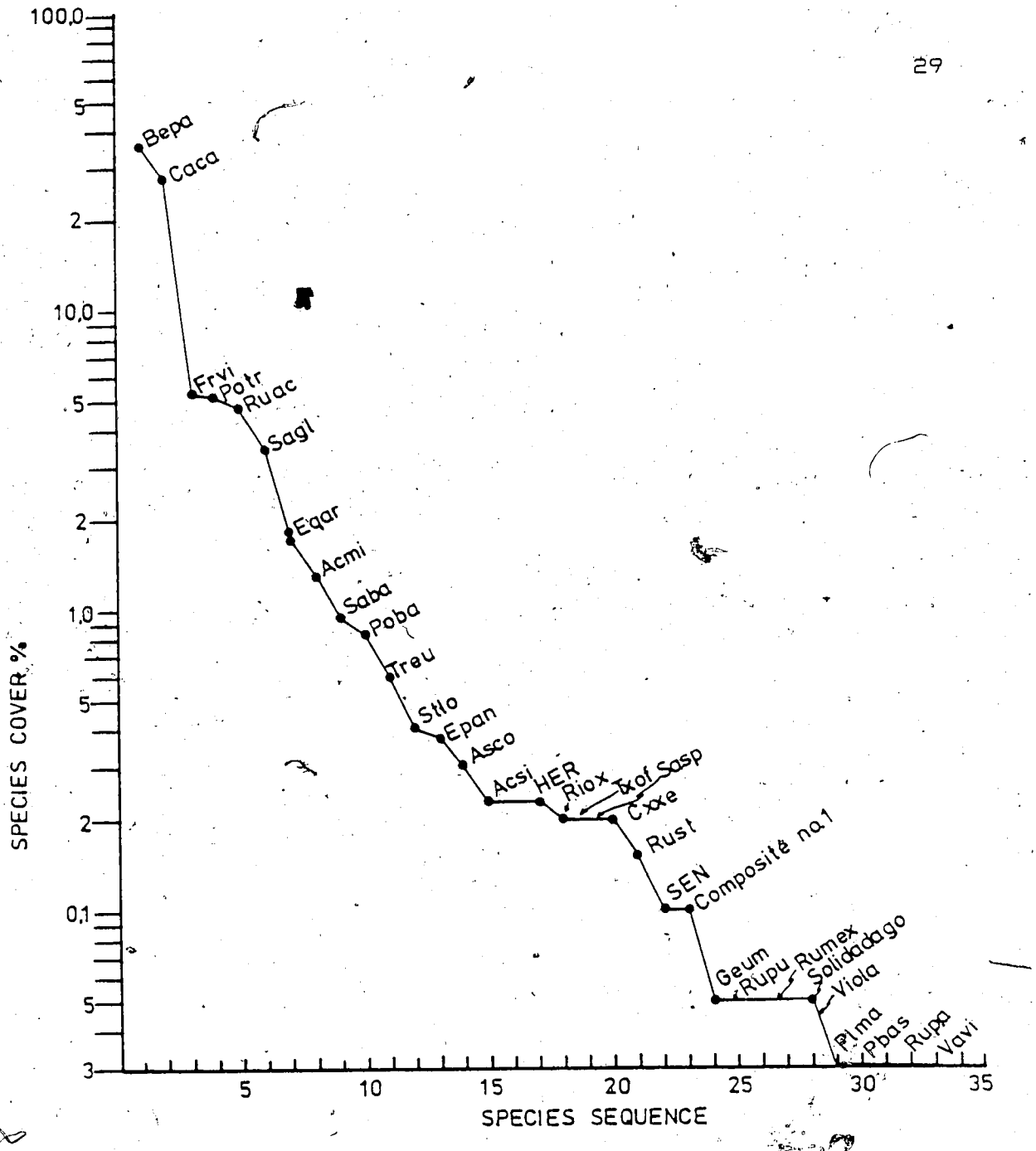


Figure 4. Dominance diversity curve and species dominance hierarchy:
Buffalo Head Prairie.
Spp. = 32
C% = 90.8

Adaptation to wet conditions must not be overlooked in attempting to understand the distribution of this low pH- and Al-tolerant woody plant. Other species common to both Moss' Tamarack vegetation and this study site include Equisetum arvense, Alnus rugosa, Vaccinium vitis-idaea, and Stellaria spp. Under this wet regime Populus tremuloides proved to be an important tree species in this study, with a rank of tenth on the species dominance hierarchy (Fig. 4). Occurrence of P. tremuloides increased toward the drier western extremity of the study site. Within Moss' Aspen Poplar Consociation, Betula papyrifera is rare or absent.

Betula is reported to be more important in Alaska. Viereck et al., (1975) outlined a vegetation unit of paper birch/alder/Calamagrostis in the Closed Deciduous Forests formation class of Fosberg. This is an abundant upland birch type which is occasionally observed on river terraces, usually after fire. Also described was a whitespruce-paperbirch / alder / Calamagrostis vegetation unit in the Closed Mixed Forests formation class, and a white spruce / paper birch / Calamagrostis / Hylocomium vegetation unit in the Open Mixed Forests formation.

In a study of plant communities in the vicinity of Sudbury, Ontario (Amiro and Courtin, 1981), Betula papyrifera was found to dominate a community forming a

spatial transition between barren areas and other communities more distant from pollution sources. This community type was frequently associated with soil pH 5.0, verifying the ability of birch to tolerate the low soil pH normally associated with Al toxicity. In the same region Freedman and Hutchinson (1980) reported Populus tremuloides and Betula papyrifera dominating in isolated forest communities existing within 3 km of an SO_2 -emitting smelter.

The dominance diversity curve (Fig. 4) illustrates an equitable distribution of species along the curve after the strong display of dominance by Betula in the tree stratum and Calamagrostis in the herb-stratum. Such a structure lends itself to the impressive distinction between these two strata (Plate 4.) In the tree stratum, Betula papyrifera is followed in total cover by Populus tremuloides, which occurs first in quadrat no. 5, gaining importance in the middle portion of the site, lending credence to the postulate that water availability plays a major role in species composition along the transect.

Salix spp. are important in the shrub stratum, indicating high moisture availability. The presence of Rubus acaulis and Populus balsamifera is interesting in that they are normally associated with alkaline conditions

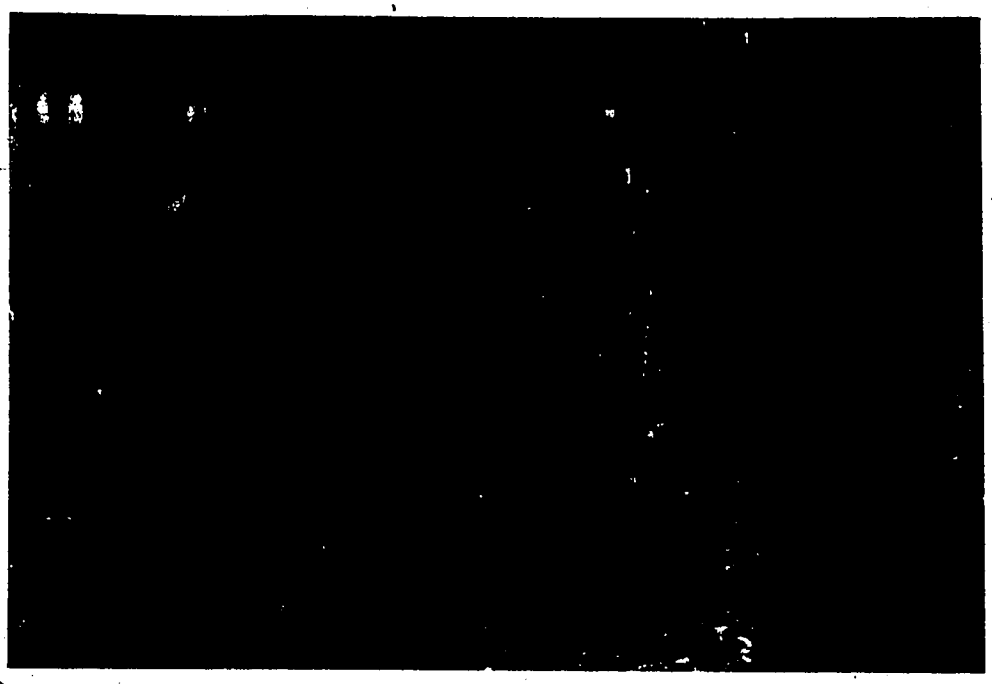


Plate 4. Tree and herb strata at the Buffalo Head Prairie study site.

(La Roi, pers comm.). Fragaria virginiana and Rubus acaulis follow the strong dominance of Calamagrostis canadensis in the herb stratum. Although bluejoint is highly important, the diversity of this stratum (spp. = 25) points to a wide variety of Al-tolerant herb species in native plant communities. C. canadensis has a wide distribution and thrives under the moist conditions of wet lowland sites (Laughlin, 1969). This grass is rhizomatous, and seldom flowers. No previous reference to Al-tolerance in this species was found in the literature.

The ecology of this birch-dominated site may be governed by fire, the combined edaphic properties of soil acidity, soluble Al and water availability. The White Spruce Association of Moss (1953) is regarded as the climax type of this region, such succession being impeded by regeneration of birch after fire by sprouts arising from stands, yet seed production is more important in making the genus a highly mobile pioneer (Lutz, 1956; Viereck et al., 1975). Birch seed is light, and readily disseminated by wind, becoming established on mineral soil in full sunlight. Furthermore, profuse seed production begins at ages as early as 10 years (Lutz, 1956). These factors have probably enabled the Betula / Calamagrostis community to become established on the moist, acidic soil of the Buffalo Head Prairie study site. Encroachment of birch sprouts into areas of high Calamagrostis cover, for example quadrat 13,

in a progression toward closed birch forest precludes development of a diverse herb stratum. Such encroachment is substantiated by a high number of stems per quadrat and their relatively small DBH (Table 3). It is unclear whether the competitive advantage of Betula over Populus is due to Al-tolerance or is governed by soil moisture content.

Table 3. Number of trees and stems of Betula papyrifera per quadrat and mean stem DBH.

	Buffalo Head Prairie	Devon
Mean no. of individuals/quadrat	7.7	1.4
Mean no. of stems/quadrat	16.8	1.7
Mean DBH (cm)	3.8	9.2

Plot composition as illustrated in Table 4 reveals a total of 32 species, with species richness increasing greatly at quadrat 7 suggesting a physical change in edaphic conditions, possibly water availability or soluble Al concentration. In general, the species composition of the Buffalo Head Prairie site is indicative of high moisture availability. Calamagrostis canadensis is typical of marshes and moist woodlands (Moss, 1953) as are Betula and Salix spp. Acid soil indicators are not prevalent with the exception of Geum sp. (La Roi. pers. comm.)

Table 4. Plant Cover Classes: Buffalo Head Prairie Transect.

Species / Plot Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Constancy
Betula papyrifera	2	4	3	4	5	6	3	6	2	4	3	2	2	4	4	4	5	4	5	4	20
Calamagrostis canadensis	+	4	5	5	3	R	4	+	3	2	5	1	6	5	2	+	+	3	2	3	20
Trientalis europaea	+	R			+		+	1	R	R			R		1	R				+	11
Epilobium angustifolium	+				R		R	R		+	R	+				R	+	+			10
Carex xerantica	R								R			R		R		R		R			6
Stellaria longipes			R		R		+		R	R			R	+		R	+	R	R	+	12
Fragaria virginiana					R	R	1	+	2	2	1	2	1	2	2	2	2	2	+	2	16
Rubus acaulis					+		1	1	2	+	+		1	+	+	+	3	2	2	3	14
Populus tremuloides					1		+	+	2	1	3	2	1	1	1	4		1	1		13
Salix glauca					+		+		1	1								3	2	1	7
Rubus strigosus						R		R		+											3
Achillea millefolium							+	R	+	+	1	1	1	1	+	+	+	1	+	+	14
Achillea sibirica							R		R	R	R						R	+	R	R	8
Equisetum arvense							R		1	1	2	1			1	2					7
Salix bebbiana							+	R			1				2		1				5
Salix sp.							+	R	+	R						R					5
Hieracium sp.								R		R	R		R	R	R	R		R	R		9
Aster conspicuous									R		R	+	R	R	+	R		R		+	9
Taraxacum officinale										+	R	+						+			4
Ribes oxyacanthoides											R	R		+		R					4
Senecio sp.									R		R		+								3
Geum sp.							R								R						2
Composite 1.												+		+							2
Populus balsamifera													2	R							2
Vicia americana												R		R							2
Rumex sp.							+														1
Rubus parviflorus							R														1
Vaccinium vitis-idaea							R														1
Pyrola asarifolia										R											1

Table 4. Plant Cover Classes: Buffalo Head Prairie Transect, continued.

Species / Plot Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Constancy
Rubus pubescens											+										1
Solidago sp.														+							1
Plantago major																				R	1
Total number of species per plot	5	3	3	2	9	4	18	12	15	17	16	13	12	15	12	15	9	14	10	11	

Total spp. = 32

A description of the plant community at the Devon site is included for completeness (Fig. 5; Table 5), although strict comparison between the two sites must acknowledge the differences in climate, soils, and drainage. Analysis of the dominance diversity curve (Fig. 5) reveals that Betula papyrifera is again the dominant vascular plant species. This is a more diverse community, with a Shannon-Weaver Diversity Index of $H' = 2.83$ versus $H' = 1.73$ for the Buffalo Head Prairie site, calculated as $H' = -\hat{E}(P_i)(\ln p_i)$ where p_i is approximated by N_i/N, where N_i is the frequency of the ith species, and N the total frequency of all species (Shannon and Weaver, 1949; Pielou, 1969) (Fig. 6).

Species richness is also greater at Devon (48 species compared with 32 species) (Table 6; Table 7). There are 15 species in common between the two sites. Propagation of Betula papyrifera at Devon is solely by seed. While fewer trees were counted (Table 3), these attained a greater mean DBH and nearly equal per cent cover in this more established site. Because of by good drainage, Calamagrostis canadensis was twenty-ninth on the species dominance hierarchy. Conditions for establishment of birch seedlings were similar on both sites. Seed beds occurred on organic substates such as moss-covered decaying logs in open areas. At Buffalo Head Prairie, seedlings also initiated growth in stands of Calamagrostis canadensis.

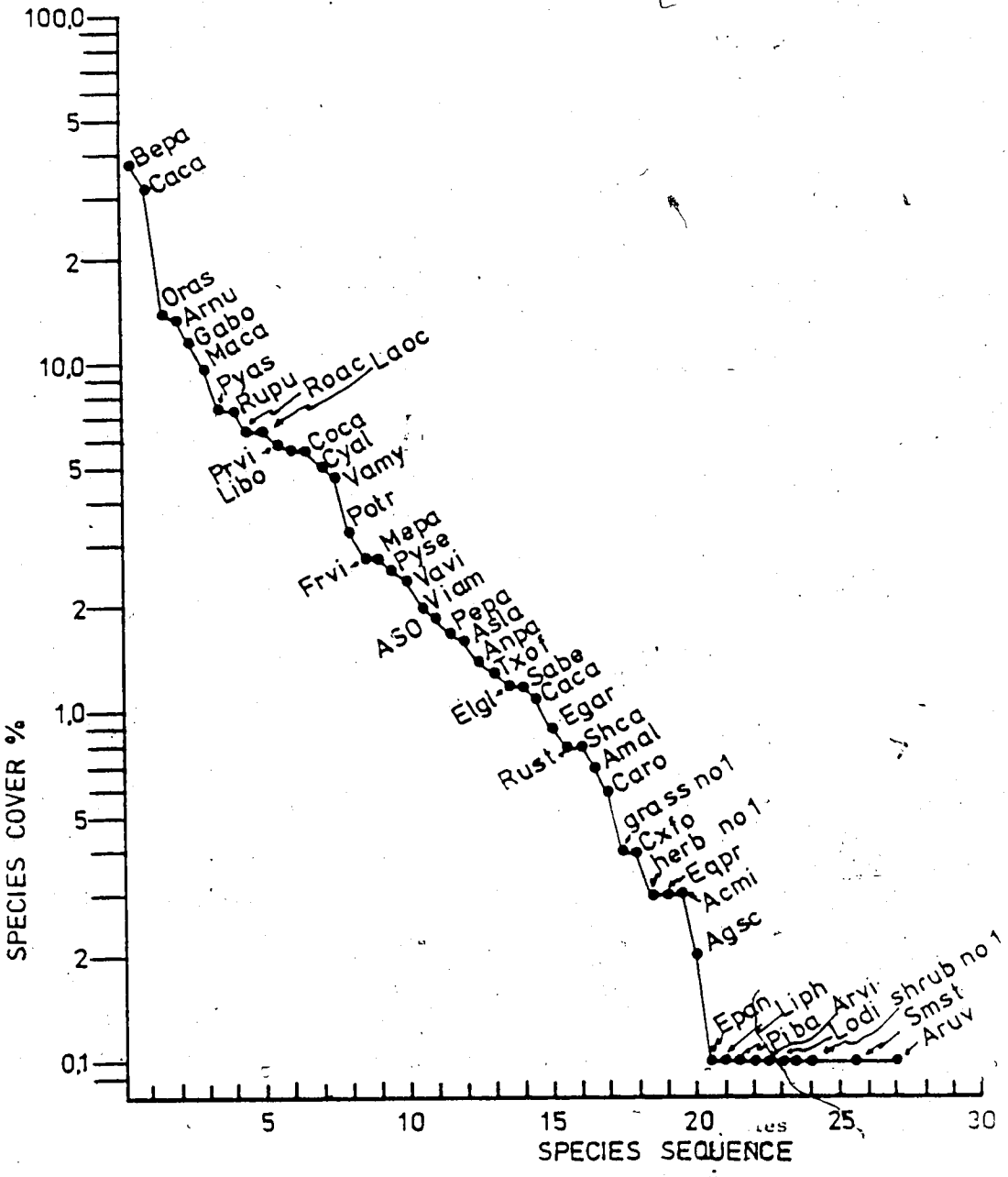


Figure 5. Dominance diversity curve and species dominance hierarchy:
Devon
Spp. = 48
C% = 207.1

Table 5. Plant Cover Classes: Devon Transect

Species	Plot Number	1	2	3	4	5	6	7	8	9	10	Constancy
Betula papyrifera		1	1	2	2	6	4	5	5	5	10	10
Corylus cornuta		3	1	+	3	5	4	5	5	4	5	10
Vaccinium myrtilloides		2	2	1	2	1	1	1	1	1	+	10
Symphoricarpos albus		+	1	1	1	+	1	1	3	1	2	10
Lathyrus ochroleucus		1	1	3	2	2	2	1	1	2	2	10
Pyrola asarifolia		2	1	2		3	1	1	1	2	2	9
Oryzopsis asperifolia		+	2		3	+	4	2	4	2	2	9
Galium boreale		+	1	1		4	3	3	2	2	2	9
Rosa acicularis		1	1		1	2	2	2	2	1	2	9
Maianthenum canadense		1	3		2	3	2	2		2	2	8
Picea glauca		+	1	3	+	2	1	+			R	8
Fragaria virginiana		1		1	1	1	1	1	R	1		8
Mertensia paniculata		1		1	R	+	+	1		1	1	8
Rubus pubescens		1		2	1	1		3	1	2	3	8
Vicia americana		+	+	1	1	1		1	1		1	8
Linnaea borealis		1	1	2	3	1			1	2	1	8
Prunus virginiana		2	+		1		3	2		1	20	7
Aralia nudicaulis		2	1		2	2	2	3	4			7
Equisetum arvense		R	+	1	+			+		+	+	7
Taraxacum officinale		R	+	+	1			1	+		1	7
Pyrola secunda		R				1	1	2	1	1	1	7
Populus tremuloides		1	1	1	2	2	1					6
Aster conspicuous		1	1	1	1		+	1			1	7
Cornus canadensis		3	2		1	1	1	2	1		1	8
Carex rostrata		R		R	R	+					1	5
Shepherdia canadensis		1	+				1		+			4
Petasites palmatus		2	1	1		R						4
Rubus strigosus			+			+			+	+		4
Carex folnea		R	+	+	+							4
Aster laevis			1		+		+			2		4

Table 5. Plant Cover Classes: Devon Transect, continued.

Species Plot Number	1	2	3	4	5	6	7	8	9	10	Constancy
Amelanchier alnifolia	1		+	1							3
Achillea millefolium	R		+	+							3
Equisetum pratense	R				+				+		3
Elymus glauca	R	2				+					3
Salix bebbiana	+		2								2
Vaccinium vitis-idaea		3				1					2
Anemone patens		2				1					2
Agrostis scabra			R				+				2
Grass 1								1		+	2
Pinus banksiana		+									1
Arctostaphylos uva-ursi		+									1
Calamagrostis canadensis	2										1
Epilobium angustifolium	+										1
Lilium philadelphicum						+					1
Smilacina stellata								R			1
Lonicera dioica	+										1
Herb 1									3		1
Shrub 1										1	1
Total number of species per plot	35	29	24	25	23	24	21	20	21	23	

Total spp. = 48

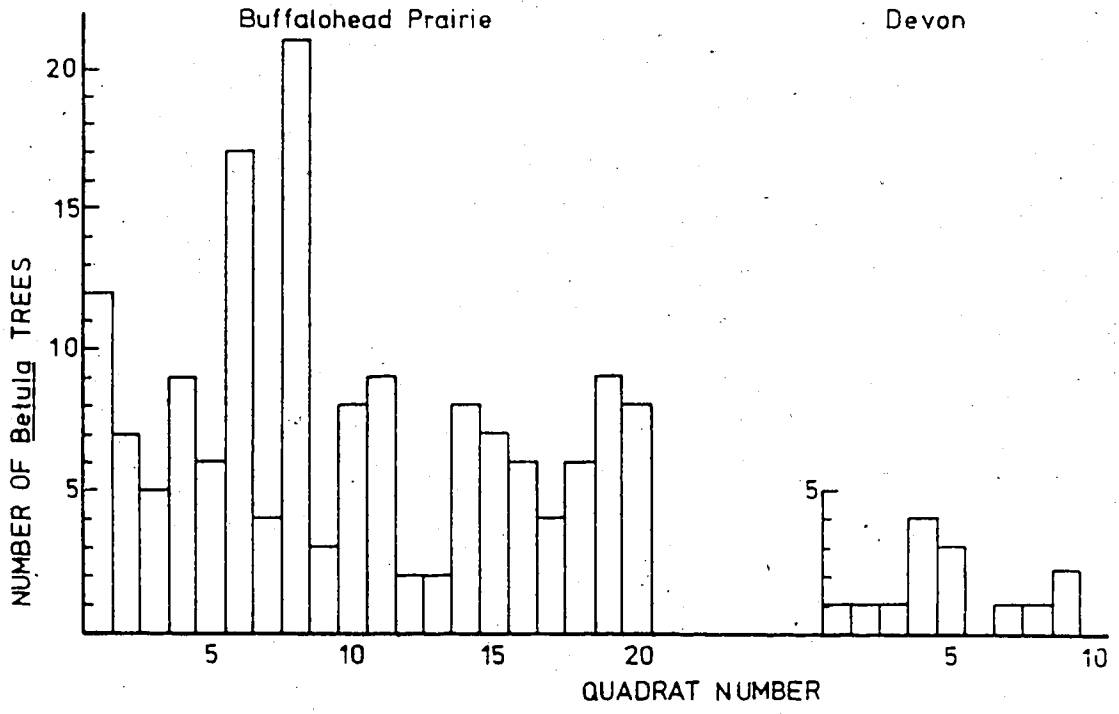


Figure 6. Frequency of Betula papyrifera trees in quadrats at Buffalo Head Prairie and Devon

Table 6. Species list: Buffalo Head Praire study site

Species	Abbrev.	Common name
Betula papyrifera	Bepa	white, paper, canoe birch
Calamagrostis canadensis	Caca	bluejoint, marsh reed grass
Trientalis europaea	Treu	star flower
Epilobium angustifolium	Epan	fireweed
Carex xerantica	Cxxe	sedge
Stellaria longipes	Stlo	long-stalked chickweed
Fragaria virginiana	Frvi	wild strawberry
Rubus acaulis	Ruac	dwarf raspberry
Populus tremuloides	Potr	aspen
Salix glauca	Sagl	willow
Rubus strigosus	Rust	wild red raspberry
Achillea millefolium	Acmi	common yarrow
Achillea sibirica	Acsi	yarrow
Equisetum arvense	Equar	common, field horsetail
Salix bebbiana	Sabe	beaked willow
Salix sp.	Sasp	willow
Hieracium sp.	Hisp	hawkweed
Aster conspicuous	Asco	showy aster
Taraxacum officinale	Txof	common dandelion
Ribes oxyacanthoides	Riox	wild gooseberry
Senecio sp.	Sesp	groundsel, ragwort
Geum sp.	Gesp	avens
Composite 1	-	-
Populus balsamifera	Poba	balsam poplar
Vicia americana	Viam	wild vetch
Rumex sp.	Rxsp	dock sorrel
Rubus parviflorus	Rupa	thimbleberry, salmon berry
Vaccinium vitis-idaea	Vavi	bog cranberry
Pyrola asarifolia	Pyas	wintergreen
Rubus pubescens	Rupu	running raspberry
Solidago sp.	Sosp	goldenrod
Plantago major	Plma	common plantain

Species collected, not occurring in quadrats

Sonchus arvensis	Perennial sow thistle
Petasites sagittatus	arrow-leaved coltsfoot
Salix pseudomonticola	willow
Crepis tectorum	annual hawksbeard
Galium triflorum	sweet-scented bedstraw
Actaea rubra	red and white baneberry
Poa sp.	bluegrass
Alnus rugosa	alder

(15 species in common between the two sites)

Table 7. Species list: Devon reference site

Species	Abbrev.	Common name
Betula papyrifera	Bepa	white, paper, canoe birch
Corylus cornuta	Coco	beaked hazelnut
Vaccinium myrtilloides	Vamy	blueberry
Symphoricarpos albus	Syal	snowberry
Lathyrus ochroleucus	Laoc	vetchling
Pryola asarifolia	Pyas	wintergreen
Galium boreale	Gabo	northern bedstraw
Rosa acicularis	Roac	prickly rose
Maianthemum canadense	Maca	two-leaved Solomon's-seal
Picea glauca	Pigl	white spruce
Fragaria virginiana	Frvi	wild strawberry
Mertensia paniculata	Mepa	tall mertensia
Rubus pubescens	Rupu	running raspberry
Vicia americana	Viam	wild vetch
Linnaea borealis	Libo	twin-flower
Prunus virginiana	Prvi	choke cherry
Aralia nudicaulis	Arnu	wild sasparilla
Equisetum arvense	Egar	common field horsetail
Taraxacum officinale	Txof	common dandelion
Pyrola secunda	Pyse	one-sided wintergreen
Populus tremuloides	Pofr	aspen
Carex rostrata	Caro	sedge
Shepherdia canadensis	Shea	Canadian buffalo-berry
Petasites palmatus	Pepa	palmate-leaved coltsfoot
Rubus strigosus	Rust	wild red raspberry
Carex siccata	Cxfo	sedge
Aster laevis	Asla	smooth aster
Amelanchier alnifolia	Amal	Saskatoon-berry
Achillea millefolium	Acmi	common yarrow
Equisetum pratense	Eqpr	horsetail
Elymus glaucus	Elgl	smooth wild rye
Salix bebbiana	Sabe	beaked willow
Vaccinium vitis-idaea	Vavi	bog cranberry
Anemone patens	Anpa	prairie crocus
Agrostis scabra	Agsc	hair, tickle grass
Pinus banksiana	Piba	jack pine
Arctostaphylos uva-ursi	Aruv	common bearberry
Calamagrostis canadensis	Caca	bluejoint, marsh reed grass
Epilobium angustifolium	Epan	fireweed
Lilium philadelphicum	Liph	western wood lily
Smilacina stellata	Smst	false Solomon's-seal
Lonicera dioica	Lidi	twining honeysuckle

Aster conspicuous	Asco	showy aster
Cornus canadensis	Coca	bunchberry
Herb 1	-	-
Shrub 1	-	-
Oryzopsis asperifolia	Oras	rice grass

In summary, plant community analysis of the high soluble Al site at Buffalo Head Prairie shows a specialized community structure strongly dominated by Betula papyrifera in the tree stratum and Calamagrostis canadensis in the herb stratum. Species diversity and richness are relatively low, yet each species obviously exhibits Al tolerance in its proliferation on this acidic site. Betula papyrifera was thus chosen as the subject for physiological investigation of Al tolerance leading to selection of Devon as a reference stand.

CHAPTER III

ALUMINUM ADSORPTION BY EXCISED ROOTS OF *Betula papyrifera*

Severe toxicity may result below pH 5.0 as Al solution increases (Magistad, 1925) such that more than half the cation exchange sites in the soil are occupied by Al (Evans and Kamprath, 1970). Given that Al shows high affinity for cell wall pectins (Joslyn and Deluca, 1957) the absorptive properties of the apparent free space (AFS) of root tissue may be critical to Al tolerance in plants. The apparent free space available to ion movement exterior to the plasmalemma is comprised of the water free space (WFS) containing ions free in solution, and the Donnan free space (DFS) where ions are bound on fixed negative charges in the root cell wall (Briggs et al., 1958).

It was hypothesized that Al tolerance might be achieved by native plants through avoidance of Al adsorption and transport, or conversely, resistance of toxicity in the presence of uptake by sequestering the ion intracellularly. The purpose of this investigation was to examine Al binding and uptake in the excised roots of *Betula papyrifera* seedlings from the two study sites of high and low soluble Al. The general tolerance mechanism of resistance or avoidance, and the degree to which it varies within the species, was assessed through a study of root affinity for the ion.

Methods

Preparation of Root Material

Two populations of Betula papyrifera were sampled. One originating from the study site of low soil pH (4.9) and high soluble Al (25.5 ppm) at Buffalo Head Prairie, was compared with the other from the Devon site soil pH with low soluble Al (0.0 ppm). It was hypothesized that plants at the low pH site may exhibit greater Al tolerance due to growth under conditions of high Al availability by binding less Al ion in the DFS.

Seedlings of the two-year age class (Plate 5) were collected from each population in May, 1981 and were grown individually in pots of their respective native soils under natural light conditions in a greenhouse at 21 - 25 ^{o}C. Upon initiation of leaf senescence, 30 seedlings were transferred to a cold chamber (-5 ^{o}C). After four weeks they were moved to a similar chamber at +5 ^{o}C for one week, then to a controlled environment chamber at 22 ^{o}C and 16 h days for the duration of the experiment.

Two days later, seedlings were carefully lifted from the soil. Roots were pruned and washed under running water for approximately 10 min. before placing the plants

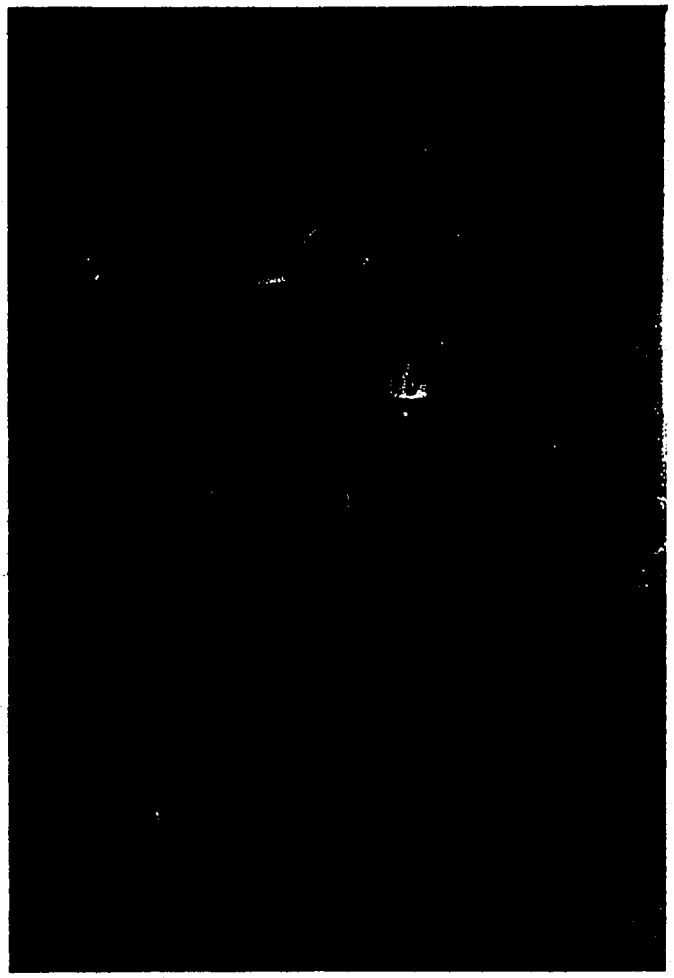


Plate 5. Betula papyrifera seedlings in native soil at Buffalo Head Prairie.

individually in two litre pots with plastic liners. The nutrient solution of aerated half-strength Hoagland solution at pH 4.01 (Hoagland and Arnon, 1955) and liners were changed after day two and subsequently at seven day intervals. Beyer and Hutnik (1969) previously demonstrated that growth of Betula pendula Roth. and B. lenta L. is not inhibited by pH 3.8 in the absence of Al.

For comparative purposes all experiments were also performed on Hordeum vulgare var. Galt. Seeds were germinated in aerated, distilled water in the dark for 24 hours and suspended upon plastic mesh over half-strength Hoagland solution adjusted to pH 4.0 in opaque 600 ml beakers after Epstein (1961). In both instances, apical 5 cm root segments were harvested after approximately 14 days at 22 ^{o}C and 16 h light.

Characterization of Desorbing Washes

To assess Al binding capacity, it was first necessary to characterize the proportion of Al desorbed over time by distilled water exchanging Al from the WFS, and by $LaCl_3$, freeing the DFS (Briggs et al., 1958). Apical 5 cm portions of newly formed roots were excised underwater from at least 6 plants within a population. Samples weighing approximately 1.00 g were accurately weighed and placed in 4 x 5 cm bags of plastic mesh sewn with monofilament line. Sets of 12 were held in a two litre container of aerated

0.5 M $CaCl_2$ for 30 minutes (Epstein et al., 1963). Samples were individually spun several times in air before submersion in beakers containing 200 ml aliquots of 0.5 M $CaCl_2$ and 50 ppm Al as the chloride salt at pH 4.0. Bathing solutions were aerated and maintained at 30 °C in a water bath).

After a 30 minute uptake period, samples were spun and transferred to Erlenmeyer flasks containing 50 ml aliquots of distilled water, 50 ppm Lanthanum (La), or 500 ppm La at approximately 5 °C in an ice bath. Desorbing washes extending to 90 minutes were terminated at 15 minute intervals. Roots were transferred to crucibles and dry-ashed (Lambert, 1976). Al concentration was determined after dilution to 5 ml, and addition of 1,000 ppm La as La_2O_3, using a Perkin-Elmer model 503 atomic absorption spectrophotometer.

Determination of Adsorbed Aluminum

Differentiation between adsorbed Al in DFS and intracellular levels was achieved by rinsing samples for 30 minutes in distilled water or $LaCl_3$ desorbing washes after exposure to the bathing solution.

A model was developed for determination of Al adsorption by excised roots (Fig. 7). Immersion of root samples, grown in the absence of Al, in uptake solutions yielded total Al as the sum of endogeneous Al plus that

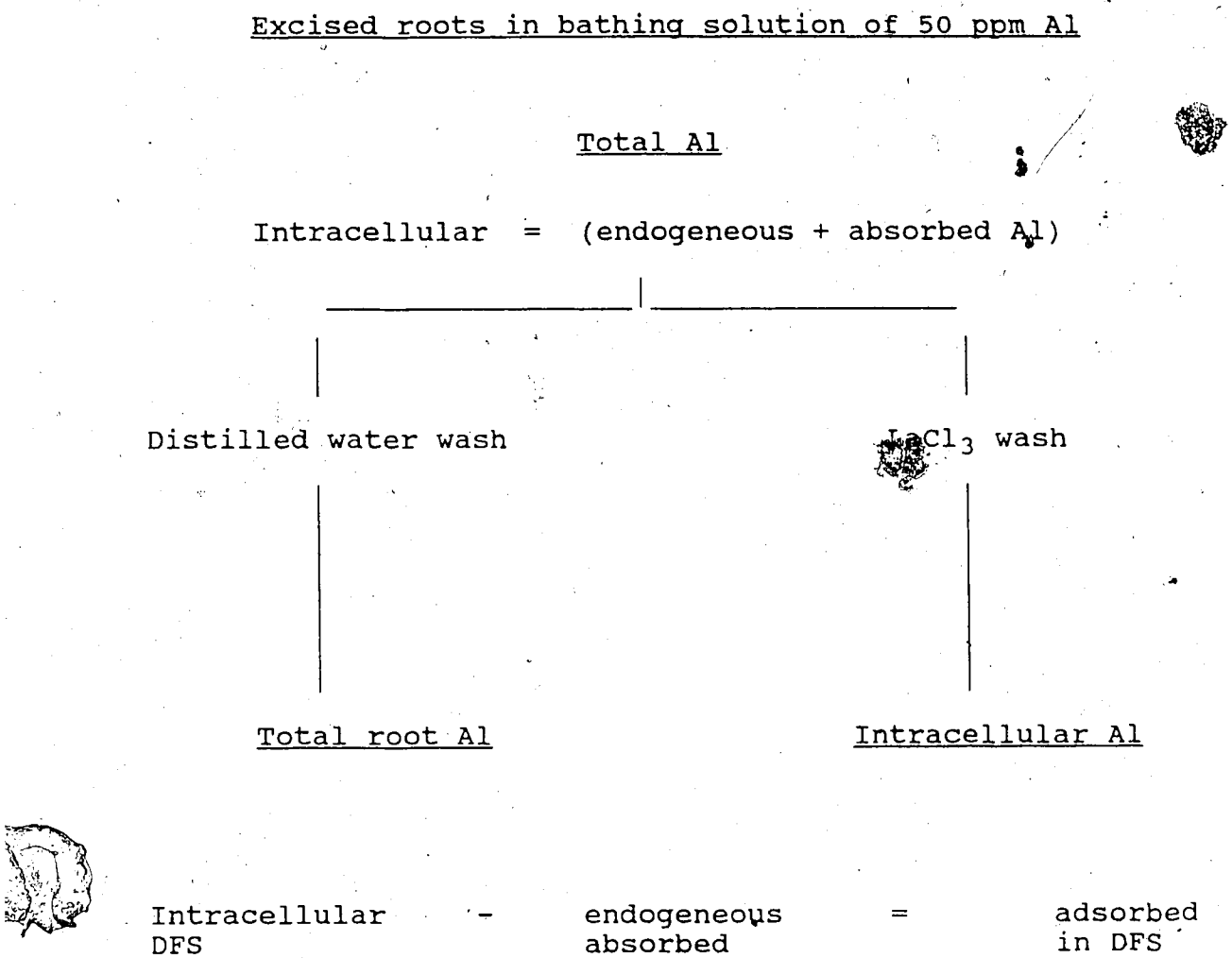


Fig. 7. Model for determination of Al adsorption by excised roots.

absorbed into non-free space or associated with the AFS. Washing in distilled water removed the WFS component, while washing in La eluted the AFS as a whole, giving total and intracellular root Al respectively. The difference was taken to be adsorbed Al in the DFS.

Cation Exchange Capacity

Apical 5 cm portions of active roots were harvested from all birch seedlings upon termination of the experiment, and from 14 day-old barley. These were dried overnight at 80 oC and ground in a Wiley mill to pass through a 40 mesh screen. Samples (200 mg barley, 100 mg birch) were then taken for determination of cation exchange capacity according to Crook (1964) with the modification of use of a magnetic stirrer.

Results and Discussion

Growth of Root Material

Vigorous root growth was achieved with both Hordeum vulgare and Betula papyrifera under the methods employed. Barley has been classified as an Al-sensitive species on acid soils (McLean and Gilbert, 1927) and the 'Galt' variety is known to acidify its growth medium to pH 3.8 (Glass et al., 1981) which is close to the levels used here. Although birch seedlings originated from sites of

differing acidity, both populations produced active roots following bud opening. This was closely correlated with leaf expansion, presumably due to auxin formation.

Aluminum Desorption over Time

After 30 minutes in uptake solution of 50 ppm Al, excised roots from Betula and Hordeum accumulated Al such that root Al was in the range of 3.9 to 16.3 ppm over the course of desorption. Each desorbing wash resulted in significant elution of Al from root tissue over the 90 minute period with the exception of 50 ppm Ca. La (MW 138.7) was selected to displace Al (MW 27.0) from the AFS due to their similarity as trivalent metals and lanthanum's low standard reduction potential of -2.37 volts compared to -1.71 volts for Al.

Distilled water washed a high proportion of Al from the AFS of Betula papyrifera from the Buffalo Head Prairie (BHP) population in the first 30 minutes of immersion, yet the remaining levels of intracellular Al and that associated with DFS were higher than in plants of the Devon population and in Hordeum vulgare (Fig. 8). Concentrations remaining in roots of barley and Devon birch were closely correlated after elution of the WFS. Blotting samples of excised Devon birch roots on Whatman No.1 filter paper after Al accumulation from bathing solutions revealed that total root Al including AFS was of the order of 12.7 ppm

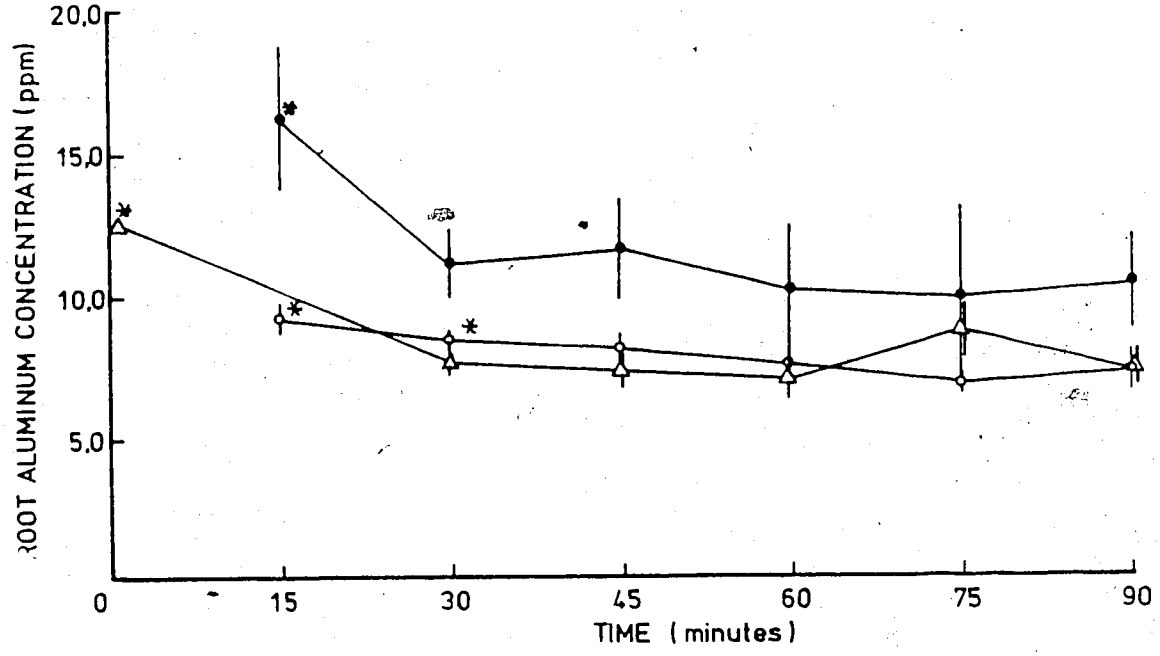


Fig Desorption of Al from excised barley (▵), BHP birch (.), and Devon Birch (o). Roots accumulated Al from 50 ppm bathing solutions for 30 min. before transferal to the distilled water desorbing wash.
*significant at 0.05 level by Duncan's multiple-range test. Results for barley and BHP birch are means of 4 replicates. Results for Devon birch are means of 2 replicates.

for that species, yielding a value of approximately 5 ppm Al in the WFS (WFS = Total Al - (DFS + absorbed Al); 5 = 12.7 - 7). Adequacy of a 30 minute distilled water wash is supported for each species as concentrations remaining after that time did not differ significantly from one another.

Desorption of Al from the AFS of excised barley roots with 50 ppm La yielded high standard errors in mean values at 15 minute intervals (Fig. 9). Comparison of the effectiveness of a two-fold increase in La concentration revealed that mean Al levels remaining after 15 minute immerson were identical (8.0 ppm), yet the 100 ppm La wash further eluted Al such that the value at 15 minutes is significantly higher than those for longer periods of washing (Fig. 10). Barley roots washed in 100 ppm La contained lower concentrations of Al than did those of BHP birch. La was shown to displace Al from the DFS, not labile with distilled water in either species. Again the length of wash was supported by statistical analysis.

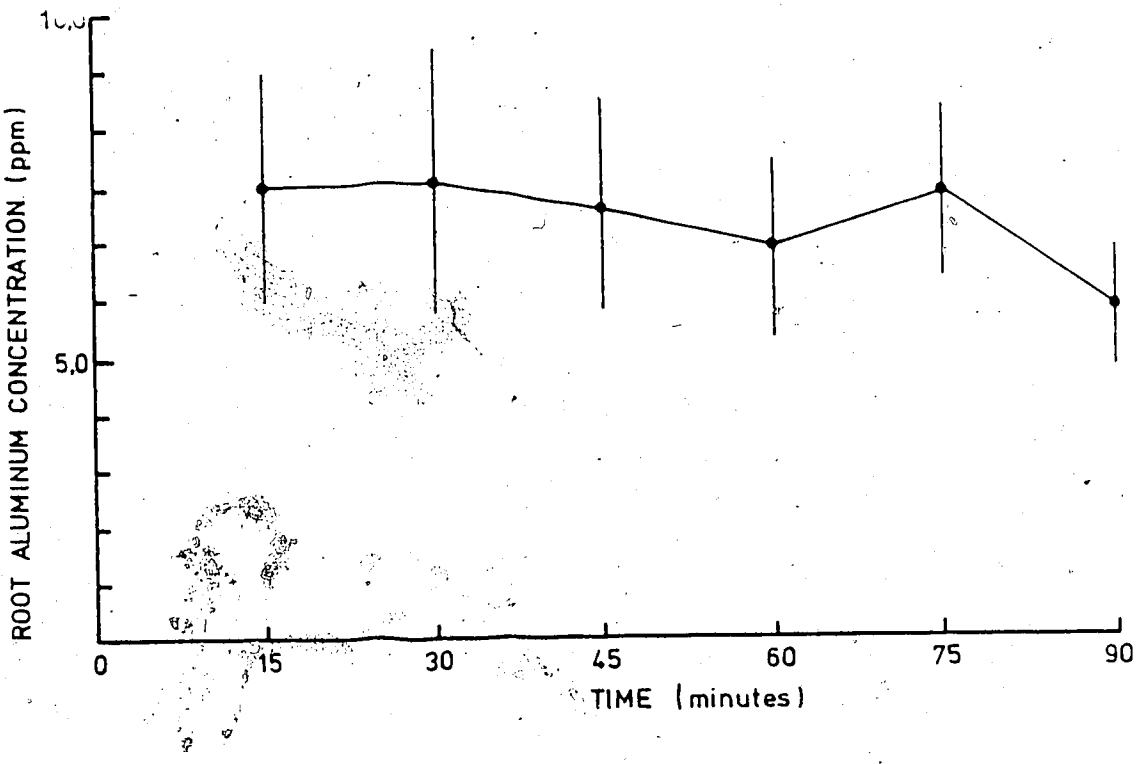


Fig. 9. Desorption of Al from excised barley roots after accumulation from 50 ppm Al bathing solutions for 30 min. The desorbing wash contained 50 ppm La. No significant difference among means at the 0.05 level by Duncan's multiple-range test. Means of 4 replicates.

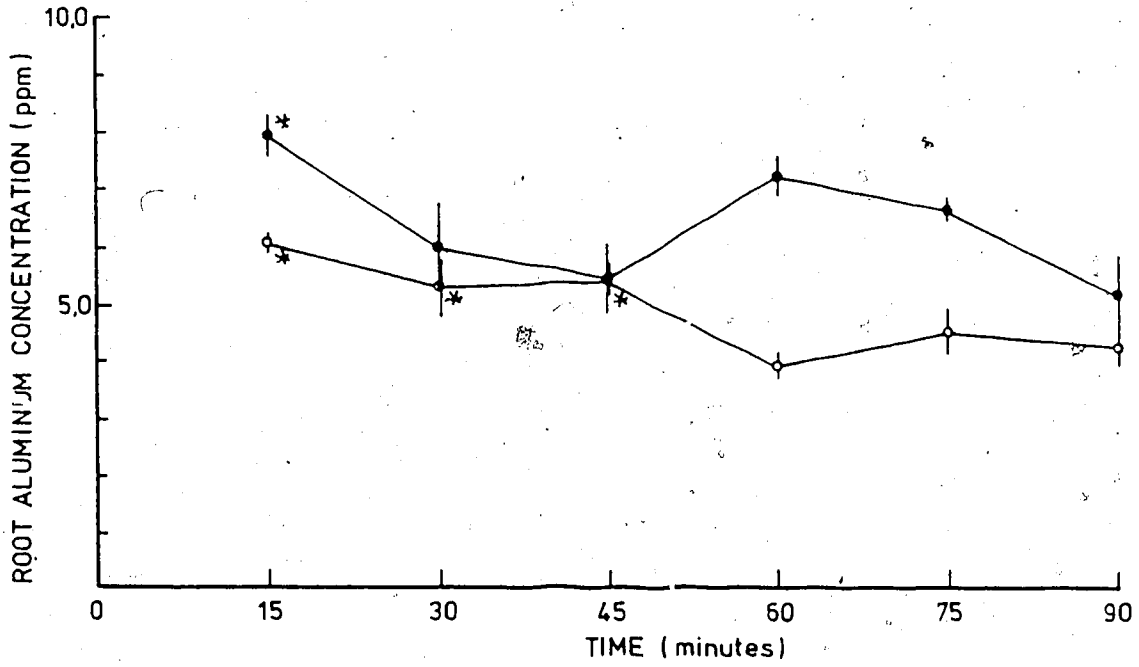


Fig. 10. Desorption of Al from excised barley (o) and BHP (.) birch. Roots accumulated Al from 50 ppm Al bathing solutions for 30 min. before transferal to the desorbing wash of 100 ppm La. *significant at .05 level by Duncan's multiple-range test. Means of 6 replicates. ± SEM.

Aluminum Desorption from the DFS

Fig. 11 summarizes the results of experiments designed to quantify total and intracellular root Al after appropriate desorption. Within each species there was a decrease in the Al concentration remaining after washing with more concentrated La, suggesting that 100 ppm La does not completely displace Al from the DFS. Total root Al, as defined, is highest (20.0 ± 2.6 ppm) in Devon birch roots and lowest in the roots of BHP birch (10.0 ± 0.9 ppm). This could be interpreted as an indication that BHP birch roots have a larger component of Al exchangeable from the WFS. Total root Al in barley was intermediate (16.3 ± 1.4 ppm). In each case, total root Al was found to be slightly lower, yet in the same range as estimated from characterization of distilled water washing (Fig. 11). Statistical analysis of Al remaining in root tissue after each type of wash showed that BHP birch retained significantly less Al than other plant types after each type of wash (Table 8). While the 500 ppm La left significantly lower levels in BHP, than either distilled water or 100 ppm La, all Al levels remaining after the three washes differed significantly from one another in Devon birch roots.

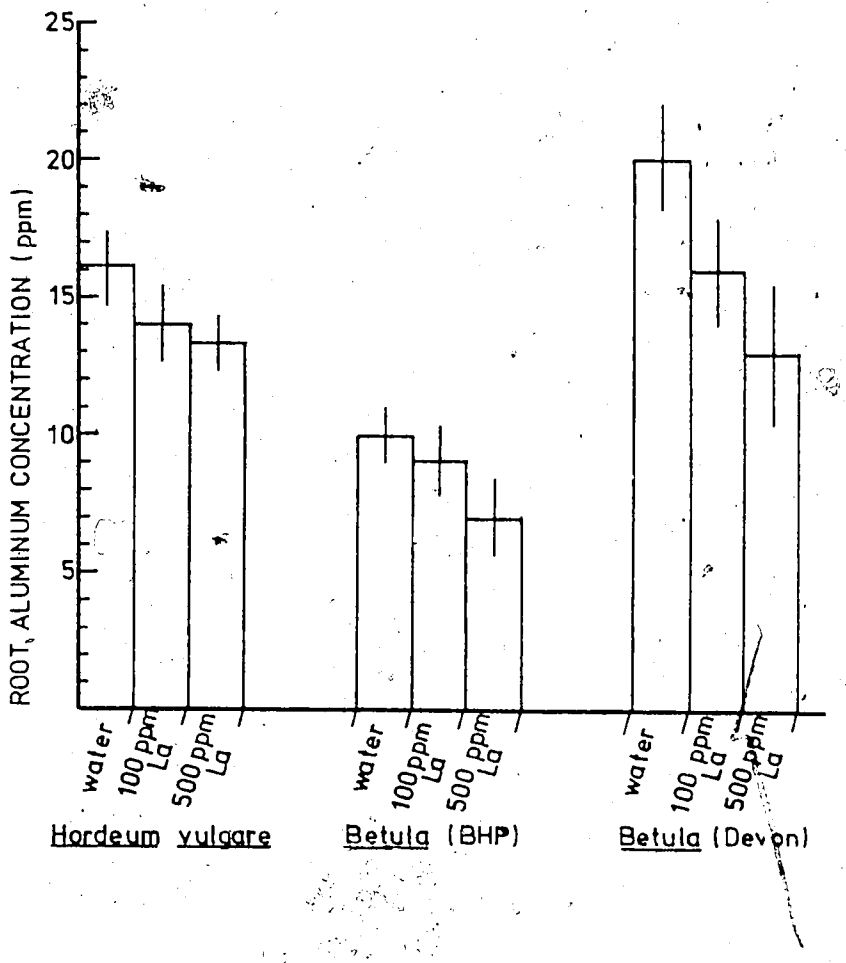


Fig. 11. Aluminum concentration in excised roots after distilled water and $LaCl_3$ desorbing washes. Means of at least 8 replicates.

Table 8. Statistical analysis of Al concentration remaining in root tissue of each plant species after desorbing washes.
*Significant at 0.05 level using Duncan's multiple-range test.

	Barley	BHP birch	Devon birch
Distilled water wash		*	
100 ppm La wash		*	
500 ppm La wash		*	

Statistical analysis of Al concentration remaining in root tissue after each desorbing wash for individual plant species.
* Significant at 0.05 level using Duncan's multiple-range test.

	Distilled water wash	100 ppm La wash	500 ppm La wash
Barley		no significant difference	
BHP birch	*	*	
Devon birch		All means significantly different	

Intracellular Al as determined after immersion in La washes was again highest in Devon birch and lowest in BHP birch. In light of the similar endogeneous levels of Al in roots of seedlings from these populations, Devon birch roots retain a higher level of Al upon exposure to elevated concentrations in accordance with lower tolerance, while Al-tolerant BHP birch bind Al less firmly.

Endogeneous Al concentrations in excised barley roots (3.9 ± 0.1 ppm) agree closely with the value of 3.8 ppm in roots of the 'Kearney' variety grown under similar conditions (Foy et al., 1969). Here there is a large difference in intracellular Al, as defined, after La wash. This Al could be accounted for by entry into the symplasm of the Al-sensitive plant root, or by irreversible binding in cell walls. In eithe case, barley is shown to retain high concentrations of the toxic metal.

Adsorbed Al as calculated from the model is summarized in Table 9. It was hypothesized that the Al adsorptive properties of plant roots may afford insight into tolerance mechanisms. BHP birch roots were shown to adsorb less Al than those from the Devon population. Since the intracellular Al concentration of 7.0 ± 1.4 ppm agrees closely with the endogeneous level of 5.8 ± 0.4 ppm, it appears that 500 ppm La effectively displaces all Al adsorbed in the DFS. Roots of the Devon plants were shown to adsorb more than twice the concentration of Al as

Table 9. Cation exchange capacity, endogenous Al concentrations, and Al adsorption in excised roots of barley, BHP birch, and Devon Birch. Excised roots were immersed in bathing solutions of 50 ppm Al for 30 minutes before transferal to desorbing washes of distilled water, giving total root Al, or LaCl yielding intracellular root Al. Adsborbed Al associated with the DFS was calculated by difference. (Means ± SEM).

plant	Endogenous Al (meq./100 g dry wt)	CEC (meq./100 g dry wt)	Desorbing wash	Total root Al (ppm)	Intracell. Al (ppm)	Adsorbed Al (ppm)
Hordeum vulgare	3.9 ± 0.1	22.0 ± 1.2	distilled water	16.3 ± 1.4		
			100 ppm La		13.9 ± 1.4	2.4
			500 ppm La		13.4 ± 0.9	2.9
Betula papyrifera (BHP)	5.8 ± 0.4	69.0 ± 3.8	distilled water	10.0 ± 0.9		
			100 ppm La		9.3 ± 1.4	0.7
			500 ppm La		7.0 ± 1.4	3.0
Betula papyrifera (Devon)	4.6 ± 0.1	61.0 ± 0.7	distilled water	20.0 ± 2.1		
			100 ppm La		16.3 ± 2.0	3.7
			500 ppm La		13.0 ± 2.5	7.0
	Means of 12 replicates	Means of 3 replicates		Means of at least 8 replicates		

determined after both types of La wash. Adsorbed Al after 100 ppm La wash (3.7 ppm) was in the range of 2.9 ppm recorded for barley roots, while the concentration of 7.0 ppm after 500 ppm La wash was higher than that of barley and BHP birch after similar desorption. Cation exchange capacities are listed in Table 9. BHP birch roots showed slightly higher CEC than Devon birch roots. Tentatively this would infer that roots of BHP birch are capable of binding more cations on a dry weight basis than plants of the Devon population. The CEC of 22.0 ± 1.2 mg/100 g dry weight for barley is in agreement with CEC for monocots cited by Crook (1964). Small amounts of adsorbed Al (2.9 ppm) determined here appear to be in line with low CEC common among grasses. Al tolerant barley is known to have low CEC and less Al accumulation in roots than sensitive varieties (Foy et al., 1969) so that low CEC may be important to the tolerance mechanism of this species.

CEC does not correlate well with adsorbed Al in Betula papyrifera. BHP birch roots, with a slightly higher CEC, adsorbed less Al than did Devon birch roots. It appears that CEC is not a good assessment of binding sites available to Al in this species.

In summary, the data presented here suggest that Betula papyrifera from sites of high and low soluble Al exhibit differential binding of Al by excised roots. Highly tolerant plants of the Buffalo Head Prairie population bind

Al loosely such that desorption of the AFS leaves low concentrations remaining in root tissue. A low number of Al binding sites may be evoked. Birch roots from Devon plants show a higher affinity for Al which could limit their tolerance. It is evident that Betula papyrifera native to acidic soil avoids Al stress by exclusion of the metal from root symplasm, and low affinity of the root cell wall for the ion.

CHAPTER IV

ALUMINUM, CALCIUM, AND PHOSPHORUS ALLOCATION IN Betula papyrifera

As outlined in Chapter I, Betula papyrifera could resist Al stress by excluding it at the level of the root, or the plant could be biochemically adapted to tolerate its presence in other plant tissues. The toxic effects of Al have been attributed to reductions in Ca and P uptake in a wide variety of crop plants (Johnson and Jackson;, 1964; Paterson, 1965; Chaisson, 1964; Foy and Brown, 1963; Ota, 1968; Long and Foy, 1980; Clarkson and Sanderson, 1971; Mugwira et al., 1980). Recent investigations extend these findings to tree species (Cummings et al., 1986). Interference with the negative charge on the pores of the free space in the root (Clarkson and Sanderson, 1971) in the case of Ca deficiency, and internal precipitation of aluminum phosphate reducing P transport (Wright and Donahue, 1953) are possible mechanisms.

To assess tolerance strategies of Betula papyrifera, it was useful to determine:

1. the concentrations of P and Ca in stems and leaves of plants from low soluble Al sites,
2. the extent to which Al gains entry into root tissues,
3. the concentration of Al in leaves, stems and catkins.

One could postulate that Al tolerance could be manifested in the ability to maintain Ca and P levels in aerial plant parts, and that such could occur either in the presence of Al excluded at the root cell wall or allowed access to plant tops.

Decreased Ca in plant tops and increased Ca in roots has been recorded by Oullette and Dessureaux (1958), MacLeod and Jackson (1965), and MacLean and Chaisson (1966). Long and Foy (1980) determined the Ca concentration in Al-tolerant 'Dayton' leaves to be more than twice that in the Al-sensitive 'Kearney' variety of barley. Jackson (1967) reported reduced Ca accumulation in wheat (Triticum aestivum). Foy et al., (1972), adding 4 ppm Al at pH 4.8, found decreased Ca concentration in root cell walls, mitochondria, and total roots of Al-sensitive Roman snapbeans. They suggested that damage could be mediated through reduction of Ca at important subcellular membrane sites. Johnson and Jackson (1964) stated that a reduction in Ca uptake in wheat seedlings caused by Al could not be overcome by supplying Ca, while Munns (1965) noted slight alleviation of Al toxicity by high concentrations of Ca in the medium.

The concentrations of root Ca and P of Al-tolerant wheat cultivars were found to be significantly below those of more sensitive plants (Mugwira et al., 1980). Foy and Brown (1963), growing cotton plants in 1.5 ppm Al at pH 4

recorded values for Al, P, and Ca in tops of 60 ppm, 0.42%, and 0.66% respectively, compared with 40 ppm, 0.64% and 2.80% in 0.09 ppm Al solution at pH 5. Chaisson (1964) banded 22 ppm P with barley seed in soil of pH 5.0 to prevent P deficiency and double yields. Munns (1965) determined Al toxicity in Medicago sativa to be associated with reduced P concentrating in both roots and tops. Reeve and Sumner (1970) concluded that Al toxicity and P deficiency were both primary, but independent growth-limiting factors. Other workers finding reduced P concentrations in tops of plants subject to Al stress include Wright and Donahue (1953), Randall and Vose (1963), Foy and Brown (1964), Macleod and Jackson (1965), Munns (1965), Clarkson (1966), MacLeod and Chaisson (1966), Lance and Pearson (1969), Naidoo (1976), and Mugwira et al., (1980). Steiner et al., (1980) reported consistent reductions in foliar Ca and in some cases P in Betula papyrifera grown in the presence of Al.

Al accumulation has been reported in the roots, but not the tops, of some Al stressed plants (MacLeod and Jackson, 1965; Foy et al., 1967). Chamura (1962) and Ota (1968) have shown Al concentrations in plant roots to be negatively correlated with crop yields under such conditions. Yet, Foy et al., (1967, 1969) found that differences in Al tolerance among wheat, barley, and soybean varieties were not associated with different Al

concentrations in plant types. Oullette and Dessureaux (1958) found that Al concentrations in tops of Al-tolera: alfalfa clones were lower, and Al concentrations in their roots were higher, than Al-sensitive clones. Otsuka (196 reported lower Al concentrations in the shoots of Al-tolerant 'Hiracki' wheat than in the Al-sensitive 'Norm 25' variety. Al concentrations in tops of alfalfa plants grown in acid soils (pH 4.0 to 5.6) were found to be highly correlated with Al extracted from the soils by 0.01 M $CaCl_2$ (Medappa and Dana, 1970). In other plants, tolerance has been associated with Al accumulation in tops (Hu et al., 1957; Moomaw et al., 1959; Jones, 1961). Differences in foliar Al concentrations between tolerant and intolerant Betula papyrifera provenances were reported by Steiner et al., (1980).

Methods

Samples of catkins, leaves, and stems were collected at both study sites from at least 25 Betula papyrifera plants of three diameter classes (0-2.5 cm, 2.5-7.5 cm, 7.5+ cm). Leaves were fully expanded; stems were taken within 1 m from the plant apex. These were rinsed for 1 hour in double distilled water, oven dried at 60 ^{o}C overnight, and ground in a Wiley mill to pass through a 40 mesh screen. Al and Ca were determined, after dry-ashing

(Lambert, 1976), using a Perkin-Elmer model 503 atomic absorption spectrophotometer. Total P was determined spectrophotometrically after Kjeldahl digestion.

Results and Discussion

Al concentrations determined for leaves, stems, and catkins of Betula papyrifera from both sites are presented in Figure 12. Pooling data between sites gives values of leaf Al = 62.9 ± 24.5 ppm, stem Al = 30.3 ± 8.4 ppm, and catkin Al = 21.8 ± 10.7 ppm. It is apparent that foliar Al concentrations are higher than those of stems or catkins. Entry of Al into root tissue and its subsequent transport to foliar tissue has been described for many plants, and Al concentrations have been found to be 272 to 1490 % higher in leaves of Betula papyrifera seedlings grown in 120 ppm Al for 14 days than in control plants (Steiner et al., 1980). However, these values are not in the range of those for aluminum accumulators, which can attain levels of more than 1,000 ppm in their tops (Moomaw et al., 1959; Hess, 1963). In fact, tissue Al levels in native Betula papyrifera are in the order of shoot concentrations of 44 to 134 ppm determined by Hoyt and Nyborg (1971) for alfalfa growing on sites adjacent to the Buffalo Head Prairie study site.

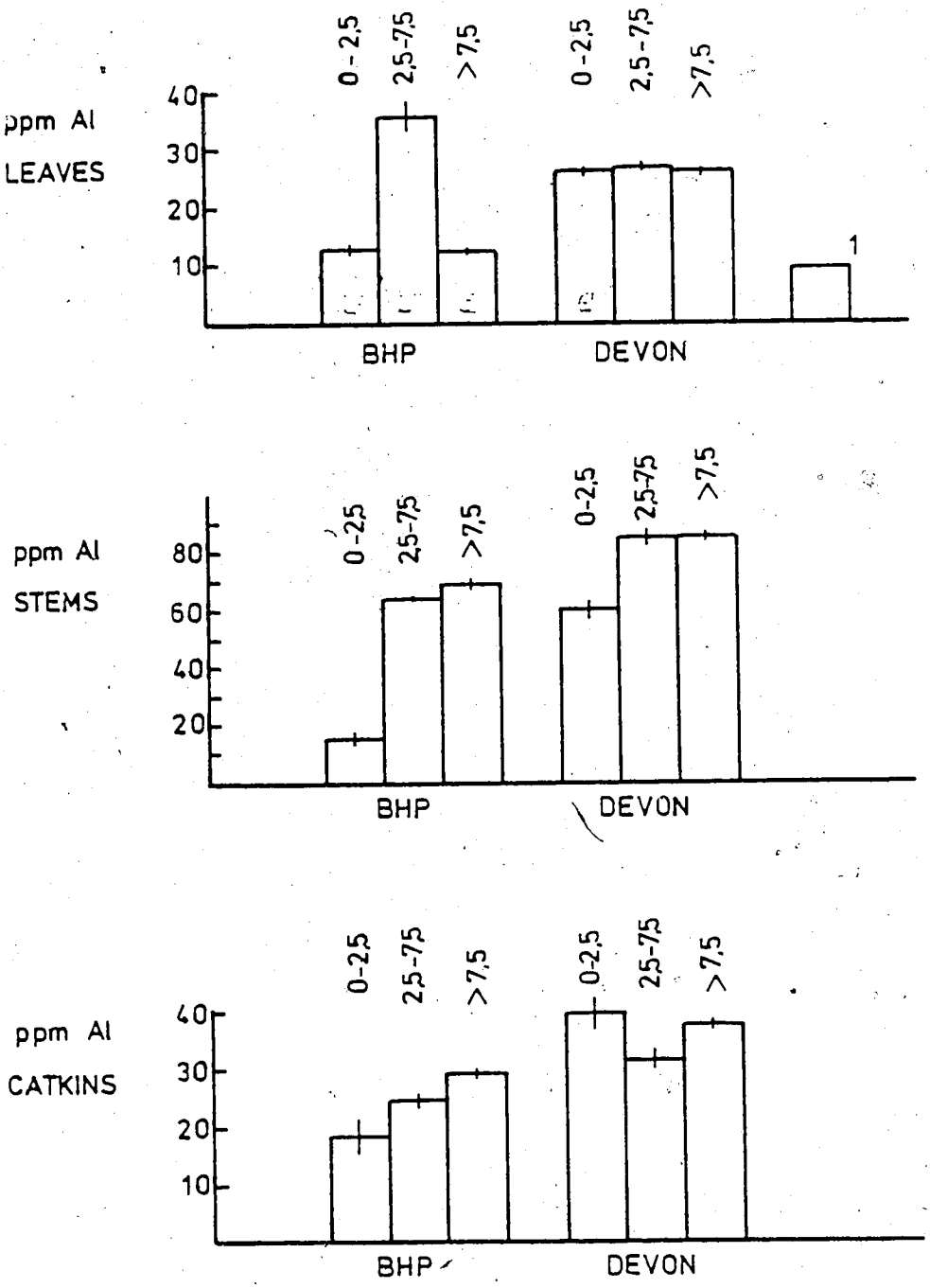
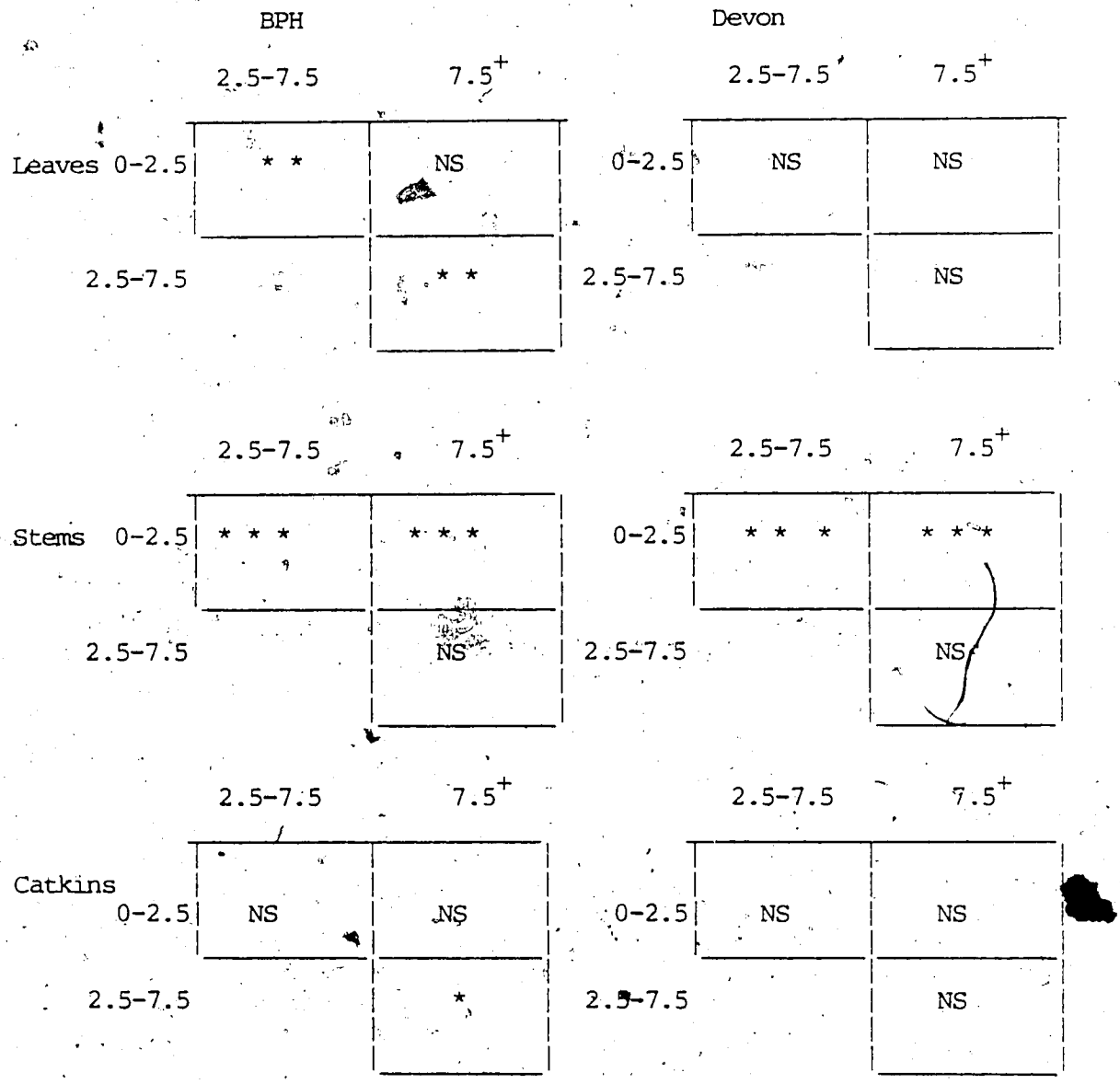


Figure 12. Al concentrations of leaves, stems, and catkins in Betula papyrifera from the two study sites. Three stem diameter classes (DBH). (Means of 6 determinations ± SEM).

Plant material was collected by diameter class to analyze the variable of age in relation to Al accumulation. A general trend toward higher concentrations with age is evident when considering stems and catkins, yet such is not the case for foliar tissues. Statistical analysis (Table 10) reveals that Al concentrations in the second diameter class are significantly greater than in the first and third classes. In contrast, Devon plants show no difference in leaves of various diameter c[illegible]is indicates that Al is mobilized to newly forming le[illegible]om stores within the plant in similar amounts regar[illegible]of age. Within diameter classes there are differences between foliar Al concentrations of populations (Table 11). Plants of the first and third diamteter class have significantly greater foliar Al levels than do their Buffalo Head Prairie counterparts. Again a discrepancy arises in the high value for the second diameter class at Buffalo Head Prairie. When comparing diameter classes between populations for stems and catkins, the less tolerant Devon population is seen to allow statistically greater concentrations to accumulate in these tissues, consistent with higher Al adsorption in their roots (Chapter III). This is in agreement with the finding of Steiner et al., (1980) that Al tolerant seedlings of paper birch provenances accumulate less Al (9.8 ppm versus 15.9 ppm) in their leaves than intolerant provenances.

Table 10. Levels of significance (1) of tissue concentration of Al in leaves, stems, and catkins of Betula papyrifera within study sites. Three stem diameter classes (DBH).



(1) Significance Levels

NS	not significant
*	significant at 0.05 confidence level
**	significant at 0.01 confidence level
***	significant at 0.001 confidence level

Table 11. Levels of significance (1) of tissue concentration of Al in leaves, stems, and catkins of Betula papyrifera of the same diameter class between Buffalo Head Prairie and Devon. (Name in parentheses indicates greater Al concentration.)

DBH Class	[Al] Leaves
0 - 2.5	*** (Devon)
2.5 - 7.5	NS
7.5 +	*** (Devon)
	[Al] Stems
0 - 2.5	*** (Devon)
2.5 - 7.5	*** (Devon)
7.5 +	*** (Devon)
	All Catkins
0 - 2.5	*** (Devon)
2.5 - 7.5	* (Devon)
7.5 +	** (Devon)

(1) See Table 10.

From statistical analysis of stem Al concentrations grouped by diameter classes within individual populations, it is evident that Al concentrations increase with increasing diameter so that values for the smallest diameter class differ significantly from the two larger classes. After Betula papyrifera trees attain a DBH greater than 2.5 cm, increases in stem Al are not significant. To summarize, leaf Al concentrations are greater than those found in stems or reproductive structures, so that Al must be readily mobilized in this deciduous species. Values are in the order of tissue concentrations of crop plants grown under similar conditions, so that Betula papyrifera is not Al tolerant via mechanisms of complete avoidance of uptake, nor Al accumulation and sequestration. Diameter class is a variable in the Al content of stems of this woody plant, as concentration increases with time.

Calcium and phosphorus concentrations determined for leaves, stems and catkins of paper birch seedlings from both Buffalo Head Prairie and Devon populations, as pooled samples from all diameter classes, are presented in Table 12. P levels in leaves and catkins were found to be higher than those in stems, as might be expected from photosynthetic tissue. No obvious differences are seen between populations. Leaf P was determined to be slightly higher than that cited by Steiner et al., (1980), however this observation is presented only for reference since it

Table 12. Concentration Ca and P in Betula papyrifera plant tissues. Means of 3 replicates.

	BHP	Devon	BHP	Devon
	P (%)		Ca (%)	
Leaves	0.27 ± 0.01	0.31 ± 0.09	0.42 ± 0.05	0.60 ± 0.11
Stems	0.09 ± 0.01	0.05 ± 0.01	0.82 ± 0.03	1.48 ± 0.25
Catkins	0.31 ± 0.01	-	0.78 ± 0.34	-
	Tolerant Provenan.	Intolerant Provenan.	Tolerant Provenan.	Intolerant Provenan.
(1) Foliage	0.24 ± 0.02	0.25 ± 0.02	0.32 ± 0.04	0.30 ± 0.08

1) From Steiner et al., (1980). Betula papyrifera seedlings were grown in 0.004 M calcium nitrate (14 days), modified Hoagland and Arnon solution (7 days), and 0.004 M calcium nitrate + 120 ppm Al (14 days).

pertains to seedlings grown in solution culture to which 120 ppm Al had been added.

Devon plants exhibited higher concentrations of Ca in both leaves and stems than did Buffalo Head Prairie plants. In addition, both populations show higher levels of Ca in tops of seedlings than those reported by Steiner's group. While average P concentrations in the 0-30 cm segment of soil profiles at the sites are similar (Devon = 30.5 ppm; Buffalo Head Prairie = 35.6 ppm), extractable Ca is nearly four-fold higher in the Buffalo Head Prairie soil (2389 ppm versus 597 ppm). Lower levels of P in Buffalo Head Prairie birch tops is most likely a reflection of P precipitation by high Al concentrations at that site.

These data are presented to better understand the tolerance strategy of Betula papyrifera under two different edaphic conditions since lack of controls makes inter-population comparisons impossible. Howeler et al., (1976) reported that Al-tolerant rice cultivars had lower concentrations of Al and higher Ca and P concentrations in their shoots than did Al-sensitive cultivars, while no relationship between tolerance and concentration of Al, Ca and P is known for wheat cultivars grown in solution culture (Foy et al., 1974; Mugwira et al., 1976). Furthermore, Steiner et al., (1980), concluded that foliar elemental concentrations are only remotely involved in the tolerance mechanism itself, thus they are not good

indicators of relative tolerance in Betula papyrifera. However it is noteworthy that paper birch plants from a site of low plant-available Al accumulate greater foliar Al concentrations than do their counterparts native to a site of high plant-available Al.

CHAPTER V

ALUMINUM ENTRY INTO EXCISED ROOTS OF *Betula papyrifera*

Localization of Al at the cellular level within the roots of plants suffering from its toxic effects has been the subject of attention for several reasons. In the first instance, knowledge of the location from which Al elicits its toxic effects is desirable. Secondly, exclusion of Al from critical metabolic sites (Antonovics et al., 1971) at the whole plant level, the tissue level (for example root meristem), or at the subcellular level of organelles (for example nuclei and mitochondria) and enzymes such as peroxidase, cytochrome oxidase, isocitric dehydrogenase, and polyphenol oxidase (Anderson and Evans, 1956; Ota, 1968), may lend insight into tolerance mechanisms. Finally, understanding Al interactions with nutrients such as P and Ca in the root necessitates knowledge of the ion's distribution.

The theory that Al precipitates P within the roots of plants was first held by Wright (1948) working with barley in culture solutions. This was challenged by Wallihan (1948) who contended that Al was not precipitated internally, rather Al and perhaps P were held to root surfaces by ionic exchange. Clarkson (1966) suggested an adsorption-precipitation reaction between Al and P at the

cell surface or in the free space of the root, which was supported by Rasmussen (1968) using electron microprobe x-ray analysis to determine the mode of Al entry, its distribution, and localization in corn roots. It was found that Al was precipitated on the surface of the root epidermal cells, but did not penetrate the cortex unless the integrity of the root surface was compromised. The epidermis prevented movement of Al into the cortex and stele such that penetration into vascular tissue was possible only at the site of lateral root emergence.

McCormick and Borden (1972, 1974) reported similar findings in barley and poplar roots using photomicrographic techniques to examine the interaction of Al and phosphate. The Al-PO_4 precipitate was found to occur as scattered globules in the mucilaginous layer along the root surface and in association with the cell wall and cytoplasmic membrane of epidermal and cortical cells. Waisel et al., (1970), investigating the localization of anionic Al in cells of bean and barley roots by x-ray microanalysis, reported only minute amounts of Al in cell walls and no correlation between the distribution of Al and phosphate. Naidoo (1976) utilized energy dispersive analysis of x-rays generated in the scanning electron microscope to determine Al distribution in snapbean. He found Al to be located in cell walls, cell contents, and nuclei of root cap and meristemic cells where it was postulated to disrupt cell

division. Use of the molybdenum blue staining technique confirmed McCormick and Borden's (1972) findings. The resultant blue stain outlined the root cap cells, epidermis, and outer cortical cells within 2 to 3 mm of the root tip, while the stele remained relatively clear. The extent to which Al is able to penetrate the root tissues of native plants may determine the mechanism of tolerance precluding the normal symptoms of disrupted root growth, decreased utilization of Ca and P, and possible Al transport to critical metabolic sites.

Aluminum has been shown by Hutchinson (1986) to accumulate in the root cap of root tips and in the epidermal and outer cortical walls of older roots of Jack Pine. This tissue distribution of Al was confirmed by scanning electron microscopy analysis, revealing its coincidental distribution with P in roots.

Methods

Betula papyrifera seedlings from the two study sites were established in solution culture as outlined in Chapter III. Ten plants from each population were then transferred to treatments of Hoagland solution + 100 ppm Al added as $AlCl_3$ at pH 4.0.

Apical 1.5 cm segments of secondary roots (Esau, 1965) were excised with fine tweezers prior to Al exposure,

and 7 and 14 days later. These were placed in glass vials containing Craf solution fixative for 24 h, washed in cold running water overnight, and dehydrated in a tertiary butyl alcohol and ethyl alcohol series. Following dehydration, the tissue was placed in toluene for 4 h before transferal to a 1:1 mixture of toluene and parafin oil for 4 h, and parafin oil for 2 h. The tissue was then infiltrated with Paraplast, a histological infiltration and embedding wax, at 62 ^{o}C for 24 h. The infiltrated root tips were then mounted in a block of paraplast and sectioned with a rotary microtome at 15 μ thickness.

Tissue sections were mounted on circular graphite stubs with Haupt's adhesive and set at 40 ^{o}C on a slide warmer over night. Parafin was removed by immersion in xylene prior to coating with nonconducting materials. Localization of Al, P, and Ca was determined by a Kevex Energy Dispersive X-ray Analyzer on a Cambridge scanning electron microscope with an accelerating voltage of 10 KEV. A 230 sec point scan was performed proximal to the meristem in the cortex and stele of root sections not exposed to Al (Plates 6 and 7). These counts were recorded as background for comparison with the same areas of treated sections.

Plate 6. Location of spot scans in cortex and stele of a cross section of a Betula papyrifera root tip from Buffalo Head Prairie (control).

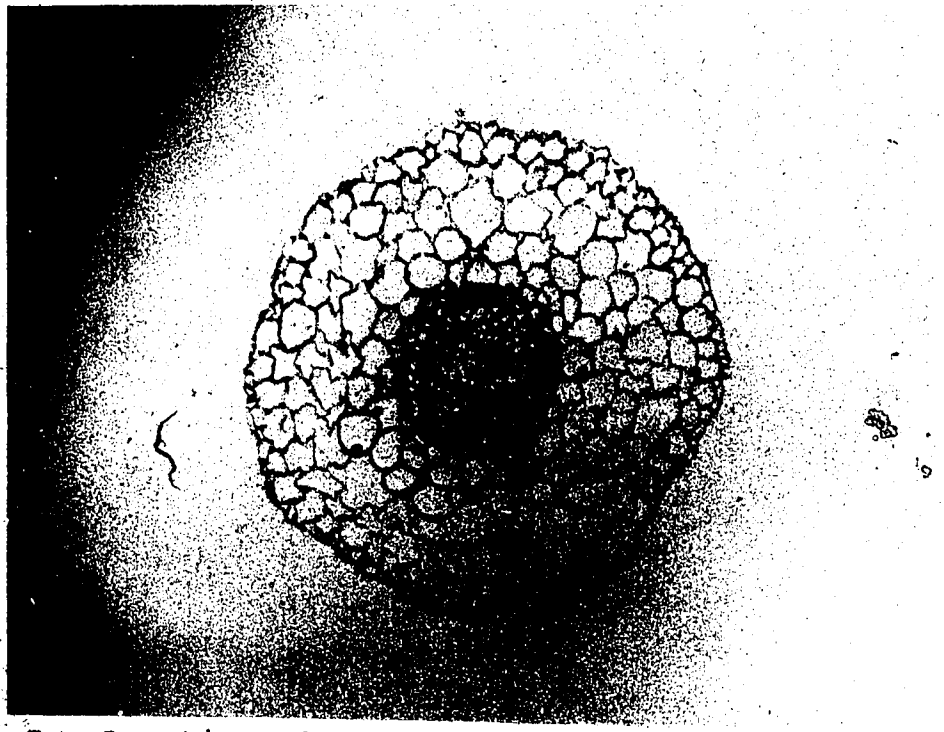


Plate 7. Location of spot scans in cortex and stele of a cross section of a Betula papyrifera root tip from Devon (control).

Results and Discussion

The areas for which point scans of Al determination by energy dispersive x-ray analysis were carried out are illustrated in Plates 8 and 9. In each case, Al was determined midway between the epidermis and the endodermis, and in the centre of the stele. Scans of controls thus involve root segments of untreated Betula papyrifera seedlings from Buffalo Head Prairie and Devon. These scans are superimposed on the elemental analyses presented in Plates 8 to 11 to indicate background levels of Al at these anatomical locations. Quantification not possible through this S.E.M. technique, however comparisons between control levels and those of Al-treated plants allow qualitative assessment of Al entry into the root at the depth of the cortex and stele.

After 14 days in 100 ppm Al, root segments of Buffalo Head Prairie seedlings revealed Al levels in both the cortex and the stele in excess of background levels in the same plant prior to Al treatment. Similar findings were made with the Devon population. Such increases in Al levels in the stele indicate that the root is not impervious to access by the ion and is thus predisposed to its dissemination throughout the plant. However the findings that excised roots of Devon plants adsorp more Al than do those of Betula papyrifera from Buffalo Head Prairie, and

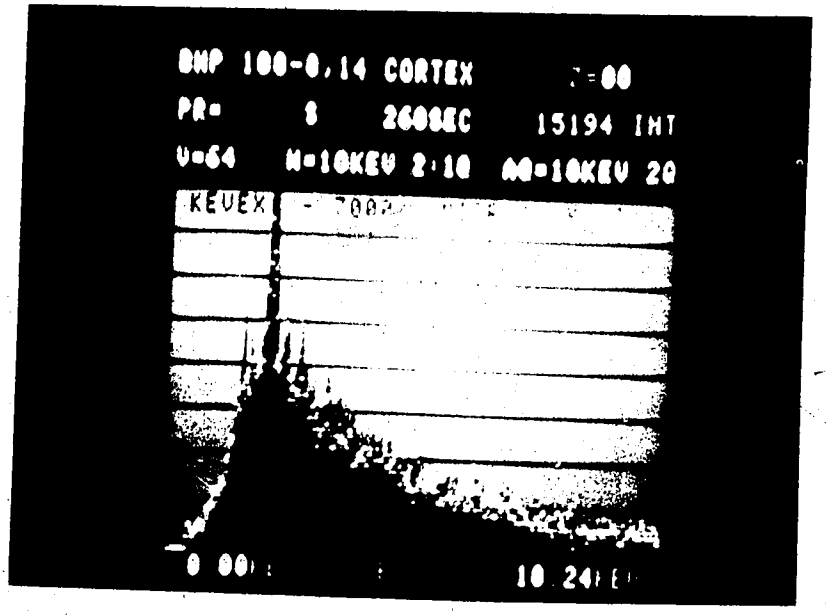


Plate 8. X-ray scan of cortex of a Buffalo Head Prairie birch root tip after 14 days in 100 ppm Al.

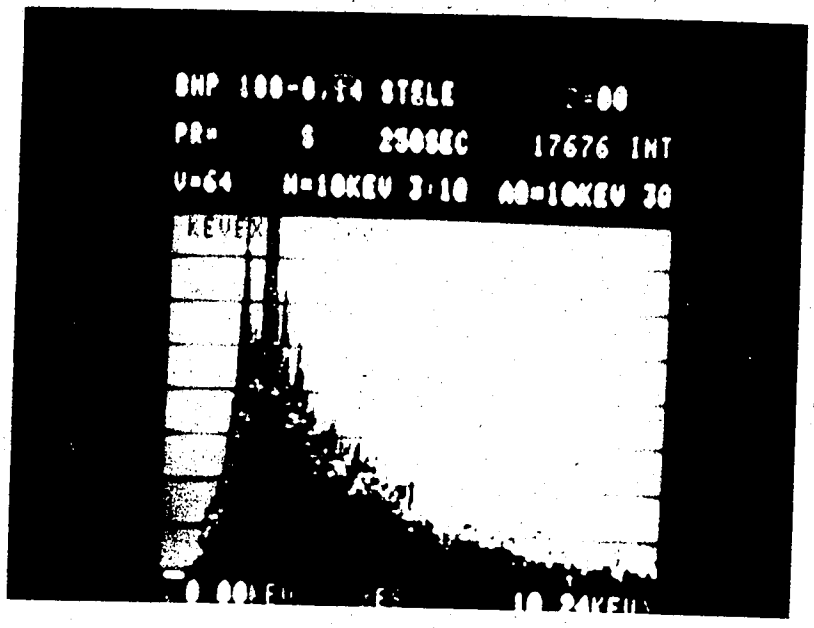


Plate 9. X-ray scan of

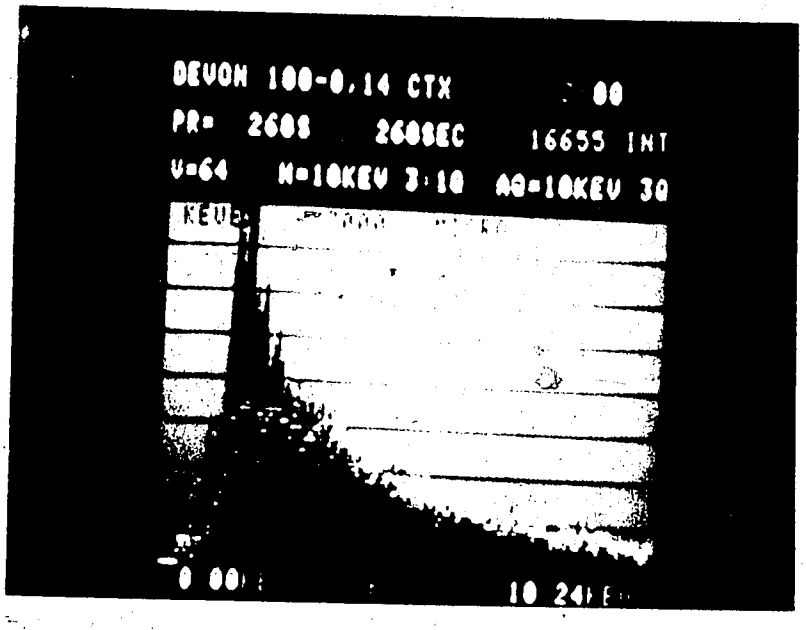


Plate 10. X-ray scan of cortex of Devon birch root tip after 14 days in 100 ppm Al.

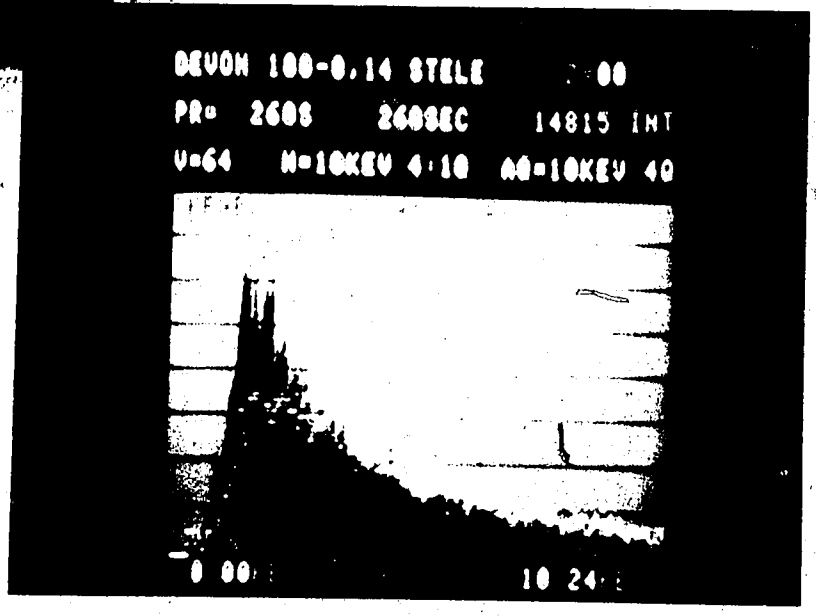


Plate 11. X-ray scan of stele of Devon birch root tip after 14 days in 100 ppm Al

that Devon plants accumulate significantly higher concentrations of Al in their leaves, stems, and catkins may still be important in the differential tolerance of these populations. The general Al tolerance mechanism of Betula papyrifera and more specifically the higher tolerance of the Buffalo Head Prairie population may have a biochemical basis and is not due to strict exclusion of the ion at the root.

McCormick and Borden (1972) examined phosphate fixation by Al in Populus sp. using a molybdenum blue staining technique and found a definite reaction in the root cap, epidermal, and cortical regions extending back from the root tip 1 to 5 mm. The Al-phosphate interaction was associated with the cell wall and plasmamembrane of epidermal and cortical cells. No reference was made to Al within the stele. In this study, Al peaks above baseline for control plants were always accompanied by high P levels suggesting an Al-phosphate interaction similar to that cited for other species (Wright, 1948; Wright and Donahue, 1953; Rasmussen, 1968; McCormick and Borden, 1972, 1974; and Naidoo, 1976; Hutchinson et al., 1986). Root Ca levels seem to be independent of Al treatments under these circumstances. In summary, these experiments illustrate that Al is not excluded from root vascular tissue of Betula papyrifera from either population over the time period and

with the Al concentrations used. While Betula papyrifera from a site of high plant-available Al may adsorb and accumulate less Al than plants of the same species from a low Al site, it is not capable of total exclusion of Al at the root.

CHAPTER VI

EFFECTS OF ALUMINUM ON ROOT GROWTH OF *Betula papyrifera*

Morphology

Effects of Al on root anatomy and morphology have been reported for crop plants (Rorison, 1958; Clymo, 1962; Hackett, 1967). Levan (1965) described severe cytological abnormalities in dividing cells of onion roots caused by inorganic Al salt solutions. Clarkson (1965) examined the abundance of mitotic figures in onion root apices via aceto-carmine squashes after Al treatments up to 10^{-3} M. Cessation of root elongation correlated closely with the disappearance of mitotic figures. No abnormalities in the mitotic cycle itself were observed, thus ruling out direct interference with the physical mechanism of the process such as spindle formation or chromatid separation. A direct effect on DNA replication at interphase was postulated. Sampson et al., (1965) found that Al-treated plants synthesize a metabolically unstable DNA of unusual base composition, and Clarkson and Sanderson (1970) put forth the suggestion that interference in DNA replication may take place by crosslinking of polymers increasing the rigidity of the DNA double helix. Al induced abnormalities were summarized by Clarkson (1965) as

1) reduction or inhibition of the growth of the main axis of the root, and

2) initiation of numerous lateral roots exhibiting reduced or inhibited subsequent growth.

Keser et al., (1975, 1977) extensively examined the influence of Al ions on the morphology of sugar beet roots and differential tolerance among cultivars. The number of laterals increased per unit of primary axis, the primary root axis curved, the root cap separated from the primary root apex, the protoplasm of the root cap cells disintegrated, and maturation of vascular tissue occurred closer to the apical zone. Al was incapable of entry through the epidermis unless mechanically broken by the emergence of lateral roots. Passage to the vascular tissue through the endodermis of newly forming laterals was thought to be possible, as Casparian strip formation lags behind endodermis development. In studies of copper, nickel, and aluminum sensitivity of White Birch and White Pine, Jones et al. (1986) described both increased Al tolerance with respect to mycorrhizal associations. Further discussion of Al entry into the root can be found in Chapter III.

Methods

Growth of plant material for study of root morphology was identical to that described in Chapter III. After 14

days in aerated half-strength Hoagland solution at pH 4.0, seedlings were transferred to similar nutrient solutions to which Al was added as $Al_2(SO_4)_3$ giving concentrations of 25 and 100 ppm Al. Solutions were maintained at pH 4.0 with H_2SO_4 and NaOH, and were changed at 7 day intervals.

Apical, intermediate, and proximal 1.5 cm root segments were excised with fine tweezers after 14 days for examination. Root tips of adventitious and secondary roots (Esau, 1965) were sampled before Al addition and 14 days after exposure. These were fixed in Craf solution for 24 h and washed in cold running water overnight. The material was dehydrated in an ethanol-t-butyl alcohol series and embedded in parafin for sectioning at 1 μ , followed by safarin and fast green staining and microscopic examination.

Results and Discussion

Morphology of newly formed roots was not affected in Betula papyrifera of either population after 14 days in 25 ppm Al (Plate 12). This concentration was chosen as an approximation of the plant-available Al of the Buffalo Head Prairie soil in the root zone (25.5 ppm). Resistance to morphological alteration in plants from the Devon site indicates a lack of ecotypic variation in this regard.

The 100 ppm Al treatment disrupted root growth in both populations (Plate 13) in the manner previously

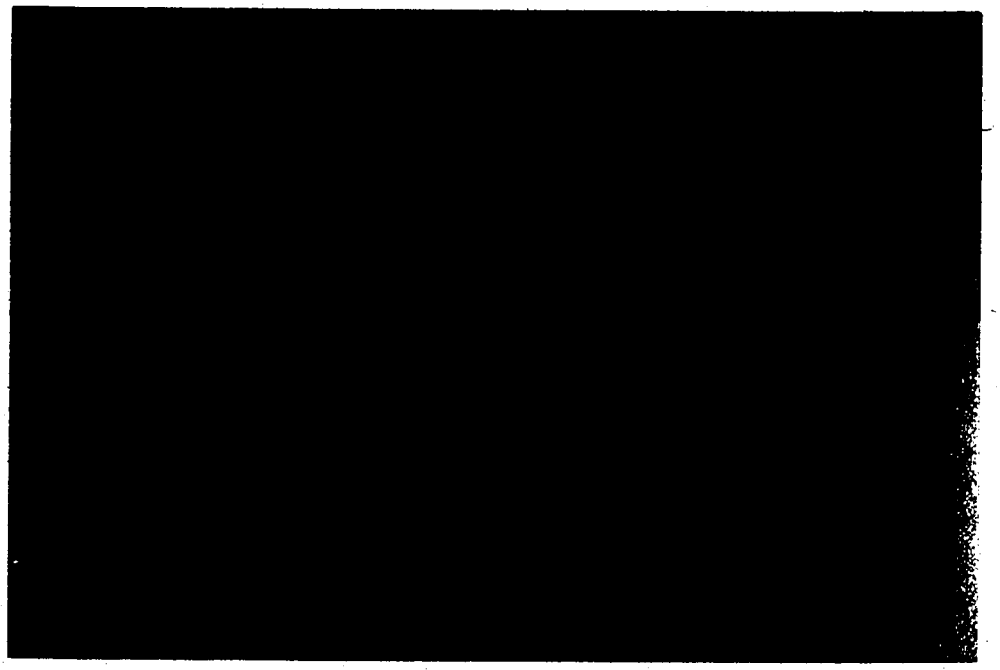


Plate 12. Root tips (apical 3 cm) of Betula papyrifera, from Buffalo Head Prairie and Devon populations after 14 days' growth in half-Hoagland's solution + 25 ppm Al.

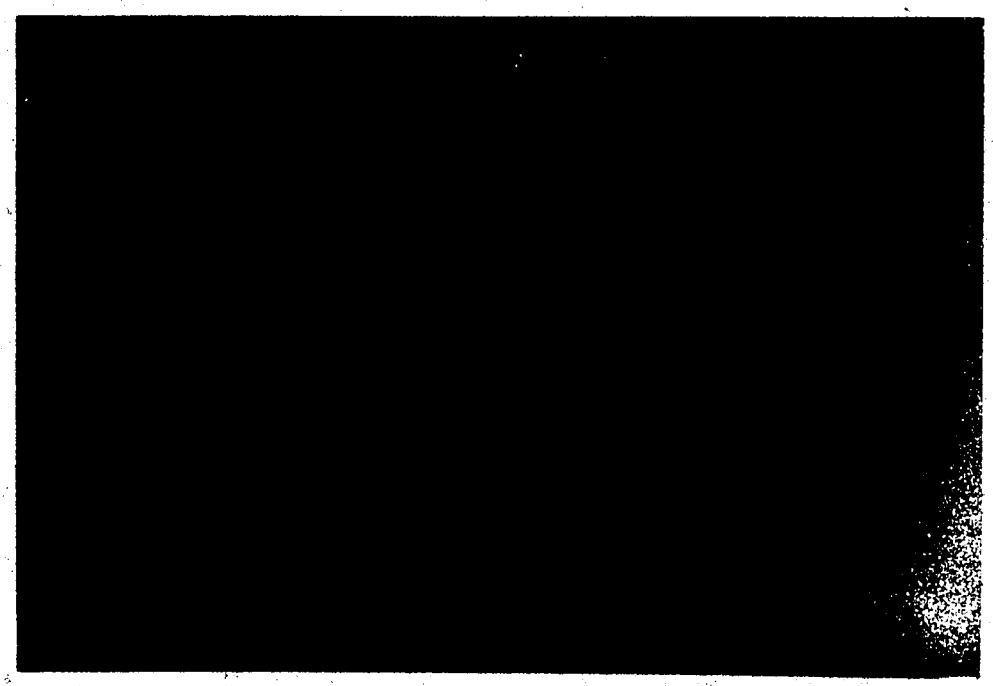


Plate 13. Root tips of Betula papyrifera after 14 days' growth in half-Hoagland's solution + 100 ppm Al.

described for herbaceous crop plants (Rorison, 1958; Clymo, 1962; Clarkson, 1965; Hackett 1962; Keser et al., 1975, 1977; Naidoo, 1976). Branching was induced in secondary roots within 1 mm of the tip. Adventitious roots did not exhibit branching, although root tips became blackened. Secondary roots browned and became brittle with increasing length of treatment. All roots were covered with a gelatinous sheath after 14 days in the 100 ppm treatment. Microscopic examination revealed disintegration of the root cap and disruption of the meristem. New laterals ceased apical growth prematurely and commonly produced laterals themselves. After 14 days in 100 ppm Al, new laterals exhibited a bulbous shape at the base, and mechanical tearing of the epidermis and cortex of the secondary root. The only other report of morphological change induced in woody plant roots by Al is that of Steiner et al., (1980) who observed suppression of fine lateral roots, browning of tips, and the presence of a gelatinous-like sheath, however the Al concentrations eliciting change were not stated.

In summary, normal root morphology in Betula papyrifera is resistant to Al intervention at levels higher than those damaging most crop plants (Keser et al., 1975). Both populations showed no altered morphology at the Al concentration of the high extractable Al site. At approximately 4 times this concentration, neither population was resistant to damage. This high threshold of

Betula papyrifera may be due to its ability to exclude the ion from nuclear sites within the meristematic region.

Root Elongation

Differential tolerance of plant species and varieties to Al has been widely cited, and Fleming and Foy (1968) postulated that differential tolerance among wheat varieties was due to resistance of root tips to morphological damage and the subsequent persistence of root growth, a theory later supported by the findings of Keser et al., (1977) in an anatomical study of sugarbeet.

A common method for quantification of Al tolerance is measurement of root elongation. Most studies have been directed toward crop plants (Foy et al., 1965, 1967) with recent emphasis being placed on screening potential.

Attention focused on root growth of native plants in work by Clarkson (1966a) on Agrostis spp. Poor growth of roots of susceptible species in critical levels of Al was attributed to inhibition of cell division in root apices. McCormick and Steiner (1978) conducted research on differential Al tolerance in eleven species of woody plants, including Betula papyrifera. Utilizing one root per plant, root elongation was determined with 12 replicates to develop a tolerance index defined as root elongation in calcium nitrate + Al / root elongation in calcium nitrate.

Hybrid poplars were found to be sensitive to 10 ppm Al, while Quercus palustris, Q. rubra, Pinus virginiana, P. rigida, P. sylvestris, Betula alleghaniensis, B. populifolia, and B. papyrifera were relatively tolerant of concentrations of 80 to 120 ppm Al. It was noted that some tree species are tolerant of Al concentrations many times higher than those resisted by most agronomic species. That the minimal Al concentration at which root growth is significantly reduced varies among species is further illustrated by the work of Schier (1985) and Thornston et al. (1986). Total dry matter production of White Pine was enhanced by Al concentrations of 5 - 20 mg/l at pH 3.8, and Jack Pine showed no decline over the same range compared to controls (Hutchinson, 1986). In contrast, spruce species exhibited higher sensitivities. Significant decline in total dry matter production was evident in White and Red Spruce at 5 mg Al/l and in Black Spruce at 10 mg Al/l.

Further to root elongation and dry matter production, Thornston et al. (1986) found that shoot growth of Sugar Maple was enhanced by aluminum concentrations of 100 and 500 mg/l at pH 4.0. Schier (1985) reported no reduction in shoot growth of Red Spruce and Balsam Fir seedlings at Al concentrations up to 200 mg/l at pH 3.8.

Investigations into intraspecific differences in Al tolerance were carried out by Steiner et al., (1980) for 13 Betula papyrifera provenances to determine if genetic

variation in Al tolerance exists within paper birch. Over a 14 day period in calcium nitrate + Al, root elongation was reduced from a mean of 9.7 to 2.2 cm. Root growth of even the most tolerant provenance was reduced to nearly two-thirds in the presence of 120 ppm Al. It was concluded that Al tolerance in paper birch varies among provenances and that those from the central and eastern portions of the range of species are most Al tolerant.

Methods

Growth of plant material for root elongation experiments was identical to that described in Chapter III. Betula papyrifera seedlings from each population remained in half-strength Hoagland solution, maintained at pH 4.0 with H_2SO_4 and NaOH, for 14 days. Eight seedlings were randomly transferred to each of 4 treatments to which had been added 0, 25, 100, or 150 ppm Al as $AlCl_3$ and $Al_2(SO_4)_3$. Three healthy, newly formed lateral roots approximately 1 cm in length were tagged with thread on each seedling and measured to the nearest mm. The position of the pots within the growth chamber was randomized at the beginning of each week to nullify environmental variation. Solutions were changed at two day intervals. Root growth was recorded for the interval of 0 - 14 and 14 - 28 days post-exposure to Al treatment.

Results and Discussion

Root elongation of Betula papyrifera seedlings subjected to elevated Al concentrations is plotted on the three-dimensional graphs of Figure 13. In each case variation about the mean was small, with SEM $\leq$ 0.7. Controls exhibited vigorous growth in the half-strength Hoagland nutrient solution with a mean value of 6.1 $\pm$ 0.3 cm for both sites over both 14 - day periods. This value is lower than that of 9.7 cm over the same period cited by Steiner et al. (1980). The difference could be explained by the use of calcium nitrate as a nutrient solution, age of seedlings, pH of the medium, light conditions, or anatomical type of root measured, whether primary or secondary. Steiner trimmed roots of seedlings to a uniform length after transplanting to solution culture. This study monitored growth in secondary roots as classified by Esau (1965). Statistical analysis of the data is summarized in Table 13. There was no significant difference in the length of root growth in the control treatment when comparing populations at 0 - 14 or 14 - 28 day intervals, nor was there any significant difference in growth between the two time periods within a single population.

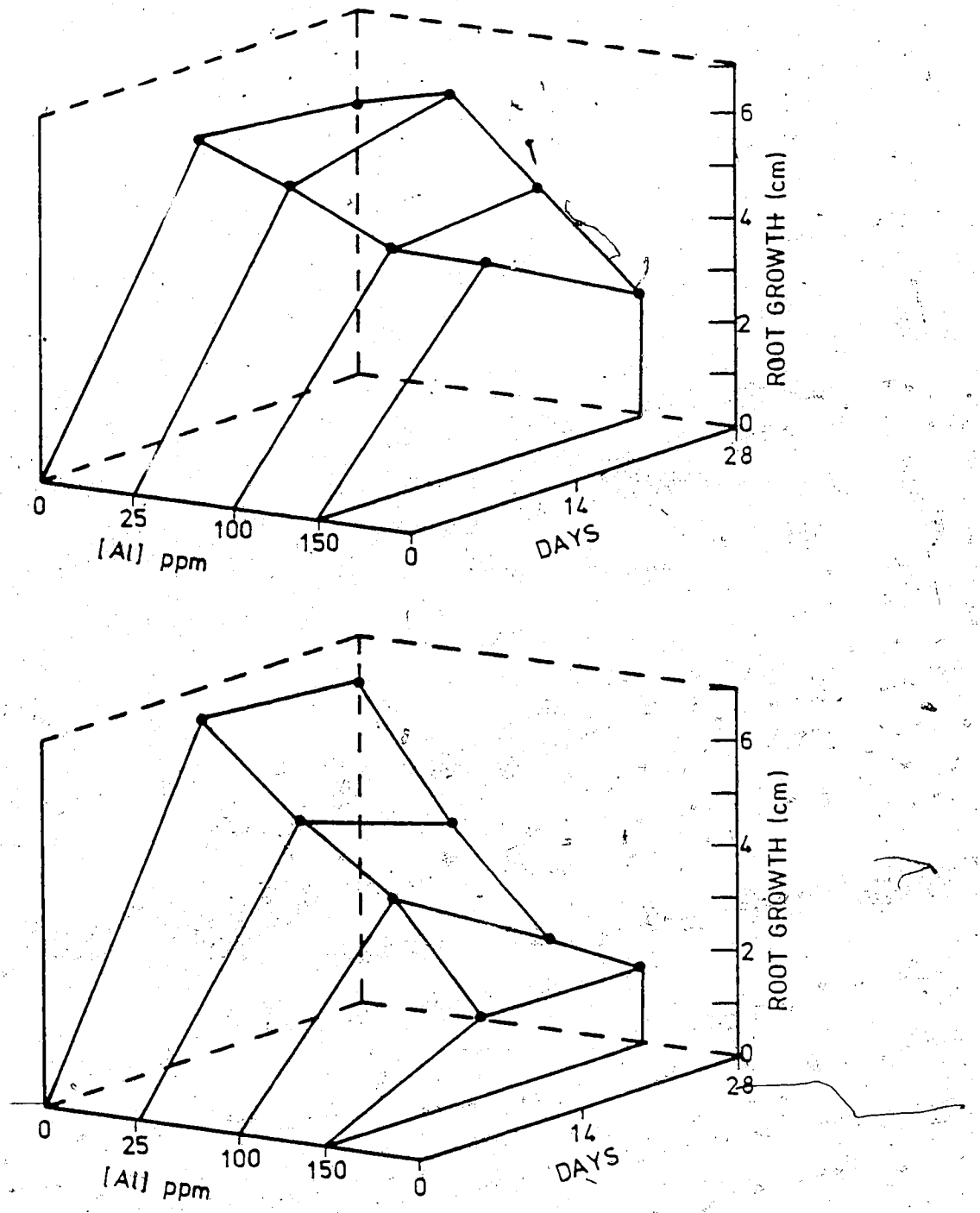


Figure 13. Root growth of Betula papyrifera during 0 - 14 and 14 - 28 day intervals after exposure to elevated Al in solution cultur (Means of 8 replicates).

Figure 13 illustrates an obvious differential response of root growth to elevated Al levels in seedlings from the Buffalo Head Prairie study site and the Devon reference site at high concentrations. The difference becomes apparent at 100 ppm Al which approximates the critical level (Table 13B) at which plants from the site in high plant-available Al supercede plants from the low Al site with respect to tolerance. This Al concentration has a similar effect on plants from both populations during the first 2 - week growth period, yet the Devon plants showed a significant reduction in root elongation in the second growth period over that of the Buffalo Head Prairie population. This would suggest cummulative damage to cell mitosis, or a threshold response over time. Conversely, as Al concentration is elevated to approximately six times that of the $CaCl_2$-extractable Al at the Buffalo Head Prairie site, plants from this population exhibit significant differential tolerance over Devon plants in the first growth period, succumbing to toxic effects after prolonged exposure, so that growth is not significantly different from that of which Devon plants are capable (Table 13A). Comparing root elongation between 2 - week growth periods within a population, it becomes apparent that growth under constant Al stress does not vary significantly over the two growth intervals. Devon plants

Table 13. Levels of significance (1) of Betula papyrifera root elongation over two, 2 - week periods after exposure to elevated Al concentrations in solution culture. Name in parentheses indicates higher value.

Treatment (Al concentration in ppm)

	0	25	100	150
A. Period 1				
	Devon			
BHP	NS	NS	NS	* * * (BHP)
B. Period 2				
	Devon			
BHP	NS	NS	* * * (BHP)	NS
C. BHP				
	Period 2			
Period 1	NS	NS	NS	NS
D. Devon				
	Period 2			
Period 1	NS	NS	* (Perio	NS

(1) See Table 10

under the 100 ppm regime are an exception in that the toxic effects of Al were greater in the first 14 days (Table 13D) such that a mean growth increment of 3.6 ± 0.6 cm was reduced to 1.7 ± .02 cm.

This again points to a progressive reduction in cell division over time, however one might anticipate a similar outcome within the 150 ppm Al treatment. Mean root growth of 2.3 ± 1.1 for both populations in the 150 ppm treatment is in agreement with the value of 2.2 cm for the same period in 120 ppm Al cited by Steiner et al., (1980).

In answer to the question of differential tolerance between Betula papyrifera plants from sites of high and low plant - available Al with respect to root elongation, the data presented here show a significant difference. However, consideration of differential tolerance and the use of tolerance indices must not lose sight of cortical concentrations necessary to give the tolerant population a physiological advantage. In this study, Al levels consistent with those of the high extractable Al site did not elicit a differential response in the two populations, although such was the case at yet higher concentrations. It should also be recognized that other strategies might intervene to determine Al tolerance when root elongation is slowed by interference with root meristem activity. With these points in mind, the study shows a less marked response of Betula papyrifera from the site of high

plant-available Al, and high Al concentrations. This could be attributed to the lower affinity of Buffalo Head Prairie birch roots for Al (Chapter III). Elongation even at 150 ppm Al supports the finding that paper birch is capable of growth at concentrations completely toxic to other plants (Steiner et al., 1980). Reduced growth at very high Al concentrations is consistent with morphological damage at the root apex described earlier in this chapter.

Frequency of Mitotic Figures

Cytological effects of Al are important in consideration of root morphology and elongation. These were first reported by Levan (1945) in a study of involvement of a number of inorganic salt solutions in disruption of dividing cells of onion roots (Allium cepa). These include "sticky chromosomes" and anaphase bridges. Clarkson (1965) cited complete inhibition of onion root elongation after 6 - 8 hours' treatment with 5.4 to 54 ppm Al applied as $Al_2(SO_4)_3$. Examination of aceto-carmine squashes of root apices showed that cessation of root elongation and disappearance of mitotic figures were closely correlated. Treatment with other trivalent metals such as gallium, indium, and lanthanum produced similar results. Clarkson concluded that cell division is highly sensitive to Al and that permanent damage to the mechanism can result from

short exposures.

In work with Agrostis spp. (Clarkson, 1966a), poor growth of roots of susceptible species in critical levels of Al was attributed to inhibition of cell division in the root apices. Absence of abnormalities in the mitotic apparatus was suggestive of interference with a mechanism operating during interphase. Growth of cotton seedling roots was observed to stop in the presence of Al concentrations greater than 0.5 ppm by Rios and Pearson (1964). Growth was not reinstated when the roots were placed in Al-free solutions. The appearance of binucleate cells in the root tip meristem indicated inhibition of root cell division. Exposing cotton roots to 1 ppm Al (pH 4.3) for 12 h also produced a high frequency of binucleate cells (Huck, 1972). At the biochemical level, Eichhorn (1962) showed that metal cations have the ability to bind to DNA in vitro, thus increasing the stability of the double helix. Clarkson and Sanderson (1970) further suggested that Al could crosslink polymers, thus increasing the rigidity of the DNA double helix. This could explain the observed interference in DNA replication induced by Al (Clarkson, 1969).

Methods

Estimation of the frequency of mitotic figures under various Al regimes was incorporated into the root

elongation experiment presented in the previous section, in which 8 Betula papyrifera seedlings from both the Buffalo Head Prairie and Devon study sites were grown in half-strength Hoagland solution to which 0, 25, 100, or 150 ppm Al was added as $AlCl_3$ and $Al_2(SO_4)_3$. Several secondary root tips were collected from each seedling in the 0 to 100 ppm Al treatments into vials of distilled water at times 0, 2, 4, 8, 12, and 24 h after exposure to Al treatments. Aceto-carmine squashes were immediately prepared following the technique of Clarkson (1965). Coverslips were sealed with nail polish to retard dehydration.

The area of the smear containing the major portion of the meristematic cells (within 1.5 mm of the root tip) was determined microscopically. Following the procedure outlined by Clarkson (1965), the abundance of mitotic figures at each stage of the mitotic cycle was determined by randomly selecting coordinates for 5 high power fields (400x) from meristematic regions of 4 replicate squashes.

Results and Discussion

The frequency of mitotic figures observed in aceto-carmine squashes under various Al treatments is presented in Table 14. No reductions are apparent before 24 h in the 100 ppm Al treatment. There is no difference in the response of Betula papyrifera from the two study sites, a finding which is in agreement with the observation that

root morphology was similarly affected in the two populations, and that no damage was apparent in the 25 ppm Al treatment.

The results serve as another indication of Al tolerance when compared with the study of Clarkson (1965) in which Allium cepa exhibited complete absence of all division after 7 hours of subjection to 54 ppm Al and that of Clarkson (1966a) in which mitotic figures in Agrostis stolonifera were absent after growth for 76 h in 0.5 mM Al at pH 4.0. As reported in these studies, no distortion of mitotic figures was observed.

Table 14. Frequency of mitotic figures in aceto-carmine squashes of Betula papyrifera root tips. Values refer to figures observed in 5 fields from 4 replicate squashes.

Hours After Al Treatment	Buffalo Head Prairie																							
	Control				25 ppm				100 ppm				Control				25 ppm				100 ppm			
	P	M	A	T	P	M	A	T	P	M	A	T	P	M	A	T	P	M	A	T	P	M	A	T
0	2.6	4.4	3.4	2.2	3.0	2.0	3.0	1.6	3.8	3.2	2.0	1.8	2.0	5.4	3.0	1.2	3.0	6.8	2.4	1.0	5.2	2.6	2.8	2.6
2	3.0	4.4	2.8	1.8	3.0	2.4	1.8	1.2	4.4	3.6	2.4	1.8	1.8	4.8	3.6	1.8	4.2	2.8	1.8	0.8	1.8	3.8	1.6	2.2
4	3.4	3.0	1.8	1.0	3.2	2.6	1.4	1.4	3.0	3.4	2.4	2.2	2.0	3.2	2.4	1.6	3.0	3.4	1.8	1.4	1.6	1.0	0	0
8	1.8	2.4	2.2	1.6	2.8	3.0	1.6	1.4	1.6	2.4	1.2	1.2	0.6	2.0	2.0	1.6	2.6	3.4	1.8	1.4	1.6	1.0	0	0
12	1.8	2.2	2.0	1.6	2.8	3.0	1.4	0.6	3.0	1.8	1.4	2.0	1.6	2.6	2.6	1.4	2.6	3.6	1.8	0.4	2.2	2.4	0.2	1.0
24	2.8	2.4	3.0	1.8	1.4	0.8	1.0	0.8	0.2	0.2	0	0.2	1.4	2.6	2.6	1.2	1.0	2.4	1.0	0.8	0.2	0.2	0	0

P = prophase
M = metaphase
A = anaphase
T = telophase

Chapter VII

INTEGRATION

It seems obvious that insight into Al tolerance might be gained through inquiry into the strategy of native plants thriving on virgin land characterized by low soil pH. The Betula / Calamagrostis association described here offers at least some answers to the question of native plant strategy under Al stress. The plant community established on the Buffalo Head Prairie study site was found to be quite species rich and diverse when edaphic factors including high soluble Al and poor drainage are considered. This draws attention to other species such as Calamagrostis canadensis and Rubus acaulis as fruitful subjects for physiological study. Most important, however, is the strong dominance expressed by Betula papyrifera, illustrating Al tolerance and the ability to compete successfully with other species on this site.

With the dominant species defined, the second objective of the study, that of assessment of Al tolerance limits and investigation of intraspecific differential tolerance was addressed. For an overview, it may be helpful to consult Figure 1. Tolerance limits of Betula papyrifera were found to vary with the parameter assessed and the population to which the plants belonged. Root elongation

persisted in the presence of Al concentrations six times the level of plant-available Al at the Buffalo Head Prairie site, although growth decreased with time and Al concentration. Differential tolerance was evident in high Al treatments, the Buffalo Head Prairie population being more Al tolerant. Morphologically, growth in solutions containing Al in concentration approximating that of the root zone at Buffalo Head Prairie (25 ppm) elicited no cytotoxic effects after 14 days. However, 14 days' growth in 100 ppm Al treatment disrupted root morphology in both populations, inferring no differential response in this respect. Finally, estimation of the abundance of mitotic figures illustrated decreased, yet persistent, cell division in root meristems after 24 h in plants from both populations exposed to 100 ppm Al.

To summarize the integration of findings to this point, it can be said that Betula papyrifera exhibits tolerance to Al concentrations approximating those occurring in the soil at the acidic Buffalo Head Prairie site. Complete interference with physiological processes is not evident at concentrations far exceeding those to which native Betula papyrifera is normally subjected. Differential tolerance is apparent between populations native to sites of high versus low plant-available Al, although this is dependent upon the parameter being assessed. Betula papyrifera is tolerant of Al

concentrations many times in excess of those toxic to most crop plants.

The final objective of the study, to investigate the tolerance mechanism of Betula papyrifera and the extent to which it is expressed in populations originating from sites of high versus low plant-available Al was first addressed in regard to Al transport within the plant. Analysis of leaves, stems and catkins revealed foliar Al concentrations to be greatest, suggesting uptake and mobilization of the element. The fact that diameter class is a variable in the Al content of stems indicates its accumulation over time.

To summarize, the tolerance mechanism of Betula papyrifera is not one of complete avoidance, nor one of Al accumulation and sequestration. This is supported by experiments with energy dispersive analysis of x-rays, showing Al levels within the stele of exposed roots exceeding levels in controls. Al adsorption experiments with excised roots demonstrated that Al adsorbed to roots was higher in plants from the low Al site, while the population originating on a high Al site adsorbed amounts similar to those adsorbed by barley roots. While cation exchange capacity was found to correlate poorly with Al binding capacity in Betula papyrifera, weak binding and exclusion of Al from non-free space could help explain Al tolerance in native paper birch, although x-ray scans and other parameters discussed above discredit exclusion.

In conclusion, a plant community, dominated by Betula papyrifera Marsh., established on a site of potentially toxic Al concentrations, has been described. Paper birch is tolerant of high levels of soluble Al through a tolerance mechanism which operates in the presence of Al uptake. It can be postulated that the answer to the Al tolerance mechanism of this native plant species lies at the biochemical level. Study of such native populations preconditioned to high plant-available Al may find practical application in revegetation of acidic soils.

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