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Title: INFLUENCE OF CATIONS ON ALUMINUM TOXICITY IN WHEAT (Triticum aestivum Vill., Host)

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The toxicity of aluminum (Al) to wheat (Triticum aestivum Vill., Host) was studied under controlled conditions using a technique designed to evaluate the recovery of root growth following a relatively brief (48 hour) exposure to nutrient solutions containing Al. When wheat seedlings were exposed to a minimum critical concentration of Al, the root primary meristem was irreversibly damaged and did not reinitiate growth when transferred into an Al-free nutrient solution. This method was quite precise and reproducible when temperature, pH, nutrient concentration and Al concentration of the solutions were rigidly controlled. The severity of toxicity was sharply increased by decreasing the concentrations of nutrients in the Al treatment solutions. Four wheat varieties of widely differing tolerance to Al all behaved similarly in this respect suggesting that tolerance is a relative rather than

an absolute varietal characteristic. The critical Al concentrations for four classes of tolerance were determined and the conditions for separating these classes by a rapid, precise, and convenient screening procedure was developed.

The inhibition of root growth by Al could be completely overcome in all four varieties by increasing the Ca, Mg, K, or Na concentration in the Al treatment solutions. These findings indicate that the effect of cations on reducing Al toxicity was nonspecific and conclusively showed that Al toxicity was not due to deficiencies of Ca, Mg, K or P as has been extensively suggested in the literature.

Influence of Cations on Aluminum Toxicity in Wheat (*Triticum aestivum* Vill., Host)

by

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DEDICATED

TO

MY FATHER

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INTRODUCTION

Soil acidity has been seriously considered by scientists for many years because of its dominant role in crop production. Today it is recognized that soil acidity is due to both hydrogen ion (H^+) and aluminum ion (Al^{+++}) and that the latter plays a major role in acid soil infertility. Because of the marked physiological effect of Al on growth of plants in general, and roots in particular, numerous concepts have been advanced to explain the observed pattern and behavior of plants growing in presence of Al. To this end, considerable research has been devoted to and Al toxicity has been variously ascribed to P, Ca, Mg, or K deficiency. These widely differing conclusions, however, are attributable to the fact that Al toxicity is profoundly influenced by genotypical as well as environmental factors and most of the earlier work was done either in soil or in solution culture without adequate control. Again, various methods and techniques have been used to evaluate the effect of Al on plants, though roots, being in immediate contact, are first to show Al toxicity symptoms. The primary effect which manifests itself in terms of stunting and thickening of the root with ultimate cessation of growth could account for all the above mentioned effects.

It was therefore, decided to further explore Al toxicity of wheat (Triticum aestivum Vill., Host) using an improved experimental technique which provides an adequate control for all the factors (pH, temperature, concentrations of nutrients, day length and light) involved and which directly measures the effect of Al and its interaction with Ca, Mg, K etc. in terms of root growth.

REVIEW OF LITERATURE

Al x P interaction

Because of the very high tendency of Al to chemically react with P under almost any conditions (in soils, in solutions, or in plants) and the consequent decrease in Al toxicity or the appearance of P deficiency, the Al x P interaction has resulted in rather extensive investigations but the conclusions are as diverse as the experimental procedures.

Availability of biologically suitable radio-isotopes of phosphorus (^{32}P) has greatly facilitated the approach to many questions which otherwise would not have been feasible. Wright and Donahue (1953) using this ^{32}P technique showed that there was less ^{32}P translocation from roots to shoots in barley plants when grown in nutrient culture in the presence of Al as compared to plants grown in the absence of it. They also found that Al inactivated P primarily within or on the roots of the plants interfering with the normal P metabolism of the plants.

^{32}P has also been used to help explain the cause of appearance of P deficiency symptoms associated with Al toxicity. Randal and Vose (1963) studied the effect of Al on uptake and translocation of P in perennial ryegrass. They reported an increase in total content as well as in concentration of P in roots at low Al levels (5 ppm)

and a decrease at high Al levels (50 ppm). This led them to conclude that Al induced P uptake was largely metabolic and P might be bound to Al after uptake causing the symptoms of P deficiency characteristic to Al toxicity. This view was further supported through the work of Medappa and Dana (1968). Using ^{32}P they observed an Al-P precipitate both inside and outside the root tissue at elevated pH (6.5). An opposite conclusion by Cruz et al. (1967) is also reported. Using ^{32}P but with a divided root technique they observed no effect of Al on migration of P into the leaves of either a sensitive or a tolerant wheat variety.

Use of nonradioactive P has also revealed many diverse effects. Ragland and Coleman (1962) reported an increase of several fold in uptake of P when excised snapbean roots were pretreated with Al. The increase of P uptake was greater when both Al and P were present in the same solution. But following almost the same procedure of pretreating barley roots with Al but using ^{32}P, Clarkson (1966b)contradicted the view of enhanced P uptake due to Al or that of the reaction between Al and PO_4 interfering with P transport. He rather suggested two types of interactions between Al and P: the first occurred at the cell surface, and resulted in fixation of PO_4 by an adsorption-precipitation reaction; the second one occurred within the cell, possibly within the mitochondria, and resulted in a marked decrease in the rate of sugar phosphorylation probably

affected by the inhibition of hexokinase. Through electron microprobe X-ray analysis, Rasmussen (1968) studied the mode of entry, distribution and localization in corn plants and found Al precipitated on the surface of the epidermal cells of the root with no penetration into the cortex as long as the roof surface remained intact. He further reported that the root cap was freely permeable, contained the highest concentration of Al and the epidermal layer behind the root cap prevented movement into the cortex and conductive tissue. Accordingly the penetration of the lateral root through the endodermis, cortex and epidermis provided a channel of entry for Al into the cortex and conducting tissues of both the lateral and the main root. He found essentially no Al in the transition zone and only small quantities in the above ground parts. From the fact that the localization of P was exactly the same as that of Al, he suggested that there was a precipitation of P by Al. As a check he ran a similar analysis for Ca and P on control plants where no such phenomenon was observed giving further support to his conclusions. However, an exactly opposite conclusion was drawn by Waisel et al. (1970) when during a study on the localization of Al in cortical cells of beans and barley roots by X-ray microanalysis they found no correlation between the distribution of Al and P and concluded that aluminum phosphates were not formed in or on the root. This conclusion is also a contrast to the assumption that Al is mostly

precipitated in the free space as Al-PO_4 (Rorison, 1964; Clarkson, (1966b). The factor that influenced the result of Waisel et al. (1970) is probably pH. While these workers used an alkaline pH (9.5), the others used an acidic pH, and the forms of Al in solution are pH dependent (cationic Al in acid pH range, $Al(OH)_3$ near neutrality and anionic Al in basic pH range). The differences in the results of the two groups using different pH can be reconciled if the existing cationic and anionic forms of Al due to pH are taken into consideration. This emphasizes the need of a very strict pH control in the study of Al x P interaction as well as Al toxicity in plants but unfortunately most of the reported experiments have failed to take this point into consideration.

Al x Cations Interactions

If there are differences in opinion as to the relationship of Al x P and the influence of one on another, that is because it has received some attention for study. The case with Al x cations is quite different. However, a search of literature almost invariably points out a trend of antagonistic effect of Al on other cations and vice versa. Since there is no reported systematic Al x cations interaction study and most observations were made along with a main study, the available literature is concerned primarily with the elements mostly studied for some other purpose.

Most of the observations regarding Al x Ca stress the decrease in Ca uptake due to Al (Horstein, 1960; Johnson and Jackson, 1964; Martin, 1965; Armiger et al., 1968). Takahashi (1963) attributed the poor growth of plants on acid, volcanic-ash soil as due to the inhibition of Ca uptake by Al. He remarked that exchangeable and not water soluble Al was responsible for this and that competition between Al and Ca on the surface of the crop roots was very likely. A decrease in growth due to Ca deficiency caused by Al has been also suggested by Armiger et al. (1968). While studying the differential tolerance of soybean varieties on an acid soil high in exchangeable Al, they suggested that the inhibition of the growth of the plants was mainly due to Al induced Ca deficiency and not due to a simple Ca deficiency. Foy et al. (1972) related the varietal tolerance of Al to the capacity of a variety to take up Ca when they found that two differentially tolerant snapbeans grown in the same nutrient solutions took up different amounts of Ca and the sensitive one was much lower in that respect. The relationship of Ca to Al injury was postulated even much earlier. Oullette and Dessureaux (1958) postulated that one of the effects of Ca was in lowering the uptake of Al by plants. A greater requirement of Ca in the presence of Al to maintain the growth of plant could be due to Ca decreasing the uptake of Al and thus maintaining the growth. Dios Vidal and Broyer (1962), Lund (1970), Clarkson

and Sanderson (1971) all found that more Ca was needed to maintain the normal growth of plants in presence of Al. The latter studied the inhibition of uptake and long distance transport of Ca by Al and other polyvalent cations in barley. From their experiments, they concluded that Al^{+++}, Sc^{+++}, and Fe^{+++} inhibit the uptake of Ca by barley plants from acid culture solutions. They reported that the inhibition caused by 25 $\mu M Al_2(SO_4)_3$ could be partially overcome if the $CaCl_2$ concentration in the medium was increased although the inhibitory effect of Al was still present. Further studies showed that the polyvalent cations reduced the amount of Ca held in the water free space (WFS) and the Donnan free space (DFS) and that the Al treated roots transported much less Ca to the shoot.

However, the antagonistic effect of Al and Ca is not as universal as might seem from the above citations. Cruz et al. (1967b) grew Al susceptible wheat in nutrient solutions by a divided root method and reported an increase in uptake of Al in leaves, stalks and roots. The significant observation however was that along with the Al content, the Ca content of the plant also increased.

Like the Ca x Al interaction, a Mg x Al interaction has also been reported (Dios Vidal and Broyer, 1962; Peive and Rinkins, 1962; MacLeod and Jackson, 1967; Kerridge, 1969; Lee, 1971). Peive and Rinkins (1962) reported the results of their study on the effect of Ca, Fe and Al on the uptake of trace elements. They

found that in neutral soils or in slightly alkaline or acid soils Al was slightly antagonistic to Zn, Mg, Mn and Mo and was strongly antagonistic to Fe, P and Ca. Kerridge (1969) confirmed a significant effect of H^+ ion concentration on the uptake of Ca, Mg, Mn and on the onset of Al inhibition of root development of wheat. He found a marked inhibition in Mg uptake and to a lesser extent that of Ca and Mn by Al. His very significant observation was that at constant pH and in the absence of Al inhibition of root elongation, the inhibitory effect of Al on nutrient uptake was generally of less magnitude than that due to an equivalent change in H^+ concentration. Accordingly, all nutrient changes were of secondary importance compared to the Al inhibition of root development.

Lee (1971) studied the Al inhibition of Ca, Mg, K, and Zn uptake by potato plants and concluded that Al tolerance of potato varieties might be related to the ability of the plant roots to absorb Mg and K.

The role of K in Al toxicity was emphasized a decade back by Rees and Sidrak (1961) who concluded that the toxicity of Al was mainly due to its effect on K/Ca balance of plants. In a series of studies with different crop plants (wheat, lettuce, turnip and radish) Aimi and Murakami (1964) observed that the inhibitory effect of a high concentration of Al on root growth could be decreased by adding K but not by adding Ca. Gangwar (1967), on the other hand,

conducting a rather elaborate study on Al absorption by crop plants (pineapple, sugarcane, corn and clover) as influenced by Ca and K, reported that the amounts of cations absorbed increased with their increasing concentrations and that the effects of one cation upon the adsorption of another cation was reciprocal. MacLeod and Jackson (1967), Lance (1968), Lee (1971, 1972), all confirmed an Al x K interaction in nutrient cultures as well as in soils.

In addition, interactions of Al with Zn, Mn, and Mo (Peive and Rinkins, 1962), Cu (Hiatt, Amos and Massy, 1963), Mn, Fe, and Zn (Paterson, 1965), Ca, Mg, K, P and NO_3 (Lance, 1968) and Ca, Mg, K and Zn (Lee, 1971) have also been reported.

In almost all of the above studies, no attempt was made to control the pH of the root medium and consequently it is not possible to separate the effects of Al from the effects of H^+. Unless the pH is purposely controlled, an increase in the Al concentration in acid solutions is always accompanied by an increase in H^+ concentration. Nutrient uptake is markedly reduced by H^+ in the pH range below 5 (Moore, 1973) and it is precisely in this pH range where Al toxicity is most evident. Consequently, reduced nutrient uptake usually ascribed to Al may be, in part, due to reduction in uptake caused by H^+. In addition, since Al severely inhibits root growth (see later section) reduced uptake of nutrients might be due to reduced root growth rather than to any direct effect of Al on

nutrient uptake.

Al x Species x Varieties

Striking differences of plant species in tolerance to Al have been recognized for many years (Hartwell and Pember, 1918; MacLean and Gilbert, 1927; Hewitt, 1948; Jones, 1961; Aimi and Murakami, 1964; Foy and Brown, 1965b; Clarkson, 1966a; Jackson, 1967; Chen, 1968; Adams and Pearson, 1970). Hartwell and Pember (1918) reported that Al was three times more toxic to barley than to rye. MacLean and Gilbert (1927) classified lettuce, beets, timothy, and barley as sensitive (sensitive to 2 ppm Al); radish, sorghum, cabbage, oats and rye as medium sensitive (depressed by 7 ppm Al); and corn, turnips, and redtop as resistant (requiring 14 ppm Al for depression). Aimi and Murakami (1964) found that lettuce was injured by 0.9 ppm Al in solution, turnip and radish by 0.9 or 9.0 ppm, and maize, rice, cucumber and squash only by Al concentrations above 90.0 ppm. Aluminum tolerance in nutrient solution was well correlated with acid soil tolerance. Jones (1961) rated barley as Al sensitive, brussel sprouts and peas as semitolerant, and S-100 white clover, mangold, mustard and Atriplex hastata as tolerant. In general, plants classified as calcifuges (acid soil plants), such as Deschampsia flexuosa and Carex demissa are more tolerant to Al than those classified as

calcicoles (calcareous soil plants), such as Carex lepidocarpa (Clymo, 1962).

Cranberry plants appear to be extremely tolerant to Al, requiring 150.0 ppm of Al added to solution for reduction of shoot growth (Medappa and Dana, 1970). Root length was reduced at Al concentrations above 2.5 ppm but root weight was not seriously decreased by even 25 ppm. In this respect the findings of Clarkson (1966a) are worth mentioning. He found that species of Agrostis (bentgrass) genus differed widely in Al tolerance. Roots of A. stolonifera showed injury at 5.4 ppm Al and A. canina at 10.8 ppm, but those of A. setacea and A. tenuis showed no root damage at 21.6 ppm in nutrient solution. Furthermore, A. setacea grew at 43.2 ppm Al which inhibited root growth of A. tenuis. But, of more interest are the recent demonstrations of intraspecific variation in tolerance to Al. Such varietal differences have been found in wheat and barley (Neenan, 1958; Foy et al., 1965a, 1965b and 1967; Mesdag and Slootmaker, 1969; Kerridge et al., 1971; Moore, 1973), alfalfa (Oullette and Dessureaux, 1958), ryegrass (Vose and Randall, 1962), soybean (Foy et al., 1969 and 1972), rice (Ota, 1968), irish potato (Lee, 1971 and 1972) and peanuts (Adams and Pearson, 1970). Kerridge (1969) has grouped more than 50 different wheat varieties into different classes depending on their Al resistance. Mesdag and Slootmaker (1969) using a different

technique have shown the existence of five different groups of wheat varieties. Moore (1973) showed that though the toxicity of Al was increased by increasing pH from 4.0 to 4.5, the sensitive wheat variety Brevor was proportionately more sensitive at all pH values than the resistant Druchamp wheat variety.

Attempts have been made to explain the cause of differential tolerance. Randall (1962) attributed tolerance to C.E.C., Clarkson (1966a) to cell wall and internal complexing capacities, Foy et al. (1966) to differential uptake due to pH changes in the root zone, Klimashevskii et al. (1972) and also Foy et al. (1972) to more rapid uptake and greater accumulation in nuclei and mitochondria. In a series of papers, Klimashevskii and coworkers (Klimashevskii, 1970, Klimashevskii et al., 1970a and 1970b) made an elaborate study of the effect of Al on specificity of the physiological activities of different cultivars of pea, wheat, corn, barley, etc. They found pronounced differences in the Al sensitive and Al resistant cultivars of the same plants with respect to organic matter content, growth during seed germination and phosphatase activity when plants were exposed to Al.

Foy, Fleming and Gerolff (1972) studied two snapbean varieties in solution culture, and found that with 8.0 ppm Al added at an initial pH of 4.8, the top and the root yield of the tolerant variety was 94% and 107% while the susceptible variety yielded 53% and

59%, respectively, in comparison to a control which contained no Al. They also studied Ca uptake of those varieties and observed that with 8.0 ppm Al added, the total Ca uptake for tolerant tops and roots were 98% and 131% and those of sensitive ones were 25% and 22%, respectively. Added Al also reduced the concentration of Ca in tops and roots of the sensitive variety markedly (30% - 50%) and only slightly in the tolerant variety.

Effect of Al on root growth

The reduction in both root and shoot growth due to the presence of Al in the growth medium was observed as early as 1925 by Magistad (1925) for barley, rye, corn, clover, oats, and soybean. Rorison (1958) correlated this reduction of growth and uptake by Al by roots. He observed that the inhibition of growth of Sainfoin seedlings occurred simultaneously with a rapid uptake of Al into the young seedling roots. The Al saturation of the roots was dependent on the external concentration of Al. He further noted that the reduction in the elongation of the tap root was directly related to the Al uptake and hence probably on the internal concentration of Al in the root. His most significant observation was, however, the peg-like formation of laterals and cessation of taproot growth. This observation on cessation of root growth suggested a cellular level effect of Al and has resulted in considerable

research interest (Clarkson, 1965; Sampson et al., 1965; Fleming and Foy, 1968; Clarkson, 1968; and Clarkson and Sanderson, 1969). Clarkson (1965) found that the morphological abnormalities of roots caused by exposing them to Al could be explained as an inhibitory role of Al on either cell division or cell extension. Working with onion roots, he observed that the root growth was completely stopped when treated with 5.4--54 ppm Al for 6-8 hours and he concluded that some mechanism associated with cell division was highly sensitive to Al and was permanently damaged. The nature of this damage was further elaborated by Sampson, Clarkson and Davies (1965) who suggested that DNA was probably the site of action of this metal and there were two types of DNA in barley roots. In an earlier work Sampson et al. (1963) had shown same two types of DNA in wheat also. In a later study Clarkson (1968) observed that Al was affecting the high molecular weight DNA fraction which was designated by Sampson and Davies (1966) as genetic DNA. According to them, the failure of genetic DNA synthesis prohibits cells from passing through the S-period and the consequent result is cessation of root growth. Since the labile DNA synthesis was not stopped due to Al treatment, the conclusion was that nucleic acid metabolism as a whole was not disturbed by Al. The involvement of Al in the mitotic cell division and the resultant cessation of root elongation was further confirmed by the work of Clarkson and

Sanderson (1969) when they compared Al and ^{46}Sc and found that both were inhibiting the mitotic cycle and that prolonged treatment disorganized the cortical cells.

The study of the root as an indication of Al toxicity was emphasized by Fleming and Foy (1968) who suggested that Al was acting as growth inhibitor of specific sites rather than as a systemic poison.

Indeed, inhibition of root growth may be the primary effect of Al toxicity (Kerridge, 1969) and many of the observed effects of Al on plants may be simply an indirect effect due to lack of root growth in the sensitive plants. In any event, root response to Al appears to be a completely reliable measure of differential varietal tolerance to Al (Kerridge and Kronstad, 1968; Reid et al., 1971; Kerridge et al., 1971).

Improvement in experimental techniques

Several workers (Foy et al., 1965; Mesdag and Slootmaker, 1969; and Reid et al., 1969) have used soil to screen large numbers of varieties for tolerance to Al. The disadvantage of using soil is that the degree of selection cannot be quantitatively controlled as the solubility of Al and the severity of its toxicity to plants are affected by many soil factors. Kerridge et al. (1971) described a water culture technique to screen wheat varieties for Al

tolerance in which the relevant variables could be readily controlled. They rigorously maintained a constant pH of a known composition nutrient culture in a growth chamber and showed quantitative differences in groups of wheat varieties. The necessity for precise pH control in studying Al toxicity cannot be overemphasized. The solubility of $Al(OH)_3$ is strongly dependent upon the pH of the system as shown by the following reaction:

$$Al(OH)_3 = Al^{+++} + 3\,OH^-$$

Raupach (1963a) gives the pK for solubility of this reaction as 32.3, and the resulting calculations based on this value show that the Al concentration rises sharply as the pH is decreased from 5.0 to 4.0 (Kerridge, 1969). For instance, the Al concentration of a saturated solution is 5 μM (0.13 ppm) at pH 5.0 and increases to 5000 μM (135 ppm) at pH 4.0. In addition, the form of soluble Al is also pH dependent as shown by the following reaction:

$$Al^{+++} = AlOH^{++} + H^+$$

The pK for this hydrolysis reaction is 5 (Raupach, 1963b), and calculations based on this value show that the distribution of the soluble Al between the Al^{+++} and the $AlOH^{++}$ forms also change rapidly in the pH range between 4 and 5. Thus at pH 4.0 the $Al^{+++}/AlOH^{++}$ ratio is 10:1 but at pH 5.0 the ratio is 1:1 (Moore, 1973). Therefore, not only does the total soluble Al change markedly

in this pH range but its form also changes. Kerridge (1969) and Moore (1973) presented evidence to show that a given amount of soluble Al was more toxic at pH 4.5 than at pH 4.0 and even suggested that the $AlOH^{++}$ form was responsible for causing the toxicity. Plants growing in the nutrient solution can change the pH of that solution due to unequal cation-anion uptake (Moore, 1973) and can thus have a significant effect on both the amount and the form of Al present. On the basis of this consideration, it can be easily understood that rigorous pH control is essential. Unfortunately, there are few reports in the literature where precise pH control was maintained.

Moore (1973) added two modifications to the basic technique described by Kerridge et al. (1971). Kerridge et al. .(1971) exposed wheat roots continuously to a constant Al concentration, and they used total length of root as the measure of root response. In the modified system, wheat plants were started in an Al free solution until root length was 3-5 cm (about 48 hours after germination). The plants were then transferred to nutrient solutions containing Al for 48 hours. The second modification was that in the Al solution P was omitted and Fe was added as $FeCl_3$ instead of as the chelate to avoid the possibility of Al being tied up. Following the 48 hour exposure to Al, the plants were returned to the original Al-free solutions and were allowed to recover for 72 hours.

The elongation of the primary root during the recovery period was used as the indicator of Al toxicity. This technique, in addition to being a very sensitive indicator of Al toxicity to the roots is also highly suitable for studying the influence of other variables on Al toxicity.

Objectives of the study

The review of literature shows that there is a considerable body of information on the effects of Al on plants. Unfortunately, much of this information is difficult to interpret because of the confounding effects of uncontrolled variables of pH, temperature, and nutrition. Furthermore, conflicting interpretations in the literature arise because of both species and variety differences which has not always been recognized.

Recent progress in experimental technique has made it possible to precisely control the variables affecting plant response to Al. This nutrient solution technique provides a direct evaluation of the primary effect of Al in inhibiting root growth. Further, the technique allows the nutrient variables to be imposed in such a way that the direct effects of nutrients on Al response can be separated from the basic nutritional status of the plant.

Wheat was chosen for this study because of the available information on differential varietal response to Al. If Al toxicity is

basically a nutrient deficiency, as suggested extensively in the literature, then varieties with different tolerance to Al should behave differently in their nutritional response under Al stress.

Specific aims of the study were:

(1) To determine the effect of Ca, Mg and K on the response of wheat seedling roots to soluble Al.

(2) To compare selected wheat varieties of widely different tolerance to Al for the effect of Ca, Mg, and K on Al stress.

MATERIALS AND METHODS

When plants are grown in soils, the soil solution is of ever-changing composition whereas an accurate control of relevant variables is a sine qua non for meaningful experimentation. The relevant variables of Al toxicity are, as described earlier, numerous and a control of them calls for nutrient culture experiment. Besides the convenience of evaluating the root, the organ which is first affected by direct exposure to Al, solution culture also provides a convenient system for precise control over the nutrient composition and pH. The basic technique used in this dissertation is the same as that reported by Kerridge et al. (1971) and modified by Moore (1973). However, a brief description of the experimental procedure is given here since neither of the above mentioned papers are detailed enough in their descriptions. The procedure given here is stepwise and follows the same sequence used during the experiments:

a. Seeds were soaked in aerated tap water at room temperature for 24 hours. At this time, the radicle was just beginning to emerge.

b. Six healthy, uniform size, sprouted seeds were placed with 'seam' down on the screen bottom of cups which were placed in holes in an acrylic cover which in turn was placed on the top of 25 litre black polyethylene waste baskets as described by Kerridge et al.

(1971). The cups were thus suspended over the nutrient solution with the screen bottom in contact with the solution.

c. The final composition of the solution which will be referred to as "base solution" is given in Table 1. The level of the solution in the baskets were such that it just touched the screen bottom of the cups thus keeping the seeds moist and providing the emerging radicles with a ready supply of nutrients. The pH of the solution had previously been adjusted to 4.0 with H_2SO_4. The solutions were continuously aerated and were in a growth chamber with a temperature of $25^{o}C \pm 2^{o}C$, a day length of 16 hours and a light intensity of about 2,000 foot-candles.

d. The plants were allowed to develop for about 48 hours by which time there were three primary roots; one longer primary root (middle one, about 4.5 cm) and the other two primary roots (a little shorter, and one in each side of the middle primary root).

e. Three out of six seedlings from each cup were selected on the basis of uniformity with respect to both root and shoot growth and the rejected three were removed from the cup. A minimum of 18 plants were used for each treatment and the reported results are an average of the 18 or more observations.

f. The length of the middle primary root of each seedling was measured. Positions of the seedlings were identified with respect to a mark on the cup so that subsequent root measurements could be

Table 1. Composition of the 'basal nutrient solution'.

Constituent	Concentration
$Ca(NO_3)_2$	4 mM
$MgSO_4$	2 mM
KNO_3	4 mM
$(NH_4)_2SO_4$	0.435 mM
KH_2PO_4	0.5 mM
$MnSO_4$	2 μM
$CuSO_4$	0.3 μM
$ZnSO_4$	0.8 μM
NaCl	30.0 μM
Fe-CYDTA	10 μM
Na_2MoO_4	0.10 μM
H_3BO_3	10.0 μM

attributed to the same seedling.

g. The cups with their seedlings were then transferred to solutions containing different treatments. The composition of the treatment solution varied according to the objective but usually contained Al. The nutrient composition of this solution was basically

the same as the initial solution except that P was omitted and Fe was added in an equivalent amount as $FeCl_3$ in place of Fe-CyDTA as described by Moore (1973). Phosphorous was omitted to avoid the possible precipitation of Al. Because of the possibility of Al-precipitation as $Al(OH)_3$, special attention was given to this point. Prior to transferring the cups into the treatment solution enough H_2SO_4 was added to bring the pH down to around 4.2 and then the requisite amount of Al as $Al_2(SO_4)_3 \cdot 18\,H_2O$ was added. The final pH was adjusted to 4.0 with H_2SO_4, thus avoiding adding any KOH which might cause Al precipitation, at least locally. The seedlings were allowed to grow for 48 hours in the treatment solution. At the end of 48 hours, the same central primary root of each seedling was measured and the cups were transferred back to original buckets containing the nutrient solution where the seedlings were grown for the first 48 hours. The difference in the root length between two measurements was, therefore, the amount of root growth which had taken place in the treatment solutions.

h. The seedlings were allowed to grow in the base (recovery) solution for 72 hours. The amount of root growth in the recovery solution was dependent on the severity of the previous Al treatment. With a toxic amount of Al, the primary roots would not regrow at all and remained thickened at the tip as a typical Al injury described by others (Rorison, 1958; Foy et al., 1965a; Kerridge, 1969). If

the toxicity was not severe enough to completely inhibit root growth permanently, the root would show growth in the recovery solution. The amount of regrowth was determined by again measuring the root length at the end of the 72 hour recovery period and subtracting the length of the root measured at the end of growth in the treatment solution.

i. During the entire experiment, the pH of both the initial/recovery solution and the treatment solution was precisely controlled by adjusting the pH at least twice a day. The maximum fluctuation in pH was always less than $\pm$ 0.01 unit because of the large volume of solution used and the repeated adjustment.

RESULTS AND DISCUSSION

The toxic effect of Al in stopping root growth of wheat varieties has been described by Kerridge (1969). He grew wheat plants in Al solutions and measured the total root length which was then used as an indicator of severity of Al toxicity. In this dissertation, a similar approach has been used except that the change in root length following pretreatment in Al has been used as the indication of Al toxicity rather than the growth of roots in Al solution (see Materials and Methods). Figure 1 shows a comparison of the two approaches where both the length of roots growing in Al solution and the length of regrowth after Al treatment has been plotted. As could be seen from the figure, the advantage of using regrowth as a measure of Al toxicity lies in the fact that an exact concentration of Al could be identified where root growth was completely and irreversibly stopped. Whereas if the growth in Al solution was used, it did not reach zero and after a certain concentration became almost constant. Thus, it is evident from the graph that 7.0 ppm of Al was toxic only when regrowth is considered but if the growth in Al solution was used, the graph does not help to differentiate after 5.0 ppm. Henceforth, only regrowth will be used for discussion though the data on growth in the Al solutions are also given in the Appendix together with the regrowth data.

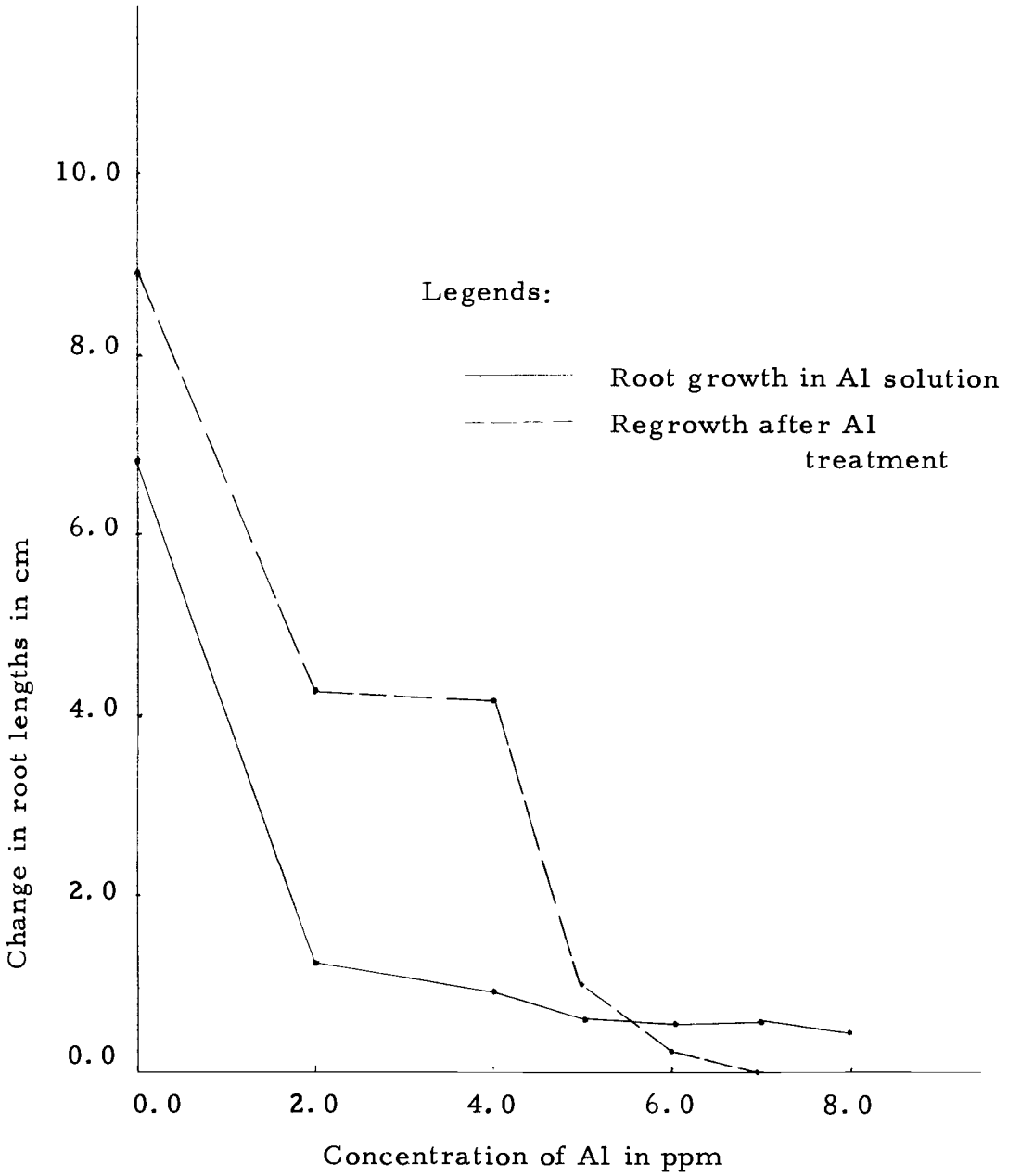

Figure 1. Effects of different levels of Al on root growth and regrowth of wheat variety Brevor grown in full strength nutrient solution.

The toxic effect of Al on root growth has long been recognized. Rorison (1958) reported the inhibitory effect of Al on root growth of legume plants. He described the toxic effect in terms of cessation of root growth and the peg-like formation of root laterals. This cessation of root growth due to Al was shown by Rios and Pearson (1964) to be the result of inhibition of cell division. They observed that an Al concentration of above 0.5 ppm prevented growth of cotton seedling roots and resulted in the appearance of binucleate cells in the meristematic regions of the root tips indicating the inhibition of cell division. This was further elaborated by Clarkson (1965) who showed the inhibitory effect of Al on mitosis and the consequent stoppage of root growth in onion due to Al treatment. Therefore, the small amount of root elongation in Al solution (Fig. 1) could be attributed to the growth of the roots after the plants were transferred to the Al solutions but before cell division was completely stopped by Al. Whether the damage was reversible or irreversible, could usually be visually distinguished depending on the degree of thickening of root tips due to Al though this was not always reliable. But the increase in root length subsequent to Al exposure was always easily distinguishable visually as well as through measurement of the change in length of roots. The observation of Fleming and Foy (1968) that Al was acting as a growth inhibitor of specific sites rather than as a systemic poison supports the observations of this

piece of work since lateral roots were seen growing normally after removing the Al stress although the root tip did not reinitiate growth once subjected to toxic levels of Al.

While the cessation of root growth has been shown to be probably the result of inhibition of cell division by Al, various authors have attributed the poor growth and the consequent toxic effect of Al to be due to the disturbance in the uptake and metabolism of P, Ca, Mg and K or other nutritional elements. Because of these uncertainties of the exact elements which might be influencing the Al toxicity, a set of experiments was designed to study the effect of concentration of nutrient solutions on the toxicity of Al. Therefore, in the next experiments the concentration of all nutrients in both the initial/recovery solution and the Al treatment solutions were reduced to half, quarter, or tenth of the strength of the nutrient solution used in the earlier experiment. Figure 2 shows the result for the variety Brevor. The most noticeable effect is the sharp increase in Al toxicity just by decreasing the strength of the nutrient solution while all the other factors, viz., temperature and pH were held constant. There was a drop from 7.0 ppm of Al needed to stop root regrowth in full-strength nutrient solution (Fig. 1) to 5.0 ppm to do the same in half-strength nutrient solution (Fig. 2), to 2 ppm in quarter strength and to 1 ppm in tenth strength.

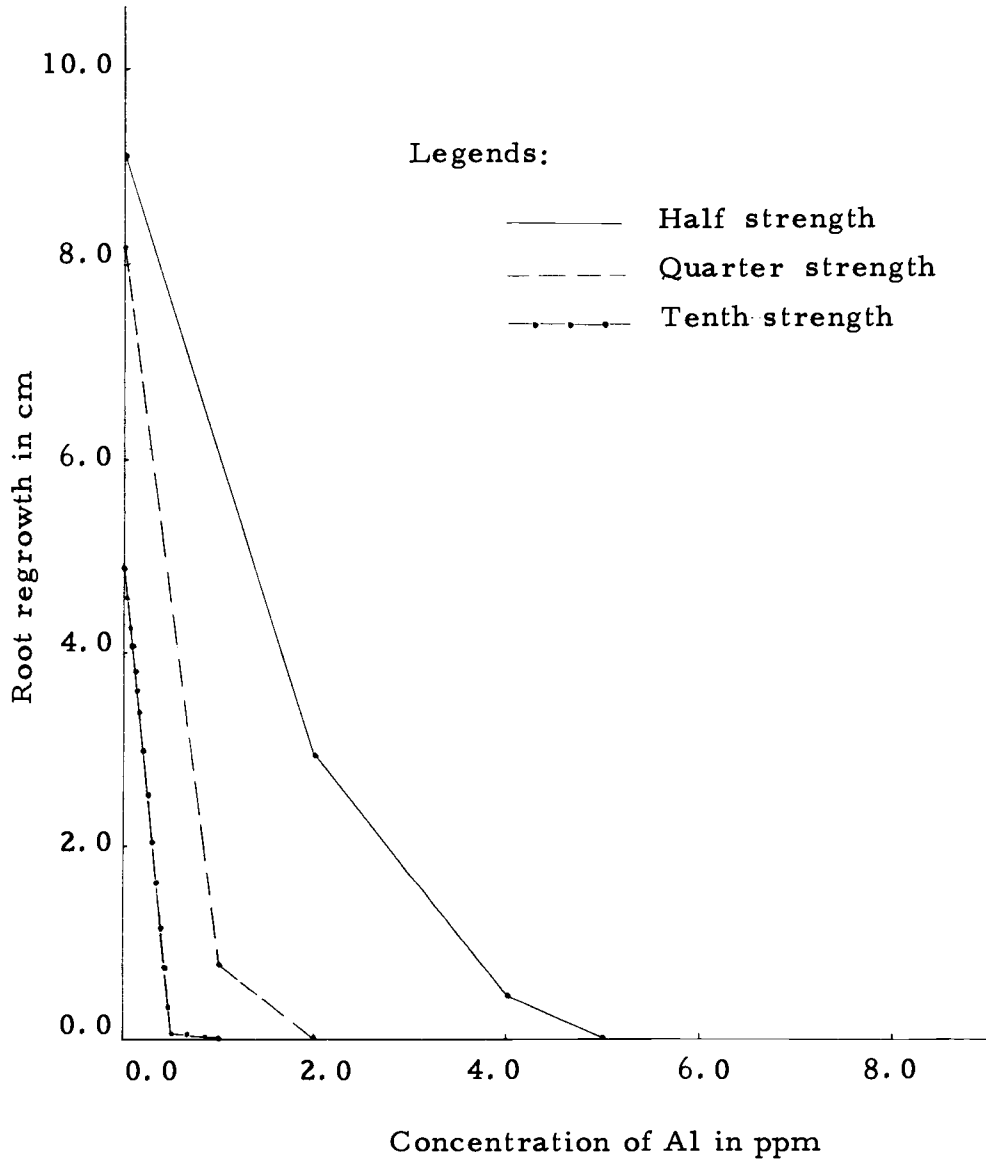

Figure 2. Effect of different concentrations of nutrient solutions (both the basal and the treatment) on Al toxicity in Brevor.

Since Brevor is representative of the most sensitive class of varieties of wheat (Kerridge, 1969), a series of experiments were conducted to see if the susceptibility to Al of the more tolerant variety Druchamp could also be increased by decreasing the strength of the nutrient solution. The results (Fig. 3) show that the same concentration of Al used for Brevor did not have much of a toxic effect on Druchamp when grown in full and half-strength nutrient solutions, though at half strength the trend towards increased toxicity is evident. However, the case is altogether different for quarter and tenth-strength nutrient solutions (Fig. 4). In both cases, a very sharp drop in root regrowth with an increase in Al concentration is noticeable. At full strength with 8.0 ppm (Fig. 3), the root length was 7.02 cm, whereas it was only 0.28 cm at the same Al concentration in quarter strength, and there was no regrowth at all even at 4.0 ppm when grown in tenth strength (Fig. 4).

The comparison between Brevor and Druchamp clearly indicates their varietal distinction with respect to tolerance to Al while at the same time showing a similar trend of increased susceptibility to Al when the concentrations of other cations in the nutrient solutions were lowered. This suggests that the mode of toxicity in both the varieties is the same and that tolerance is relative rather than absolute.

In each of the above experiments, both the initial/recovery

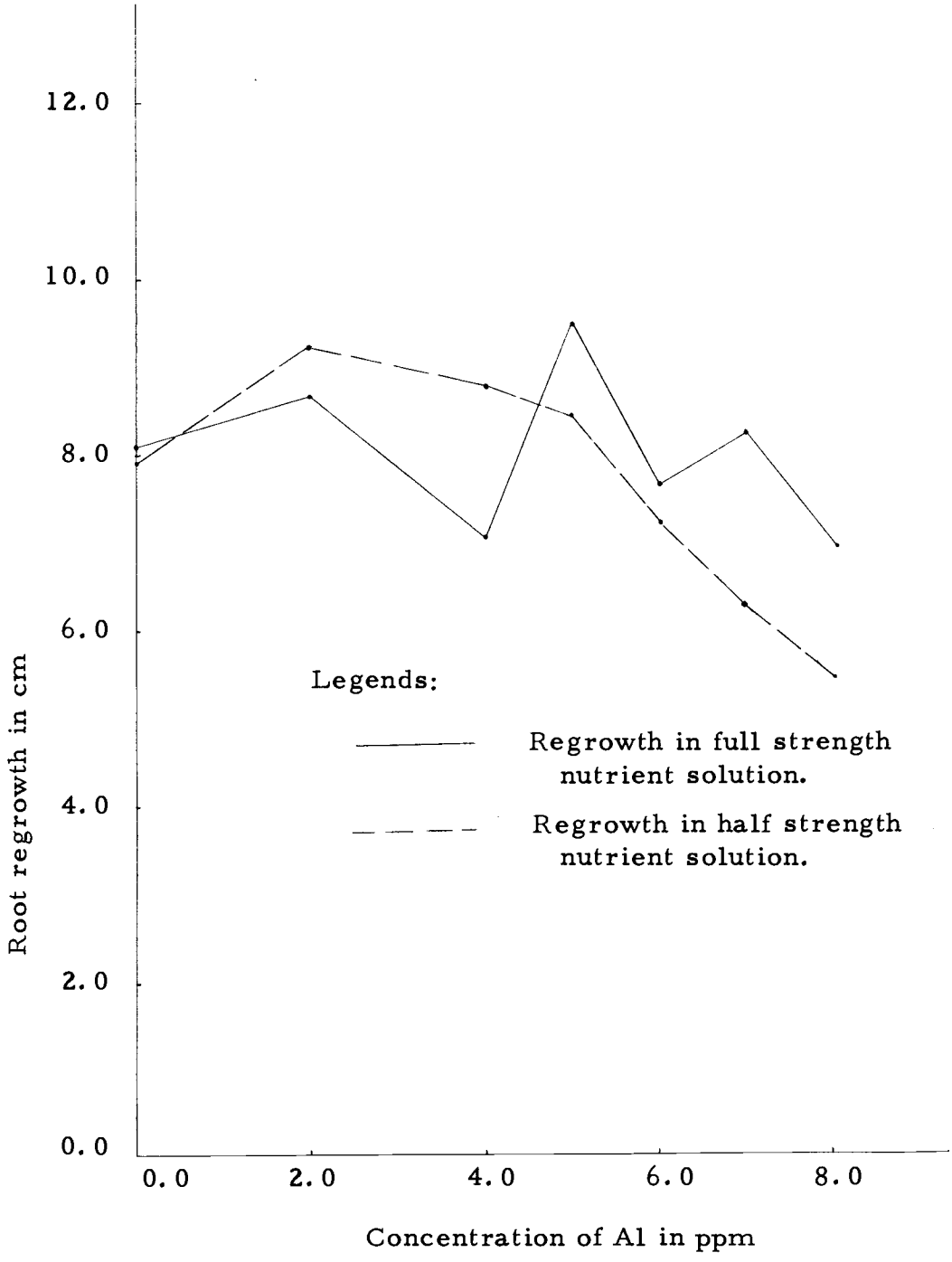

Figure 3. Effect of different concentrations of Al in full and half-strength nutrient solutions on root regrowth of Druchamp.

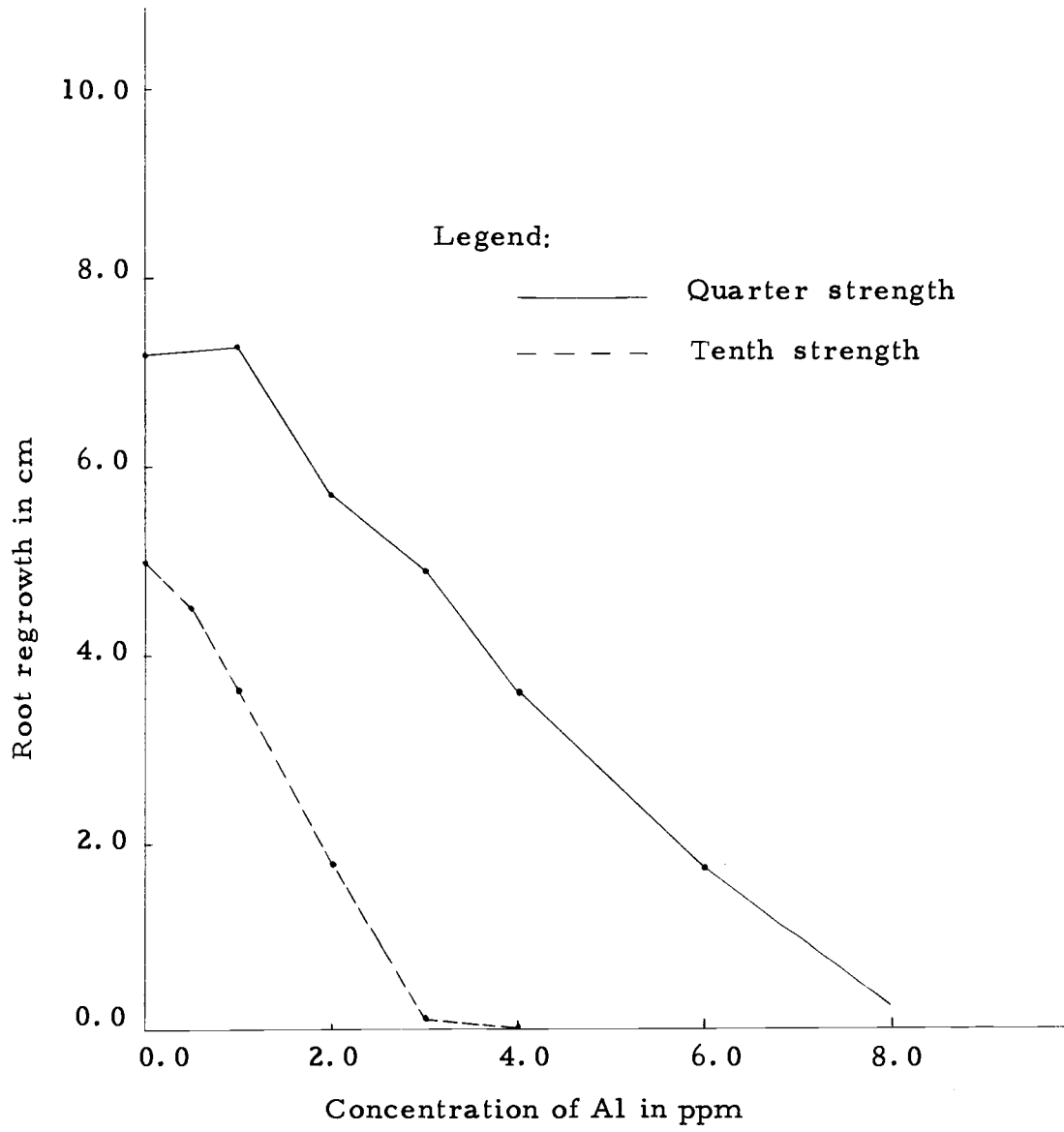

Figure 4. Effect of different concentrations of nutrient solution (both basal and treatment) on Al toxicity in Druchamp.

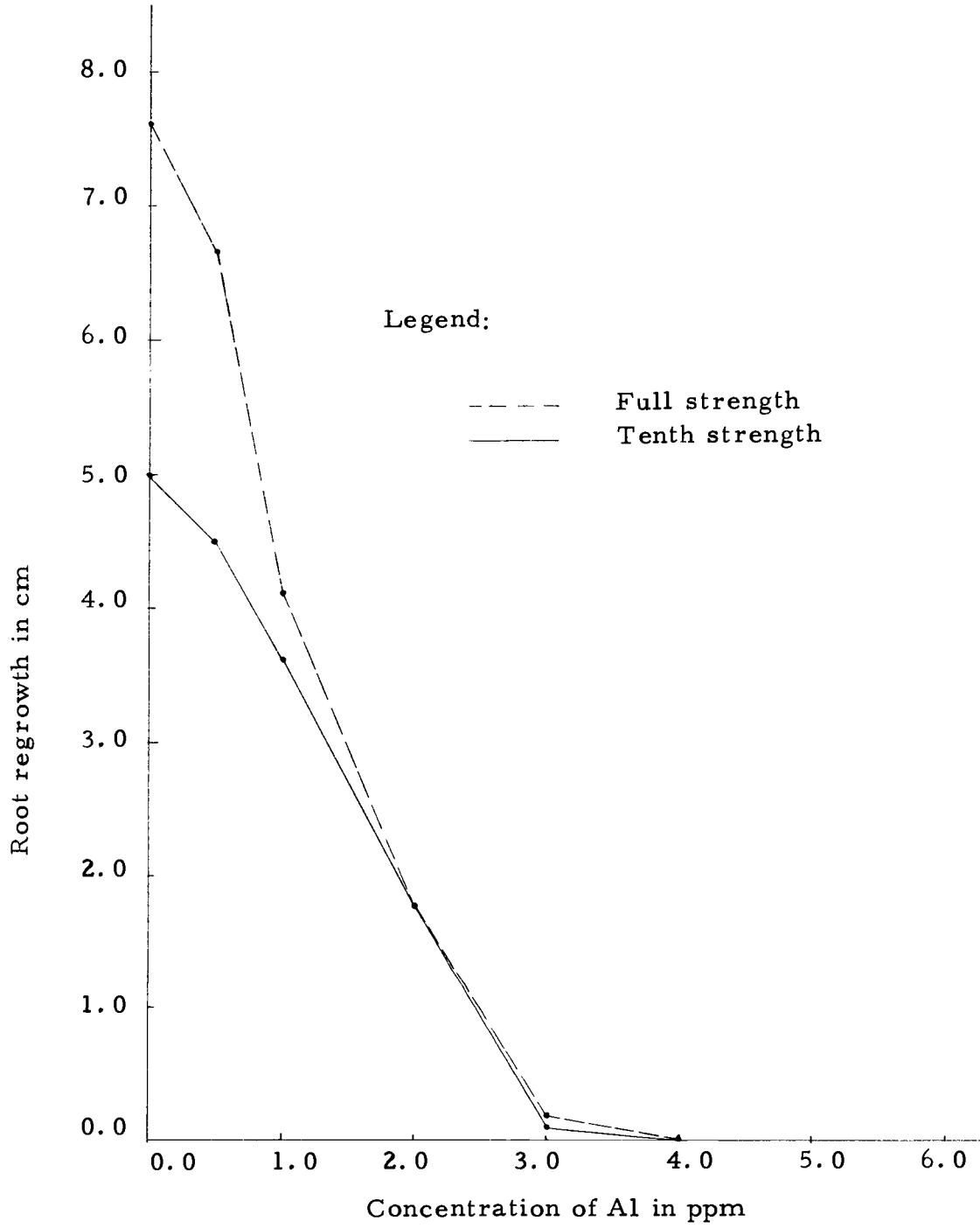

Figure 5. Comparison of Al toxicity in Druchamp at different concentrations of basal solution. (Treatment solution tenth strength in both cases.)

solution and the Al treatment solutions were maintained at the same strength. For example, in the experiment in the tenth-strength solution, plants were started in tenth-strength solution, transferred to tenth-strength solution containing Al for 48 hours, then transferred back to the initial tenth-strength solution for the regrowth period. Therefore, it was not clear how much of the increase in toxicity of Al, as the strength of nutrient solution was decreased, could be attributed to the conditions in the Al treatment solutions and how much could be attributed to the effect of the strength of the initial/recovery solutions. That the strength of the recovery solution may have had an effect on root regrowth can be seen by comparing the various strengths of solution at 0 Al for the variety Druchamp. Root regrowth in the full, half and quarter-strength solutions was around 7 to 8 cm in the 72 hours recovery period (Figs. 3 and 4). However, when the solution strength was reduced to a tenth, the root regrowth was reduced to about 5 cm (Fig. 4).

In order to separate the possible effects of the initial/recovery solutions from the Al treatment solutions, an experiment was conducted to compare root regrowth in full versus tenth-strength solutions. The Al treatments were set up in tenth strength rather than in full strength to provide maximum nutrient stress if this was the responsible factor. The results for the variety Druchamp (Fig. 5) show that the critical Al concentration needed to completely inhibit

root regrowth was the same (4.0 ppm) regardless of the strength of the initial/recovery solution. A similar result was obtained for the variety Brevor, thus the conditions in the Al treatment solutions primarily determine the toxicity of Al. On the basis of these results, all subsequent experiments were standardized using full strength initial/recovery solutions with the solution variables being applied only to the Al treatment solutions. This modification served to further reduce the possibility that the plants were suffering from nutrient stress.

In order to more accurately define the critical Al concentration for completely inhibiting root growth in the variety Brevor, the quarter strength and tenth strength experiments were repeated using more closely spaced Al treatments than previously. At quarter strength, the critical Al concentration was found to be 1.5 ppm and at one tenth it was 0.4 ppm (Fig. 6). These results agree quite well with the earlier results and serve to confirm that it was the composition of the Al treatment solution that was crucial rather than the composition of the initial/recovery solutions.

While Brevor and Druchamp both were more susceptible to Al damage as the strength of the nutrient solution was decreased, these varieties maintained their relationship to one another at each nutrient solution strength. In other words, Druchamp was always more tolerant than Brevor under comparable conditions. It is

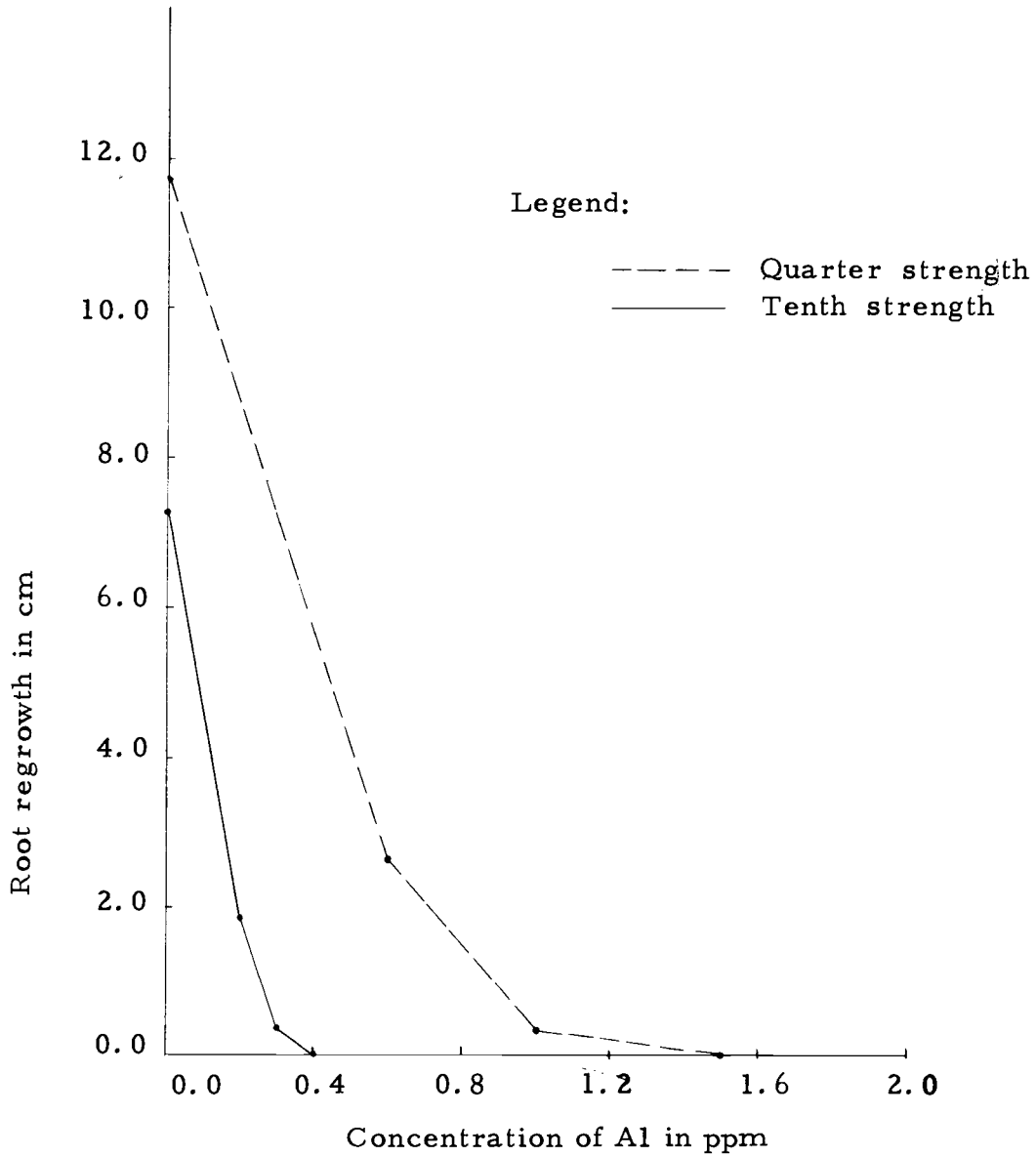

Figure 6. Al toxicity in Brevor when grown in quarter- and tenth-strength treatment solutions. (Basal solution full strength in both cases.)

interesting to note, however, that Brevor was more tolerant at full strength and half strength (Figs. 1 and 2) than Druchamp was at tenth strength (Fig. 5). This suggests that tolerance to Al is indeed relative. As a further test of this, two varieties having even greater tolerance to Al than Druchamp, i.e., Chinese Spring and Atlas 66 (Kerridge, 1969), were evaluated. Earlier results* in full strength solutions with these varieties showed that about 45 ppm was required to completely inhibit root regrowth in Chinese Spring and about 120 ppm in Atlas 66. Figures 7 and 8 show the effect of Al concentration in tenth-strength nutrient solution for these two varieties. Under these conditions, the critical Al concentration to completely inhibit root regrowth was 6.0 ppm for Chinese Spring (Fig. 7) and 30.0 ppm for Atlas 66 (Fig. 8). Thus the four varieties representing four distinctly different levels of Al tolerance all behaved similarly with respect to changes in the strength of nutrient solution. Taking the concentration of Al needed to stop root regrowth of Brevor in tenth-strength solution (0.4 ppm) as 1, Druchamp was 10 times more tolerant than Brevor while Chinese Spring was 15 times more tolerant and Atlas 66 was 75 times more tolerant.

Figure 9, showing the amount of Al needed to stop regrowth of Brevor at different concentrations of nutrient solution, indicates

* Personal communication with Dr. D. P. Moore, Prof. of Soils, O.S.U., Corvallis.

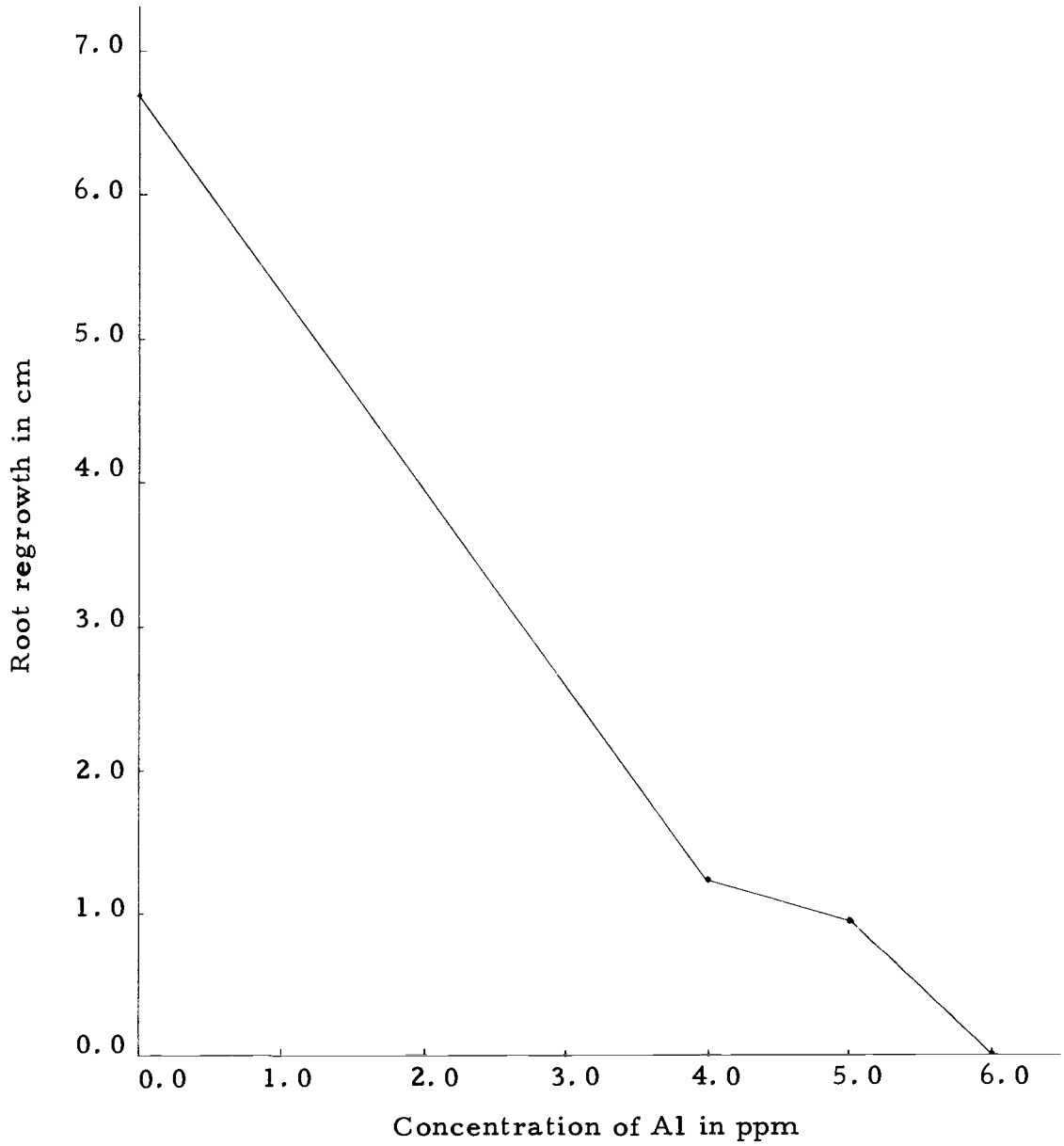

Figure 7. Effects of different concentrations of Al in tenth-strength nutrient solution on the root regrowth of Chinese Spring.

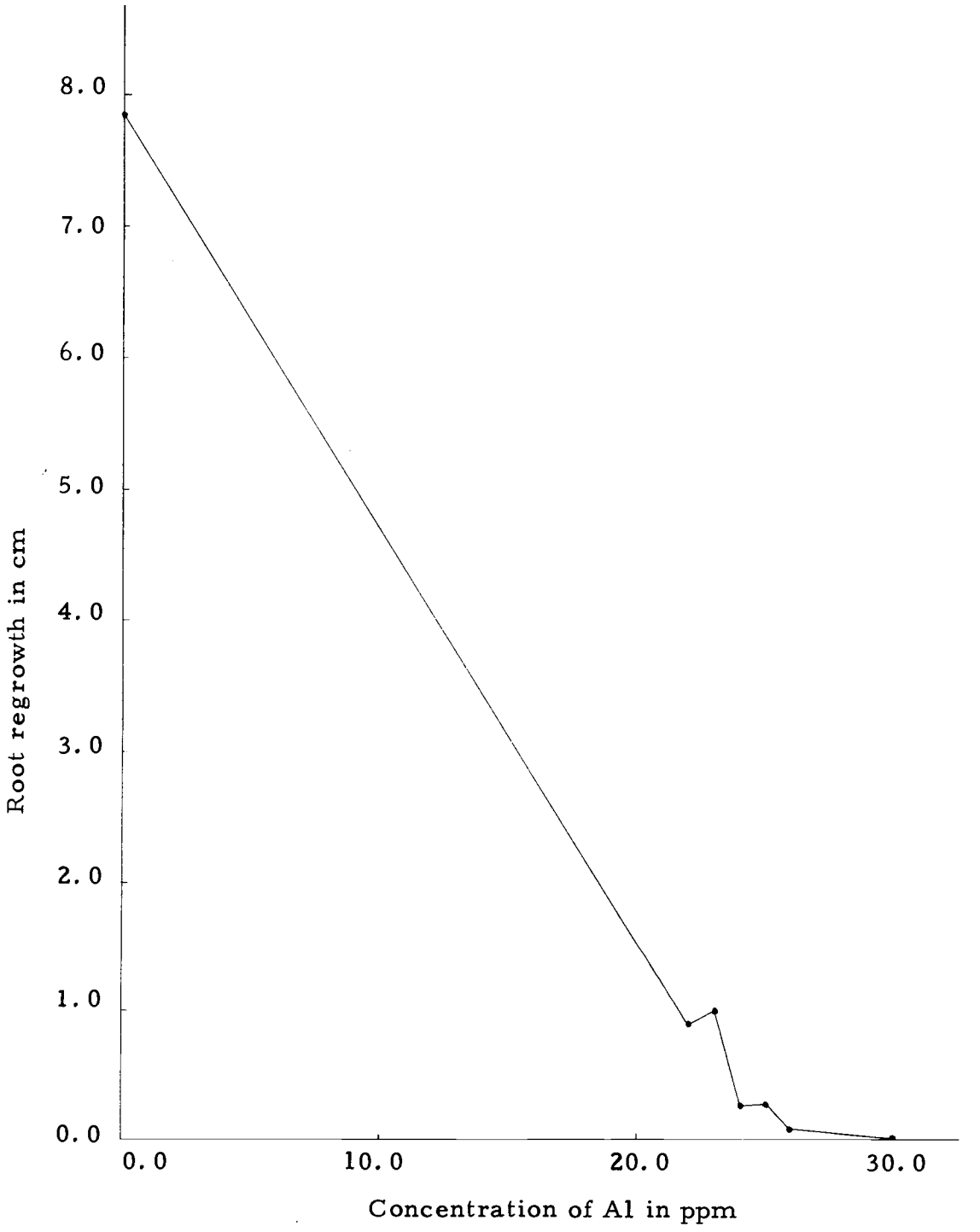

Figure 8. Effect of different concentrations of Al in tenth-strength nutrient solution on the root regrowth of Atlas 66.

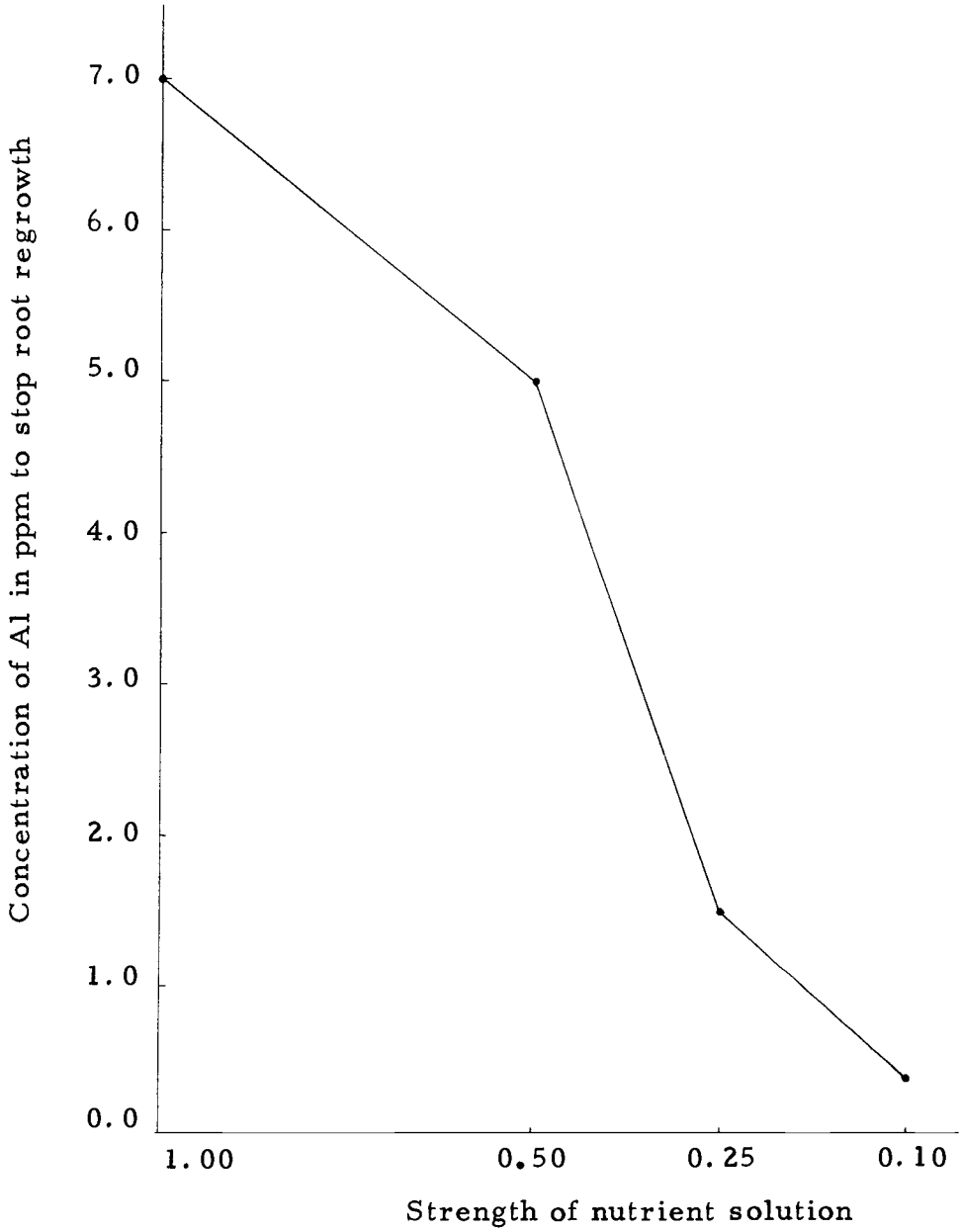

Figure 9. Increase in Al toxicity with decrease in the strengths of nutrient solutions used (variety Brevor).

a sharp increase in Al toxicity with reduced nutritional competition. Here keeping pH, temperature, variety and time constant, Al toxicity was increased by a factor of 1.4, 4.7, and 17.5 at half, quarter and tenth-strength solutions, respectively. Note that the toxicity of Al increased more rapidly at lower nutrient concentrations. A similar effect of salt concentrations in decreasing Al toxicity was reported by Cate and Sukhai (1964) when they observed that Al concentrations as low as 1-2 ppm in the absence of nutrient cations was toxic to rice plants, but when soluble nutrient salts were present, this toxicity could only be seen at much higher concentrations of Al.

The striking effect of nutrient solution strength on Al toxicity led to the design of the next set of experiments where the objective was to evaluate the effects of Ca, Mg, and K individually and in combinations on the influence of Al toxicity, since the above observed effects could have been due to some specific ion.

The concentrations of Al needed to stop the growth of roots of Brevor in quarter and tenth-strength nutrient solutions (Fig. 6) were used as the basis for this series of experiments. The present experiments were designed to grow plants in treatment solutions containing tenth-strength concentrations of nutrients, add enough of any one cation or a combination of cations as their sulfate salts to bring up the strength of that particular cation(s) to quarter strength

and to study the effect of this increase in concentrations of the cation(s) on Al toxicity. Sulfate salts were used to bring the particular cation(s) from tenth strength to quarter strength since sulfate is generally nontoxic to plants. In a preliminary experiment where all macronutrient cations of Table 1 and Al were used either as sulfates or as chlorides in the treatment solutions, the same concentration of Al was slightly more toxic when the salts were used as sulfates rather than as chlorides (Appendix Table 5). This rules out the possibility that the decreasing of the Al toxicity was due to sulfate. An increase in tolerance due to an increase in the concentration of a cation could, therefore, be safely attributed solely to the effect of that particular cation. Since all the four varieties behaved similarly with respect to varying the solution strength above, it was decided to use Brevor alone to study the effect of the individual cations and their combinations. Treatment solutions were chosen at tenth strength for these experiments to accentuate any nutritional effects if these were involved in Al toxicity.

From Table 2 it is seen that each of the three cations, viz., Ca, Mg, and K does have a decreasing effect on Al toxicity. In tenth-strength solutions, the critical Al concentration was 0.4 ppm (Fig. 6) but when Ca was increased to quarter strength (in a base of tenth-strength solution) the critical Al concentration increased from 0.4 ppm to something over 1 ppm. The critical Al

concentration was certainly less than the 2.0 ppm shown in Table 2, since the critical concentration for quarter strength of all salts was only 1.5 ppm (Fig. 6). The critical concentration of Al needed to completely inhibit root regrowth when Mg was increased to quarter strength was 1.0 ppm whereas that needed for K was 0.6 ppm. While the decrease in Al toxicity is absolute, the comparison between the strengths of these cations in the solutions, needs more careful examination. A look at Table 1 shows Ca and K to be of equal concentration in the basic solution while Mg is half of their concentration, i.e., Ca and K are each 4 mM and Mg 2 mM. Accordingly in tenth-nutrient solution, the concentration of Ca and K is 0.4 mM and 0.6 mM is added so that the final concentration of these cations is 1.0 mM when they are increased to quarter strength. Mg was only 0.2 mM in tenth strength and 0.3 mM extra Mg was added to bring the strength of Mg to quarter strength resulting in a total concentration of only 0.5 mM. The decrease in Al toxicity by Mg given in Table 2, therefore, was due to 0.3 mM added Mg. Nevertheless, the decrease in toxicity was substantial and more than that caused by 0.6 mM additional K. It can, therefore, be inferred that at 0.6 mM concentration Mg might have been at least equal to if not more effective than Ca in overcoming the toxicity of Al and surely was more effective than K.

Results of Table 3 show the same trend as revealed in Table 2

Table 2. Effects of increasing concentration of Ca, Mg, or K in the treatment solution to 1/4 strength (in 1/10 strength solution) on decreasing Al toxicity in the variety Brevor.

Cation	Growth in	Concentration of Al in ppm										
		0	0.2	0.4	0.5	0.6	0.8	1.0	2.0	3.0	4.0	5.0
Ca	Treatment solution (cm)	4.22	--	--	1.03	--	--	0.49	0.41	0.34	0.29	0.26
	Recovery solution (cm)	8.50	--	--	3.44	--	--	0.12	0.00	0.00	0.00	0.00
Mg	Treatment solution (cm)	1.66	--	0.72	--	0.51	0.43	0.41	--	--	--	--
	Recovery solution (cm)	8.29	--	3.30	--	1.43	0.03	0.00	--	--	--	--
K	Treatment solution (cm)	0.78	0.96	0.59	--	0.47	0.37	--	--	--	--	--
	Recovery solution (cm)	6.91	3.75	0.28	--	0.00	0.00	--	--	--	--	--

Table 3. Effects of increasing Ca+Mg, Ca+K, or Mg+K in the treatment solution to 1/4 strength (in 1/10 strength solutions) on decreasing Al toxicity in the variety Brevor.

Cation	Growth in	Concentration of Al in ppm										
		0	0.6	0.8	1.0	1.2	1.4	2.0	3.0	4.0	6.0	7.0
Ca+Mg	Treatment solution (cm)	4.26	--	--	0.66	--	--	0.43	0.40	0.41	0.34	0.32
	Recovery solution (cm)	8.59	--	--	2.47	--	--	0.00	0.00	0.00	0.00	0.00
Mg+K	Treatment solution (cm)	3.13	0.60	0.52	0.55	0.42	--	--	--	--	--	--
		8.60	1.12	0.33	0.06	0.00	--	--	--	--	--	--
Ca+K	Treatment solution (cm)	3.53	--	0.68	0.52	0.48	0.48					
	Recovery solution (cm)	7.03	--	1.22	1.11	0.04	0.00					

in addition to the fact that the cation strengths are additive where overcoming of Al stress or toxicity is concerned. The joint effect of Ca+Mg is greater than the joint effect of Mg+K or Ca+K. The result with Ca+Mg is a bit misleading. At quarter strength of the treatment solution, the critical Al concentration needed was 1.5 ppm (Fig. 6). If cations were reducing Al stress, the critical Al concentration cannot be 2.0 ppm just by bringing the strength of Ca and Mg alone to quarter strength as shown in Table 3. Unfortunately, there was no intermediate levels of Al between 1.0 and 2.0 ppm and whether the exact concentration of Al needed was 1.5 ppm or more or less could not be confirmed with this experiment. None the less, it is clear that all three cations, Ca, Mg, and K were each capable of protecting the roots from Al injury.

On the basis of these results, it was decided that a complete study of Ca, Mg and K variables needed to be made. In order to determine the critical Al concentration, Al must be a variable. If Ca, Mg, and K were also variables, this approach would require a large number of experiments to achieve the desired information, since only one such experiment could be accommodated in the available growth chamber space at one time. Therefore, a new approach was taken.

It was shown earlier that 1.5 ppm Al in quarter-strength solution was <u>just</u> sufficient to completely and irreversibly inhibit

root growth for the variety Brevor (Fig. 6). Apparently this concentration of Al under these conditions was lethal to the dividing cells of the primary meristem (Clarkson, 1965). Therefore, if a cation were able to protect the root from Al injury, increasing the concentration of that cation (under these specific conditions in the treatment solutions) to some higher level should reduce the toxicity sufficiently so that 1.5 ppm Al no longer would be inhibitory. Some regrowth of the roots should occur when the plants were transferred back into the recovery solution. Furthermore, the amount of regrowth as compared to a parallel control treatment containing no Al should be a measure of the amount of the injury from Al. Figure 10 shows the results of such an experiment for the effect of increasing the concentration of Ca (as sulfate) above the quarter-strength level for the variety Brevor.

On the basis of these results, it is evident that increasing Ca by itself had no effect up to 9.6 mM concentration, since the root growth without Al was essentially constant. The figure shows that as the concentration of Ca was increased, it increasingly relieved the stress of Al on the roots. The increase in Ca levels by adding 9.6 mM $CaSO_4$ resulted in the almost complete elimination of toxicity caused by 1.5 ppm Al. This complete protection of roots from Al toxicity by Ca resulted in plants that were indistinguishable from the controls. Also shown in Figure 10 is an identical

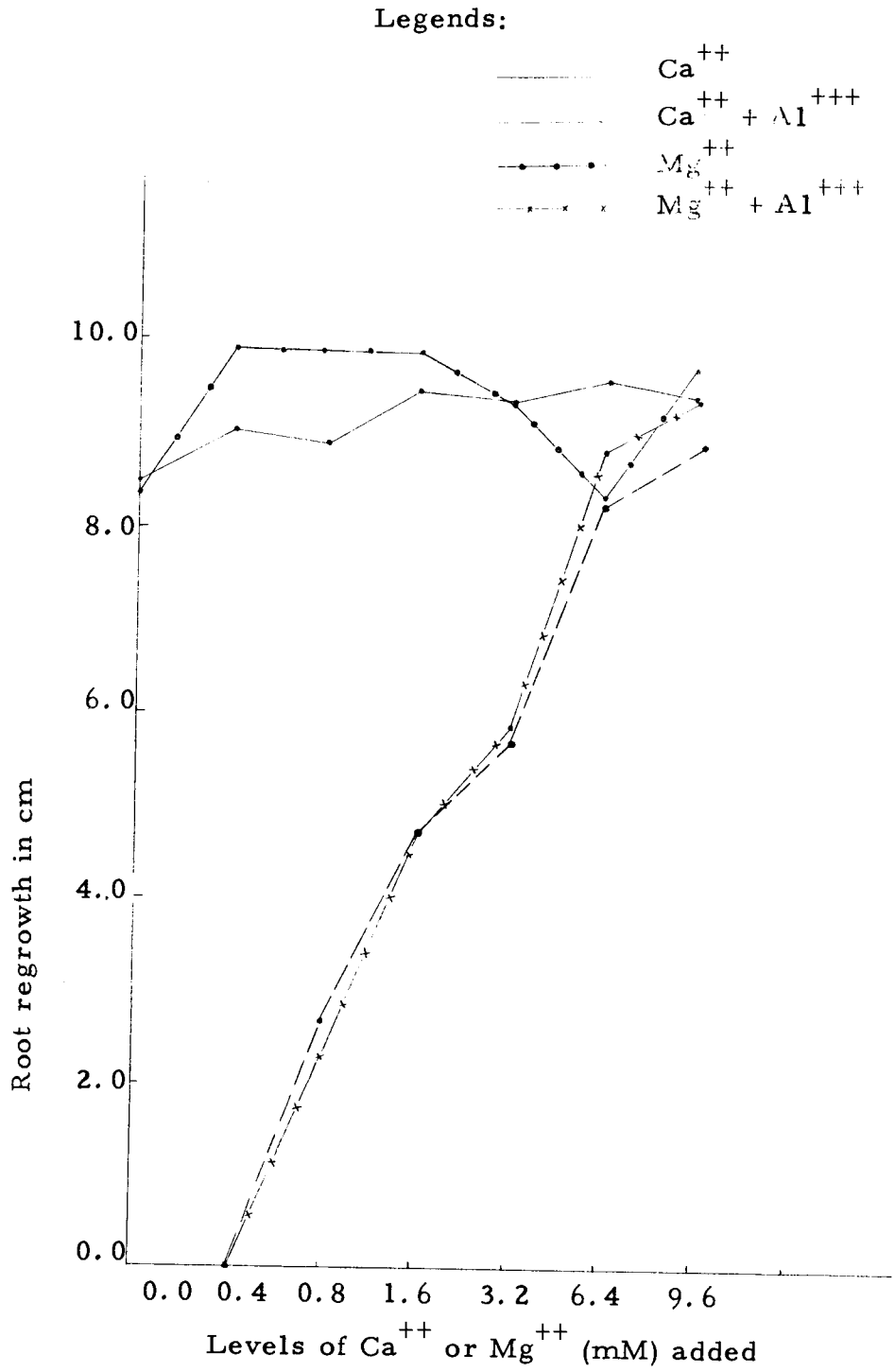

Figure 10. Effects of Ca^{++} and Mg^{++} added above the quarter-strength level in overcoming root growth inhibition due to 1.5 ppm Al (variety Brevor).

experiment with $MgSO_4$. Interestingly, the graphs for Ca and Mg are essentially identical, suggesting their similar roles and equal effectiveness in overcoming Al toxicity. This also shows that the protection of the root from Al injury is not specific to either Ca or Mg, since perfectly normal plants could be obtained by increasing either cation. Therefore, K was the next element to be tested. It was found that K was as effective as Ca or Mg in overcoming Al toxicity but only at higher concentrations (Fig. 11). Since essentially complete protection was obtained with Ca, or Mg, or K, the role of elements tested seemed to be nonspecific. Na was then tested to see if the nonspecific protection was nonnutritional too. The results of this experiment are shown in Figure 11 together with K. Na too eliminated the Al toxicity almost completely, suggesting that the protection by cations to Al injury on wheat seedling roots is a nonspecific as well as a nonnutritional phenomenon. Furthermore, Na and K appeared to have almost identical effects on Al toxicity except that K itself seemed to slightly reduce root growth especially at high K concentrations. This could be due to the very strong competitive effect that K has on Mg uptake (Moore, 1964).

Since the results obtained with Ca, Mg, K, and Na for the variety Brevor clearly indicated that Al toxicity could be overcome by certain amounts of any cation, viz., Ca, Mg, K, or Na, the next attempt was to investigate if other wheat varieties with wide differences in

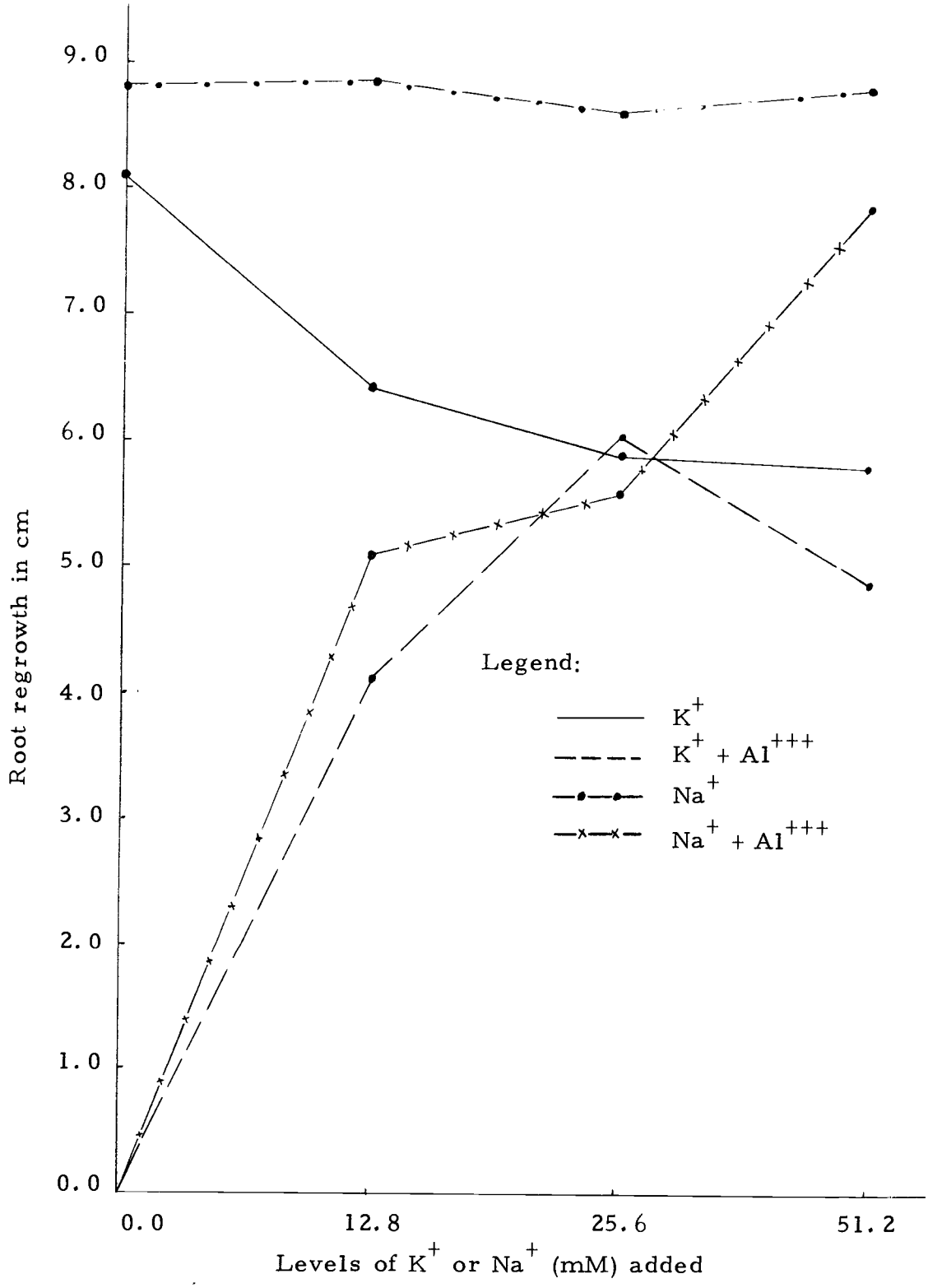

Figure 11. Effects of Na^+ and K^+ added above the quarter-strength level in overcoming root growth inhibition by 1.5 ppm Al (variety Brevor).

tolerance to Al follow the same trend. These experiments were set up in exactly the same way as the Brevor experiments except that the Al concentration required to just completely inhibit root growth was necessarily different with each variety because of their inherent differences in Al tolerance. Earlier experiments had shown that in tenth-strength solution 4.0 ppm Al was the critical Al concentration to irreversibly inhibit root growth for Druchamp, 6.0 ppm for Chinese Spring, and 30.0 ppm for Atlas 66. Each of these concentrations created an approximately equivalent Al stress in the respective varieties as was obtained for Brevor at 1.5 ppm in quarter-strength solution. To insure that a toxic level of Al was present, the Al concentration was increased slightly in each case to 4.5 ppm for Druchamp, 7.0 ppm for Chinese Spring and 35.0 ppm for Atlas 66.

Instead of using the complete range of concentrations of Ca, Mg, K, and Na as was done for Brevor, only the highest concentration was used in each case, i.e., 9.6 mM for $CaSO_4$ and $MgSO_4$ and 51.2 mM for K_2SO_4 and Na_2SO_4. The purpose of the experiments was to see if Ca, Mg, K, and Na would completely protect the roots of these varieties from a concentration of Al that completely inhibited root growth.

Table 4 shows the results of the experiment with the variety Druchamp. The increase in growth due to the extra cations Ca,

Table 4. Effect of different concentrations of cations on Al-toxicity of wheat variety Druchamp. (Treatment solution concentration 1/10; Ca or Mg 9.6 mM; K or Na 51.2 mM.)

Al status (ppm)	Growth in	Cations added				
		None	Ca	Mg	K	Na
0.0	Treatment solution (cm)	3.41	6.29	6.15	3.44	3.48
	Recovery solution (cm)	7.06	8.37	8.51	8.48	8.16
4.5	Treatment solution (cm)	0.40	6.72	6.08	2.18	3.88
	Recovery solution (cm)	0.00	9.33	9.47	6.59	8.13

Mg, K and Na added over the control in the sets without any Al (3.41 cm) once more confirms the earlier conclusion drawn from Figure 4 that the nutrient solution was more dilute than the optimum concentration of the nutrient needed for growth of the wheat plant at pH 4.0.

The almost equal growth in extra added K and Na solution in comparison to check (3.44 cm and 3.48 cm vs. 3.41 cm) indicates that the amount of these two cations present even in tenth-strength solution is enough for the growth and they are not the limiting factors. The increase in the root length due to added Ca or Mg (6.29 cm and 6.15 cm for Ca and Mg, respectively, as compared to 3.41 cm for check) indicates that the nutrient solution with tenth strength is lower than needed for these two nutrients. This stimulation of root growth in the treatment solution by Ca or Mg may be

related to the divalent cation protection against H^+ damage (Moore, 1973).

The results in the same Table 4 where 4.5 ppm Al had been added in all treatments (described above) show a different trend. The root length measurement shows that there was no regrowth in the tenth-strength solution with 4.5 ppm Al added and therefore this amount of Al was indeed toxic. However, when 9.6 mM $CaSO_4$ and $MgSO_4$ were added to the treatment solution, the growth in the recovery solution was equivalent to the root growth when Al was not present. Therefore, these cations completely protected the roots of Druchamp from Al damage as they did for Brevor. The results with K are somewhat different. While K did protect the roots considerably from the damage by Al, the root growth both in the treatment solution and the recovery solution was less than the comparable results for the 0 Al treatment. Thus K_2SO_4 at 51.2 mM did not afford the complete protection with Druchamp as it did with Brevor. It is likely that this high a K concentration had an adverse effect on Mg, especially since the treatment solutions were only a tenth strength. On the other hand, adding 51.2 mM Na_2SO_4 to the treatment solution resulted in complete protection of the roots from injury by 4.5 ppm Al.

The experiment with Chinese Spring was of a similar nature and varied only in that the amount of Al added was 7.0 ppm. Results are

Table 5. Effects of different concentrations of cations on Al-toxicity of wheat variety Chinese Spring. (Treatment solution concentration 1/10; Ca or Mg 9.6 mM; K or Na 51.2 mM.)

Al status (ppm)	Growth in	Cations added				
		None	Ca	Mg	K	Na
0.0	Treatment solution (cm)	2.42	6.33	6.41	3.60	3.38
	Recovery solution (cm)	7.94	9.32	9.07	8.17	9.08
7.0	Treatment solution (cm)	0.35	5.76	4.77	0.98	3.56
	Recovery solution (cm)	0.00	9.50	9.12	5.09	8.48

shown in Table 5. It is interesting to note the similarity in the results of Druchamp and Chinese Spring (cf. Table 4 vs. Table 5) with respect to each cation, both with and without Al, and the higher effectiveness of Na compared to K. The concentration of Al is different (4.5 ppm vs. 7.0 ppm) but still the similarity in the results could only confirm the earlier statement that the mechanism involved is the same for the varieties.

Table 6 shows the results with Atlas 66. These results confirm those with the earlier two varieties (Druchamp and Chinese Spring) including the slight superiority of Na over K.

It can, therefore, be safely concluded that Ca, Mg, K, or Na protected all three varieties in the same way as with Brevor and that tolerance to Al was not due to differential response to Ca, Mg, K, or Na but that it varied in degree while the varieties maintained

their relative position on the tolerance scale. These findings further confirm the earlier conclusions that the cation effect in protecting roots against Al toxicity was nonspecific and furthermore not a nutritional response.

Table 6. Effects of different concentrations of cations on Al-toxicity of wheat variety Atlas 66. (Treatment solution concentration 1/10; Ca or Mg 9.6 mM; K or Na 51.2 mM.)

Al status (ppm)	Growth in	Cations added				
		None	Ca	Mg	K	Na
0.0	Treatment solution (cm)	2.33	5.94	6.04	3.48	2.02
	Recovery solution (cm)	8.43	9.10	9.16	9.14	9.28
35.0	Treatment solution (cm)	0.35	3.56	1.22	0.42	0.88
	Recovery solution (cm)	0.00	10.24	8.57	5.23	8.17

GENERAL DISCUSSION

Screening for tolerance to Al

Since Neenan (1960) first showed the differential tolerance of Al among wheat varieties, Foy and co-workers (1965a, 1965b, 1967) have repeatedly demonstrated striking differences among both wheat and barley varieties. Differences have also been observed for wheat and barley varieties by Ikeda et al. (1965), for oat and wheat varieties by Anon. (1967) and for wheat by Fleming and Foy (1968), Kerridge and Kronstad (1968), Kerridge et al. (1971) and Moore (1973).

Fleming and Foy (1968) found that the differential Al tolerance of Atlas 66 and Monon wheat varieties was characterized by internal disorganization and the appearances of binucleate cells in the more sensitive variety (Monon). They further observed that the greater Al tolerance of Atlas 66 (as compared with Monon) was associated with greater ability to continue root elongation and resist morphological damage to root tips and lateral roots when under stress, and to initiate new lateral roots when the stress was removed. On the basis of these observations, they suggested that the inhibition of root development may be a useful biological indicator of Al toxicity on acid subsoils.

Foy et al. (1965a) used soil to screen large numbers of wheat and barley varieties. Reid et al. (1969) also used soil for screening large numbers of barley varieties according to Al tolerance. But the observation made in the result and the discussion section of this dissertation shows that Al toxicity is dependent, besides various recognized factors such as pH, Al concentration, duration of treatment, temperature and variety, on the kind and concentrations of different salts present in the solution. This adds to the disadvantage of using soil where the chemical variables cannot be quantitatively controlled and considerable experimentations must be carried out to insure that Al is the only toxic factor in the particular soil.

Use of nutrient solution for this purpose, has, therefore, many advantages. In addition to allowing an immediate observation of the first obvious effects of Al injury, i.e., the inhibition of root elongation, it insures that the toxicity can be ascribed solely to Al. However, an adequate control of pH and P status of the nutrient solution generally has been overlooked. Kerridge et al. (1971) were first to take into consideration these factors. Their results were limited to measuring total root length in Al solution rather than the regrowth which is much more definitive as pointed out during discussion of Figure 1. Moore (1973) has used recovery of the root following an Al treatment as an

indicator of Al toxicity and has pointed out the necessity for strict pH control because Al toxicity was strongly affected by changes in pH. However, pH control is greatly complicated by hydrolysis, especially at higher Al concentrations. For this reason, the results of this study provide a means of greatly simplifying pH control in screening genetic materials and varieties for tolerance to Al. Other data (Kerridge, 1969; and unpublished data from Moore) show that wheat varieties fall into four groups: sensitive like Brevor, moderately sensitive like Druchamp, moderately tolerant like Chinese Spring and tolerant like Atlas 66. But the above groupings were all done at full strength nutrient solutions. From a practical standpoint, there is no problem in setting up Al solutions for Brevor group or Druchamp group since amount of Al needed is not high. However, the Chinese Spring group required 45-50 ppm Al to inhibit root growth completely. The case with Atlas 66 is even more acute, since 120 ppm Al was required for the same purpose. It is very difficult to control the pH of Al solutions of these concentrations, because of the hydrolysis of Al. Since the addition of this much Al lowers the pH below 4.0, to bring it back to pH 4.0 requires the addition of large amounts of base which results in localized high pH before it is completely mixed even in vigorously agitated solution. This localized high pH causes precipitation of $Al(OH)_3$ which does not readily redissolve.

Alternatively, dilute base could be added to prevent the localized high pH effect and precipitation of $Al(OH)_3$ but maintenance of exact volume and concentrations of nutrient elements in the final solutions becomes a problem. Furthermore, adjusting the pH in this way is a very time consuming process. In addition to this, a concentration of 120 ppm Al is almost approaching the solubility limit of $Al(OH)_3$ at pH 4.0 and the pH adjustment even with dilute base results in precipitation. These problems can be easily overcome by using lower strength of nutrient solutions where the Al concentration needed is greatly reduced. Also pH adjustment does not require use of base in the dilute solutions but rather acid is used to bring pH down to 4.0. The results of this dissertation, therefore, can be used in the following way for screening wheat varieties.

To separate sensitive (Brevor) from moderately sensitive (Durchamp), seedlings can be exposed to 3 to 6 ppm Al for 48 hours in 1/4 nutrient solution at pH 4.0 and 25°C. Roots showing no regrowth in the recovery solution are sensitive (Brevor) and roots that grow are moderately sensitive or higher (Druchamp, Chinese Spring or Atlas 66 types). This is because 1.5 ppm is the critical concentration for the Brevor type and about 8 ppm is for the Druchamp type. Therefore, any concentration in between should separate these two types simply on the basis of whether the roots

regrow or not in the recovery solution. Alternatively, at 1/10 strength of nutrient solution, keeping other factors constant, separation can be made at 1 to 3 ppm Al since the root growth of Brevor is inhibited at 0.4 ppm and Druchamp at 4.0 ppm. An additional advantage of this approach is that the root length does not need to be measured. It is necessary to only separate those roots that recover from those that do not by simple visual inspection.

Similarly, the separation of moderately sensitive types (Druchamp) from moderately tolerant types (Chinese Spring) could be done at 1/10 strength, 25°C, pH 4.0 and a concentration of Al of about 5.0 ppm since this would completely inhibit Brevor and Druchamp types but not Chinese Spring and Atlas 66 types.

Following the same rationale, moderately tolerant Chinese Spring types could be separated from tolerant Atlas 66 types when the plants are grown in 1/10 solution, pH 4.0, 25°C, and 10 to 20 ppm Al. All that regrow would be tolerant like Atlas 66 and all that did not grow would be Brevor, Druchamp and/or Chinese Spring types.

The same information can be used in an identical manner to look for more sensitive types than Brevor. Any plants whose roots fail to recover when exposed to an Al concentration below the critical concentration for Brevor would be more sensitive than Brevor. For instance, one could use 1.0 ppm Al in 1/4 strength

solution at pH 4.0 and 25°C. One can use 1/10 strength too, but in that case the amount of Al needed is less than 0.3 ppm, and so the first one, that is the 1/4 nutrient solution, is preferable because the Al concentration could be more accurately and easily maintained. A higher strength of nutrient solution with a higher corresponding critical level of Al could also be selected; e.g., with full strength, the critical concentration is 7.0 ppm and so a selection of 5.0 ppm should serve the same purpose.

In a likewise manner, types more tolerant than Atlas 66 could be identified when roots are found growing even at, say, 35.0 ppm in 1/10 nutrient solution at pH 4.0 and 25°C. The procedure could as well be used to identify intermediate types just by varying Al concentration in an appropriate range to establish the reaction of the roots to Al. It cannot be overemphasized, however, the necessity for maintaining strict control of pH, Al concentration, temperature, and salt concentration if this procedure is to be reproducible. Also, it does have the advantage of being adaptable to large populations.

Nutritional relationships

The other main contribution of this dissertation is to show that the roots can be completely protected from the adverse effects of Al by increasing the salts concentration. The effect of salts

appears to be nonspecific since Ca, Mg, K, or Na were all effective. All varieties behaved the same way and maintained their position relative to one another. The symptoms of Al toxicity (inhibition of root growth) could be obtained by either increasing the Al concentration or by decreasing the salt concentration in all the varieties. This similarity suggests that the mechanism of tolerance could be overcome by more Al or less salts. This also suggests that it is the amount that gets into the meristematic cells which is important in determining the damage done. The amount that gets into these cells is presumably dependent on the concentration of Al in the solution, salt concentrations, pH, temperature, duration of treatment and the variety.

There are two possible explanations of how the salt concentrations can affect the amount of Al which gets into the root meristematic cells: (a) Permeability of the cell membrane, and (b) Nonspecific cation competition. A brief consideration of these are dealt with here separately.

(a) Permeability of cell membrane: It has been well established that the cell membrane maintains a low permeability to many solutes as long as the cells are healthy and rapidly metabolizing. If the cells are injured in any of a number of ways, e.g., by heat, ionizing radiation, extreme pH changes, excess electrical stimulation or by placing them in improperly balanced salt

solutions, the permeability rises. Experimental evidence suggests that a portion of the root is readily accessible to the external solution and that ions may move by diffusion and mass flow into these areas. This space has been defined as "free space" and includes both the "water free space" and the "Donnan free space" (Briggs et al., 1958). It has been suggested that ions are free to move passively into portions of the root especially into the meristematic cells of the root tip from the external medium (Handly and Overstreet, 1963). The epidermis may constitute a partial barrier (but is probably incomplete) and ions can move into the cortex along the wall and through the intercellular spaces. The cell wall and the interstices of the cortex are freely accessible to ions, but the cytoplasm (and vacuoles) of mature cells probably must be excluded except under conditions where the permeability of the plasmalemma is increased. Arisz (1963, 1964), Handly and Overstreet (1963), Hiatt and Low (1967), all have demonstrated that the permeability of the plasmalemma is dependent on the ionic composition of the bathing medium and, therefore, may become permeable under some experimental conditions.

Hydrogen ion has a marked effect on the permeability of cells (Moore, 1973) and under conditions of low pH, cells are known to become quite "leaky" (Fawzy et al., 1954; Jacobson et al., 1957; 1960; Moore et al., 1961a, 1961b). Ca, Mg and other polyvalent

cations tend to offset the injurious effects of H^+ and exert a major influence on maintaining the integrity of cell membranes and the selectivity of the ion absorption process (Fawzy, et al., 1954; Epstein, 1961; Brandt and Freeman, 1967). All of the experiments reported in this study were done at pH 4.0 and under these conditions the meristematic cells might be relatively permeable to Al especially in the dilute solutions such as tenth and quarter strength solutions where Al was shown to be the most toxic. Increasing the strength of nutrient solution or increasing the Ca, Mg, K or Na individually might decrease the permeability of the cells and reduce the amount of Al entering the meristematic cells. The greater effectiveness of the divalent cations, Ca and Mg, is consistent with the common recognition that polyvalent cations generally decrease protoplasmic permeability (Batiste, 1935). The permeability hypothesis, however, is not consistent with the idea that monovalent salt solutions, e. g., K or Na, tend to increase the permeability of tissue (Baptist, 1935), rather than to decrease it. It should be kept in mind, however, that in the present study, the cations were being increased in an otherwise balanced nutrient solution and might have behaved differently than in a single salt solution. For instance, Li and Na had a much different effect on K uptake by barley roots when Ca and Mg were present in the solution than when they were absent (Jacobson et al., 1960).

(b) Nonspecific cation competition: An interesting aspect of Al toxicity is its interaction with other nutrient elements especially those taken up in cationic forms. Although Jones (1961) proposed an anionic form of Al, viz., aluminate being taken up from high pH medium (pH > 7), the solubility and the cationic form suggests its being taken up as a cation in acid pH range. A strong support of these postulates comes from the studies of Rorison (1958), Clarkson (1966a) and Rasmussen (1968) on one side and Waisel et al. (1970) on the other side. The first three workers have used acidic pH in their experiments where Al is present in cationic form and as such it formed a precipitate with the PO_4 groups. Rasmussen (1968) using electron microprobe X-ray analysis, studied the mode of entry, distribution and localization of Al in corn plants. He found Al precipitated on the epidermal cells of the root. From the fact that the localization of P was exactly the same as that of Al, he inferred that there was a precipitation of P by Al. As a check, he ran a similar analysis for Ca and P on control plants and no such localization was observed giving further support to the assumption made. Waisel et al. (1970), on the one hand, using pH 9 but the same X-ray microanalysis technique, found no correlation between the distribution of Al and P and concluded that Al PO_4's are formed neither at the free space nor in osmotic space. These observations, though apparently contradictory, become meaningful when the form of Al at different pH is taken

into consideration.

In the acidic pH range, where Al is present in the cationic form, its depressing effects on the uptake of almost all cations have been reported. Horstein and Fiskell (1961), Takahashi (1963), Johnson and Jackson (1964), Lance and Pearson (1969), Clarkson and Sanderson (1971), Foy et al. (1974), all reported a decrease in uptake of Ca by plants due to Al. Peive and Rinkins (1962) reported a decrease in the uptake of almost every essential cation, viz., Ca, Mg, Zn, Mn and Mo. Similar were the observations for Ca, Mn, Fe and Zn by Paterson (1965); for Ca, Mg and K by Macleod and Jackson (1967); and for Ca, Mg, K, and Zn by Lee (1971). Hiatt, Amos and Massy (1963) reported that Al concentrations as low as 0.1 ppm markedly reduced total Cu uptake by excised wheat roots. The reverse effect of cations on Al toxicity is also well documented. Oullette and Dessureaux (1958), Lund (1970), and Clarkson and Sanderson (1971) observed similar detoxifying effects of Mg on Al. A K-effect was reported by Aimi and Murakami (1964), an Fe-effect was reported by Tanaka and Navasero (1966), and Otsuka (1968), while Cate and Sukhai (1964) reported the Al toxicity was generally reduced by neutral salts.

From these many diverse effects reported it might be reasonable to conclude that Al and a number of nutrients might be competing with one another for entry into the plant's roots.

Mutual competition between pairs of cations for entry into plant roots is a common phenomenon (Epstein and Hagen, 1952; Jacobson et al., 1960; Moore et al., 1961; Epstein, 1962; Moore, 1964; Alam et al., 1965a, 1965b; and Lee, 1971) and if Al is taken up in a manner similar to other cations, competition could occur. Lee (1971) showed that potato roots accumulated Al when grown in Al solutions. Chen (1968) observed an increase in Al content of rice roots, straw and leaves increased with increasing concentrations of Al in the media. Otsuka (1968) observed an increase in Al content in the roots of wheat, barley and rye varieties with the increase of Al concentration in the nutrient solutions. In the light of these observations of increased uptake of Al with the increase of its concentrations in the growth media, it is reasonable to assume that the root growth was inhibited because of greater uptake of Al into the meristematic cells when its concentration was increased in the nutrient medium. A nonspecific mechanism of cation competition would explain why when the strength of the nutrient solution was reduced, Al became more toxic. The decrease in the strength of cations as a whole when the strength of the nutrient solution was reduced would allow Al a greater chance to enter the root and thus cause more damage. The same reasoning applies to the decreasing effect on Al toxicity of increasing Ca, Mg, K and Na individually. These results lead to the conclusion that Al uptake and hence the

toxicity might be a nonspecific, reversible cation competition phenomenon, a conclusion already reached by Gangwar (1967) when he studied the adsorption of Al, Ca, and K on the roots of crop plants and reported that the adsorption of these cations were reversible and nonspecific in nature.

The results showing the need for 9.6 mM Ca or Mg and 51.2 mM K or Na to eliminate the toxicity caused by the same amount of Al do suggest that the valence of the cations protecting the roots from Al injury is an important factor in keeping Al out of the root through competition though Al toxicity may be governed by a non-specific cation competition principle.

In many of the studies reported by others, pH control has not been adequate and it is not possible to separate the H^+ variable introduced with the Al variable. Al undergoes hydrolysis, and unless the pH is deliberately controlled, H^+ will always be a variable. H^+ has a marked effect on cation uptake by plants (Arnon et al., 1942; Fawzy et al., 1954; Jacobson et al., 1957; Rains et al. 1964; Marschner et al., 1966; and Kerridge, 1969) and many of the Al effects on cation uptake could be due in part to the H^+ variable. In addition, since Al inhibits root growth many of the reported effects of Al on nutrient uptake could be due to this reduced root growth. This is especially true where tolerant varieties have been

compared with sensitive varieties. It is not at all surprising that nutrient uptake is less affected by Al in a tolerant plant than in a sensitive plant when it is recognized that the roots of the tolerant plant continue to grow and the sensitive one is inhibited. This criticism, however, does not apply in the present study. There was rigorous control of pH in all the solutions and, in addition, the criterion of Al toxicity used in this study was its effect on root growth. This is the primary effect of Al toxicity and all the reported nutritional effects could be an indirect result of the damage done to the root system by Al.

The results of this study provide strong evidence that the primary effect of Al toxicity is not due to an interference in uptake of Ca, Mg, or K (i.e., to a deficiency of these nutrients) as has been proposed (Rorison, 1958; Maclean and Chiasson, 1966; Lee, 1971 and Foy et al., 1972). If the inhibition of the root growth was due to Al interference in Ca uptake, then increasing Mg, K or Na would not protect the roots from the adverse effects of Al. By the same token, the effect cannot be due to a Mg deficiency since the roots could be protected by increasing the Ca, K or Na. Finally, the root inhibition cannot be due to K deficiency since the inhibition could be overcome by increasing the Ca, Mg or Na concentration. Such an argument does not rule out that once the Al is inside the cell, it could interfere in some process where Ca, Mg or K

played an essential role. However, it would not seem possible to overcome the adverse effect of Al on, for instance, Ca utilization inside the cell in some indispensible reaction by increasing the supply of Mg, K or Na. The most logical interpretation is that Ca, Mg, K or Na prevents Al damage to the roots by preventing Al from getting into the meristematic cells and reaching some critical site.

Phosphorus-aluminum interaction

Because of the appearance of Al toxicity as P deficiency in plants grown on acid soils or in nutrient solutions (Foy and Brown, 1963, 1964) and the effectiveness of excess P to precipitate and detoxify Al (Munns, 1965), an Al-P interaction has often been proposed as the mechanism responsible for Al damage to plants. Wright and Donahue (1953) using ^{32}P and barley plants grown in solution culture with and without Al, concluded that Al inactivates P primarily within the roots of the plants and thus interferes with the normal P metabolism of plants. In earlier papers, Wright (1948, 1952) had reported that Al precipitated P internally in barley roots and thereby caused P deficiency in tops. A similar conclusion was reached by Mcleod and Jackson (1967) who reported that P appeared to be immobilized by Al in barley roots. Rasmussen (1968) using electron microprobe X-ray analysis observed the same localizations for Al and P and suggested an Al-P precipitation in

plants. Clarkson (1966b) proposed two types of possible Al-P interactions in the plant system: one occurred at the cell surface and resulted in fixation of PO_4 by an absorption precipitation reaction while the other occurred within the cell, possibly within the mitochondria and resulted in a marked decrease in the rate of sugar phosphorylation probably affected by the inhibition of hexokinase. Along with these reports of Al-P precipitation, there are reports suggesting increased P uptake and translocation due to Al (Ragland and Coleman, 1962; Randal and Vose, 1963, Medappa and Dana, 1968). On the other hand, using a divided root technique with two wheat varieties (one sensitive and the other resistant) Cruz et al. (1967a) showed that Al did not have any effect on the enhancement of P uptake. Clarkson (1966b) also reached a similar conclusion stating Al did not have any effect on the enhancement of P uptake. Waisel et al. (1970) using X-ray microanalysis found no correlation between distribution and localization of Al and P in plants. Of course, their findings have severe experimental limitations and that is of high pH (about 9).

Because of these uncertainties about the Al-P relationship and the effect of P on Al-toxicity, the present investigation was designed to eliminate any direct contact between Al and P in the nutrient solutions. In the light of the findings of this dissertation where Al toxicity was directly dependent upon the concentration of

Al in the solution (Figure 1), variety (Appendix Table 1) and cation concentration of the solutions (Figures 10 and 11) even in the absence of P, it could be stated that Al toxicity was independent of the presence of P and its uptake from the nutrient solution. The same conclusion was reached by Ruschel et al. (1968) when they suggested that Al toxicity was due to Al and not due to its action on P. Furthermore, the suggestion that a phosphorous pump is one of the mechanisms responsible for controlling Al-toxicity (Sivasubramanian and Talibuddin, 1971) does not appear to be reasonable because the Al-toxicity was evident even in absence of P.

Using the same reasoning as before, since Ca, Mg, K or Na were nonspecifically able to relieve the stress produced by Al, it can be argued that Al toxicity cannot be due to a phosphorous deficiency per se or to an interference in P uptake. Again, this does not rule out the possibility that once Al is inside the cell it may interfere with the metabolism of P at some specific site.

Mode of action

The toxicity of Al in terms of inhibition of cell division and consequent root elongation can be better understood when viewed through its site and mode of action. Most of the evidence regarding the site of action is indirect but convincing. On the basis of biochemical results, Clarkson (1968) suggested two possible sites in

cells where Al might be acting, viz., mitochondria and nucleus, both DNA rich sites. Klimashevskii et al. (1972) working on the genotypical specificity of localization of Al in pea root cells also reported that Al ions have two main inhibitory regions--one in the mitochondria and the other in the nucleus, where metabolism is disturbed by Al in susceptible plants. Foy et al. (1972) working on two widely differing Al tolerant snapbean varieties, fractionated the subcellular constituents of the roots. They showed that though grown in the same nutrient solutions, the cell walls, nuclei and mitochondria of sensitive varieties took up more Al than tolerant ones and concluded that these actions of Al on subcellular levels might be influencing the varietal resistance. Even less is known about the mode of action of Al directly. Lance (1968) postulated that Al might be acting through the replacement of Ca and causing the alteration in the structural configuration or macromolecules. The macromolecules, according to Moratwetz (1972) are of two broad classes. The crucially important class for living organisms have their molecular chains folded in a highly specific manner, so that the molecules assume a well-defined shape. Enzymes, RNA and DNA fall under this category. The double helical structure of DNA, as first described by Watson and Crick (1953), endows it with its unique role as a code which allows the genetic message to be handed down with high precision through a large number of cell

divisions. DNA is stable in aqueous solution under physiological conditions, but the range of its stability is limited by temperature, pH and the ionic environment. As with polypeptides, the helix-coil transition of DNA is a strongly cooperative process, so that "melting" takes place rather sharply with a change of an external parameter such as temperature or pH or ionic environment. The effect of Al could be seen in terms of changing the ionic environment inside the cell. DuPraw (1970) noted that DNA supercoiling is markedly influenced by the ionic environment. One of the primary mechanisms for this ion effect is the fact that each DNA phosphate carries a negative charge; in the absence of positively charged counterions, the negative charges repel one another forcing the double helix into an extended (unpacked) configuration (or even inducing separation of the two complimentary strands). When positively charged counterions are present, they bind to the phosphates and confer electrical neutrality, however, monovalent cations such as Na^{+} are thought to form complexes of simpler structure than divalent cations such as Ca^{++}. According to Anderson and Norris (1960) the divalent cations Ca^{++} and Mg^{++} induce or preserve a condensed state, whereas the monovalent cations Na^{+} and K^{+} induce swelling. Similar was the observation of Huberman and Attardi (1966) who observed that the HeLa chromosome tended to contract as the concentration of divalent cations was raised, or as the pH was lowered; this contraction can be reversed in the first case by

removing the ions with a chelating agent, and in the second case by raising the pH. All these observations suggest that the vicinity of DNA is freely approachable to cations and the configuration of it is highly dependent on the charges of these cations. On the basis of the evidences provided by Eichhorn (1962) that metal cations bound to DNA increase the stability of the double helix, Clarkson and Sanderson (1969) have suggested that the observed interference in DNA replication induced by Al takes place by cross-linking the polymers which increases the rigidity of the DNA double helix.

A mechanism whereby Al interfered with normal DNA processes could account for the observation in this study that a toxic level of Al resulted in a complete and irreversible inhibition of root growth. If once the Al were inside a meristematic cell, it interfered with DNA replication, the net result would be an inhibition of root growth. Again, the nonspecific protection against the Al damage by Ca, Mg, K or Na would suggest that the Al is prevented from getting inside rather than preventing the damage once the Al is inside the cell.

Cause of varietal tolerance to Al

Attempts have been made to explain varietal differences in terms of damage caused by Al to the roots, its uptake and transport, plant induced pH changes in root zones, other cations uptake and

utilization by plants as well as genetically controlled exclusion and complexing of Al together with or without greater sensitivity to same amount of Al. Fleming and Foy (1968) found that greater Al tolerance in Atlas 66 wheat (as compared with Monon, a sensitive variety) was associated with a greater ability to continue root elongation and resist morphological damage to root tips and lateral roots when under stress, and to initiate new lateral roots when the stress was removed. Differential Al tolerance of Atlas 66 and Monon wheat was also characterized by greater internal disorganization and the appearances of binucleate cells in the more sensitive variety (Monon). Their comment that the varietal differences observed resulted from a series of events that started at cellular levels though highly appropriate, the disorganization of the root structure seems to be the result rather than the cause of Al sensitivity as discussed presently.

Differential Al tolerance among wheat varieties has also been attributed to the ability to alter the pH of their root zone. Foy et al. (1965b, 1967) showed that the Al sensitive Monon wheat variety induced lower pH values in the growth media than did the Al tolerant Atlas 66. Otsuka (1968) reported identical observation where Al tolerant wheat variety (Hiraki) raised the pH of its growth media while an Al sensitive variety (Norin) lowered it. Similar results have been confirmed for rice (Subramoney and Sankaranarayanan,

1964) and cotton (Adams and Pearson, 1970). But the suggestion that a lower plant induced pH in the root zone increases the solubility and therefore the potential toxicity does not seem to apply for soybean or snapbean varieties when Foy et al. (1969) and also Foy et al. (1972) showed that Al tolerance in these crops was independent of Ph changes induced by the plants in the nutrient cultures.

The results reported in this thesis would tend to rule out pH changes brought about by the activity of the roots as being a factor in tolerance to Al. First, the pH was rigidly controlled at 4.0 and yet the varieties exhibited substantial differences in tolerance. Secondly, the amount of Al in solution was well below the solubility limit of $Al(OH)_3$ and pH changes could not affect the amount of soluble Al as was the case in other studies where pH was allowed to fluctuate. In comparing tolerant and sensitive varieties, pH changes brought about by root activity would no doubt be affected by the amount of root growth. It would not be surprising that the tolerant variety would increase the pH more than the sensitive variety as reported by Foy et al. (1965b, 1967) since the tolerant variety's roots were still growing well in the Al solution and the sensitive one's were not. Such a mechanism of tolerance does not seem reasonable in light of the cation effects reported here since tolerance was shown to be relative and not absolute. For instance, by the proper choice of nutrient solutions it would be possible to

show that a given amount of Al was more toxic to a tolerant variety than the same amount of Al would be to a sensitive variety in another strength of solution.

The findings of Kerridge and Kronstad (1968) that the Al tolerance of Druchamp over Brevor was due to a single gene has led Moore (1973) to suggest that the gene might be acting in keeping Al from entering the cell or an exclusion phenomenon. The Al tolerance of barley has also been shown by Reid et al. (1968) to be simply genetically controlled. The observations that reducing the competing cations or increasing pH or temperature increased Al toxicity for all varieties proportionately suggest that it is the amount of Al which gets into the meristematic cells that is the controlling factor. This would also suggest that the varieties differ in some inherent way in the amount of Al that gets into these cells, that is, the tolerant varieties tend to exclude Al. Foy et al. (1965b, 1967) have shown that the varietal Al tolerance is due to differences in uptake rate. Klimashevskii et al. (1972) showed that genotypes of peas varying in their Al tolerance have differential capacities to accumulate Al in mitochondria. They observed that the sensitive varieties assimilated and accumulated Al at least $\simeq$ 50 percent more intensively than did the resistant plants and that the root mitochondria of the same differed more widely ($\geqslant$ 3 fold). Similar was the observation of Foy et al. (1972) who found greater Al in

mitochondria of a sensitive snapbean variety than in the tolerant one though grown in the same nutrient solutions. The suggestion that the inhibition of root elongation by Al in alfalfa was associated with rapid uptake of Al led Rorison (1958) to suggest that the tolerant plants might take up Al more slowly. Besides uptake, the translocation of Al from root to tops also seems to be involved in Al resistant. Macleod and Jackson (1967), Foy et al. (1967) reported that Al accumulated more in the roots but not in the tops of sensitive plants. Almost opposite was the conclusion of Oullette and Dessuraeux (1958) who found that Al tolerant alfalfa clones contained lower concentration of Al in their tops and higher concentrations of Al and Ca in their roots than did Al sensitive clones. Ca was believed to reduce the Al toxicity. Foy et al. (1969) reported that Al tolerant Perry and sensitive Chief soybean varieties accumulated Al equally in their roots.

In addition to uptake and transport, Al-toxicity has also been postulated to be a function of internal complexing. Clarkson (1969) while studying the toxic effect of trivalent cations, viz., Al, Ga, Y and La observed that though all of these cations produced similar inhibitory results on cell division, the resistance to Al in Agrostis setaceae and Secale cereale was ion specific, and they suggested that these genera have developed some kind of complexing mechanism within the cells for Al ions specially. This complexing as

suggested earlier by the same author (Clarkson, 1967) is mostly confined within the cell wall and would presumably keep Al from entering the cell. Foy et al. (1972) have also shown that cell wall and not the intracellular materials (excluding mitochondria and nuclei) can complex Al in a resistant variety. It is difficult to see, however, how complexing of Al can explain the substantial shifts in Al tolerance due to changes in the ionic strength of the nutrient solution. If Atlas 66 is tolerant because it complexes more Al than the other varieties, then this should not be influenced by changes in the concentration of competing cations in the treatment solution.

SUMMARY AND CONCLUSIONS

Aluminum toxicity to four varieties of wheat (Brevor, Druchamp, Chinese Spring and Atlas 66 representing different classes of Al tolerance) was studied in the growth chamber under rigidly controlled conditions. All the experiments were conducted in nutrient solution at pH 4.0 and root recovery following an exposure to Al-containing solutions was used as an indicator of the degree of Al toxicity. This root regrowth was a much more sensitive measure of Al damage than other procedures reported so far and allowed a precise selection of the amount of Al needed to cause irreversible damage to root growth. The results showed that Al toxicity was dependent on the concentration of the nutrient ions and the toxicity of Al increased with a decrease in the concentration of the nutrients in the solution. The same trend of increasing Al toxicity with decreasing concentration of nutrients was observed for all four differentially tolerant varieties suggesting that wheat varietal tolerance to Al was a relative rather than absolute characteristic.

The results also showed that Al toxicity could be almost completely overcome by adding extra Ca, Mg, K, or Na in the nutrient solution. The effect of Ca or Mg was practically identical with respect to overcoming the toxicity and both were more effective

than either K or Na which were almost identical in their effectiveness. This indicated that the valence of the cations was of primary importance in overcoming Al toxicity. These findings suggest a nonspecific cation phenomenon and show that Al toxicity could not be due to a deficiency of Ca, Mg, K, or even P as reported in the literature.

It was postulated that the effect of cations in overcoming Al toxicity was due to a mechanism of keeping Al out of the meristematic cells through either nonspecific cation competition or a nonspecific reduction in cell permeability.

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APPENDIX

Appendix Table 1. Effects of different concentrations of Al on growth and regrowth of root of the variety Brevor at different strength of nutrient solutions. (Both treatment and recovery solutions of same strength.)

Growth in	Al concentrations in ppm									
	0.0	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
				Full strength						
Treatment solution (cm)	6.79	-	-	1.23	-	0.88	0.59	0.51	0.57	0.45
Recovery solution (cm)	8.93	-	-	4.28	-	4.14	0.98	0.22	0.00	0.00
				Half strength						
Treatment solution (cm)	6.79	-	-	0.61	-	0.48	0.39	0.33	0.38	0.29
Recovery solution (cm)	9.08	-	-	2.94	-	0.43	0.00	0.00	0.00	0.00
				Quarter strength						
Treatment solution (cm)	5.29	-	0.64	0.38	0.34	0.34	-	0.22	-	0.23
Recovery solution (cm)	8.12	-	0.75	0.00	0.00	0.00	-	0.00	-	0.00
				Tenth strength						
Treatment solution (cm)	2.96	0.36	0.25	0.25	0.23	0.29	-	0.37	-	-
Recovery solution (cm)	4.93	0.025	0.00	0.00	0.00	0.00	-	0.00	-	-

Appendix Table 2. Effects of different concentrations of Al on growth and regrowth of roots of the variety Druchamp at different strength of nutrient solutions. (Both treatment and recovery solutions of same strength.)

Growth in	Al concentrations in ppm									
	0.0	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
				Full strength						
Treatment solution (cm)	7.18	-	-	5.35	-	4.66	5.15	3.14	3.65	2.71
Recovery solution (cm)	8.08	-	-	8.68	-	7.07	9.49	7.69	8.27	7.02
				Half strength						
Treatment solution (cm)	5.96	-	-	5.22	-	4.58	3.84	2.57	1.74	1.16
Recovery solution (cm)	7.92	-	-	9.22	-	8.80	8.45	7.26	6.32	5.51
				Quarter strength						
Treatment solution (cm)	5.52	-	4.47	2.64	1.75	0.76	-	0.44	-	0.26
Recovery solution (cm)	7.18	-	7.23	5.67	4.87	3.60	-	1.74	-	0.28
				Tenth strength						
Treatment solution (cm)	3.09	2.07	1.11	0.39	0.30	0.36	-	0.28		
Recovery solution (cm)	5.00	4.50	3.62	1.78	0.11	0.00	-	0.00		

Appendix table 3. A comparison of tenth strength vs. full strength initial/recovery nutrient solutions on Al toxicity of varieties Brevor and Druchamp.

Strength	Growth in	Al concentrations in ppm						
		0.0	0.5	1.0	2.0	3.0	4.0	6.0
					Brevor			
Tenth	Treatment solution (cm)	2.96	0.36	0.25	0.25	0.23	0.29	0.37
	Recovery solution (cm)	4.93	0.025	0.00	0.00	0.00	0.00	0.00
Full	Treatment solution (cm)	2.47	0.52	0.32	0.27	0.26	0.27	0.22
	Recovery solution (cm)	8.71	0.00	0.00	0.00	0.00	0.00	0.00
					Druchamp			
Tenth	Treatment solution (cm)	3.09	2.07	1.11	0.39	0.30	0.36	0.28
	Recovery solution (cm)	5.00	4.50	3.62	1.78	0.11	0.00	0.00
Full	Treatment solution (cm)	3.15	2.19	1.12	0.40	0.46	0.48	0.31
	Recovery solution (cm)	7.63	6.66	4.13	1.76	0.18	0.00	0.00

Appendix Table 4. Effects of different concentrations of Al on growth and regrowth of roots of the variety Brevor at quarter and tenth-strengths nutrient solution. (Initial/recovery solution full strength.)

Growth in	Al concentrations in ppm							
	0.0	0.2	0.3	0.4	0.5	1.0	1.5	2.0
			Quarter strength					
Treatment solution (cm)	2.09	-	-	-	1.43	0.79	0.78	1.21
Recovery solution (cm)	11.69	-	-	-	2.66	0.34	0.00	0.00
			Tenth strength					
Treatment solution (cm)	1.03	0.92	0.67	0.62	0.64			
Recovery solution (cm)	7.28	1.86	0.36	0.00	0.00			

Appendix Table 5. Effects of adding macronutrients and Al as chlorides or as sulfates on Al toxicity in the variety Brevor at quarter-strength nutrient solution.

Growth in	Al concentrations in ppm					
	0.0	1.25	1.50	1.75	2.00	2.25
			Chlorides			
Treatment solution (cm)	3.50	2.52	1.68	1.43	1.30	1.16
Recovery solution (cm)	6.44	4.10	3.80	3.47	3.12	3.28
			Sulfates			
Treatment solution (cm)	2.89	0.88	0.69	0.76	0.62	0.72
Recovery solution (cm)	6.86	2.77	1.80	1.47	0.79	0.00

Appendix Table 6. Effects of different levels of Ca and Mg on root growth and recovery from Al toxicity. (Variety, Brevor; nutrient solution, quarter strength.)

Al (ppm)	Growth in	Levels of cations added (mM)						
		0.0	0.4	0.8	1.6	3.2	6.4	9.6
					Calcium			
0.0	Treatment solution (cm)	4.02	5.31	5.18	5.55	6.02	6.52	5.29
	Recovery solution (cm)	8.50	9.04	8.93	9.47	9.37	9.59	9.42
1.5	Treatment solution (cm)	0.37	0.47	0.63	1.15	2.58	5.39	4.18
	Recovery solution (cm)	0.00	0.00	2.65	4.72	5.69	8.21	8.86
					Magnesium			
0.0	Treatment solution (cm)	3.66	5.42	-	6.0	5.54	6.81	4.57
	Recovery solution (cm)	8.38	9.92	-	9.89	9.35	8.35	9.87
1.5	Treatment solution (cm)	0.40	0.44	-	0.82	1.82	4.07	5.08
	Recovery solution (cm)	0.00	0.00	-	4.65	5.88	8.82	9.36

Appendix Table 7. Effects of different levels of K and Na on root growth and recovery from Al toxicity. (Variety, Brevor; nutrient solution, quarter strength.)

Al (ppm)	Growth in	Levels of cations added (mM)			
		0.0	12.8	25.6	51.2
			Potassium		
0.0	Treatment solution (cm)	5.41	4.96	5.43	3.82
	Recovery solution (cm)	8.09	6.45	5.89	5.82
1.50	Treatment solution (cm)	0.42	1.34	2.14	3.28
	Recovery solution (cm)	0.00	4.13	6.05	4.87
			Sodium		
0.0	Treatment solution (cm)	4.86	4.73	4.89	4.70
	Recovery solution (cm)	8.81	8.86	8.61	8.82
1.50	Treatment solution (cm)	0.35	0.95	1.78	3.91
	Recovery solution (cm)	0.00	5.08	5.58	7.85