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I am submitting herewith a dissertation written by Gonasageran Naidoo entitled "Aluminum Toxicity in Two Snapbean Varieties." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant and Soil Science.

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ALUMINUM TOXICITY IN TWO SNAPBEAN VARIETIES

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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Gonasageran Naidoo

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ABSTRACT

The mechanism of Al tolerance in plants was investigated in a series of greenhouse experiments which compared two varieties of snapbeans (Phaseolus vulgaris L.) that were differentially tolerant to Al. The more tolerant 'Dade' variety was compared to 'Romano' with respect to growth, mineral nutrition, pH, total acidity, Al fixation by root macerates, organic acid content, morphological effects of Al, and to location of Al and P in roots.

To compare the two varieties with respect to growth and mineral nutrition, seedlings were grown in one-fifth strength Hoagland's nutrient solution (pH 4.8) at 0, 4, 8 and 12 ppm added Al for 10 days. The Al treatments reduced yields and the concentrations of P and Mg in roots and tops of both varieties. In both varieties, the Al treatments generally increased the K concentration in roots and tops. In the Romano variety Al decreased the Ca concentration in roots and tops. The Al-tolerant Dade variety had a significantly higher root weight than Romano at 12 ppm added Al.

Comparison of the pH, titratable acidity, Al fixing capacity of root macerates and citric and malic acid concentrations revealed no major differences between varieties.

Plants of both varieties treated for 12 days at 20 ppm Al at pH 4.6 exhibited typical symptoms of Al toxicity. These included upward curling of roots; root discoloration; significant reductions in root and top growth; inhibition of lateral root growth; abnormally dark green leaves

and gelatinous root tips. The Romano variety also showed purple coloration of stems and petioles. Anatomical changes induced by Al included swelling of root tips; curling backward and detachment of root caps; disorganization of cells in the root cap and meristem and loss of cell contents of root cap cells. The relative degree of Al injury was greater in the susceptible Romano variety.

Elemental distribution in Dade and Romano snapbean roots, treated for 12 days with 20 ppm added Al, was determined by staining with Mo and by energy dispersive analysis of X-rays generated in the scanning electron microscope. The results of both techniques revealed that young meristematic tissues of the root tip are the sites of Al action. The location of Al was identical to that for P, suggesting the precipitation of P by Al. High concentrations of Al and P were found on the root surface and within the root cap. There was no relationship between the distribution of Al and that of Ca, Mg or K. Aluminum was located in cell walls, cell contents and in nuclei of root cap and meristematic cells. It is suggested that Al disrupts the cell division process in the nucleus.

The ability to maintain adequate levels of P in tops, and the capacity to exclude a major part of the Al from sensitive root tissues by regeneration of root cap cells probably account for the greater tolerance of the Dade variety to Al.

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CHAPTER I

INTRODUCTION

Hartwell and Pember (1918) were the first to suggest that Al was the probable cause of the infertility of many acid soils. Since then numerous workers (Magistad, 1925; Rorison, 1958; Foy and Brown, 1964; Clarkson, 1965; MacLean and Chiasson, 1966; Lance and Pearson, 1969) have verified the harmful effects of soluble Al on plant growth.

Plant species differ widely in their tolerance to Al, both in nutrient solutions and in acid soils containing high levels of exchangeable or soluble Al (McLean and Gilbert, 1927; Jones, 1961; Foy and Brown, 1964). The nature of this differential tolerance is, as yet, not understood. Available evidence indicates that inhibition of root growth is the primary symptom of Al toxicity in susceptible species. Inhibition of root growth is generally associated with decreases in the uptake and utilization of P (Wright, 1943; Randall and Vose, 1963; Munns, 1965; Clarkson, 1966a; Andrew et al., 1973) and Ca (Johnson and Jackson, 1964; Munns, 1965; Foy et al., 1969; Lance and Pearson, 1969). In addition, the accumulation and distribution of various other mineral elements are often strongly affected by Al (Harward et al., 1955; MacLeod and Jackson, 1967; Ota, 1968; Lee, 1971). However, the manner in which Al influences the accumulation and distribution of mineral elements is not known. The exact location of Al injury within the roots has been subject to much controversy. The study of the uptake and distribution of Al in plants has been limited by the absence of a suitable radioactive isotope and by

the imprecision of analytical methods (Jackson, 1967). In many instances reports on the effects of Al on plant growth are conflicting. Contradictory results may be attributed to the use of differing techniques, to the use of species of differing tolerances to Al, and to inadequate control of pH and P and thus Al concentration (Rorison, 1958; Kerridge et al., 1971).

Numerous workers (Ota, 1968; Foy et al., 1969; Adams and Pearson, 1970; Lee, 1971; Foy et al., 1972) have shown that plant varieties within species also differ widely in their tolerance to Al. These varietal differences provide valuable tools for fundamental studies on the physiological nature of Al injury. Studies of this kind may assist plant breeders in screening for Al tolerance (Foy, 1974), since tolerant varieties are desirable for deeper rooting and for efficient water utilization in acid soils. Phaseolus vulgaris L. varieties 'Dade' and 'Romano,' which were shown to differ in Al tolerance (Foy et al., 1972), were selected for the studies to be reported in this dissertation. Possibly, there are some biochemical differences between these two snapbean varieties that confer on Dade a greater degree of tolerance to Al. By determining such differences one might learn more about the mechanism of Al toxicity. The objectives of this investigation were:

1. To characterize Al tolerance in Dade and Romano snapbean varieties with respect to growth and mineral nutrition;
2. To compare the two varieties with respect to pH, total acidity, Al fixing capacity of root macerates and to organic acid concentration;

3. To identify morphological and anatomical changes induced by Al, and
4. To identify by chemical staining and X-ray spectroscopy the sites of Al-phosphate interaction in bean roots.

The effects of Al on these parameters were investigated in an attempt to learn more about the differential response of the two snapbean varieties to Al in solution.

CHAPTER II

LITERATURE REVIEW

Aluminum in solution is an important growth-limiting factor in many acid soils. This was first suggested by Hartwell and Pember (1918), who discovered that Al was of much greater significance as a toxic factor affecting the growth of barley in acid soils than was hydrogen ion concentration. Magistad (1925) demonstrated that there was a qualitative relationship between plant growth and soil solution Al concentration. He was probably the first to suggest that poor plant growth in high-pH soils, as well as low-pH soils was caused by toxic concentrations of Al. Generally, at low pH (< 5) Al is present almost entirely as the free trivalent ion. As the pH rises it is generally precipitated out as hydroxides but appears again in solution as aluminate under alkaline conditions (Magistad, 1925). The solubility of Al in soils is not only determined by soil pH but also by the type of clay mineral, concentrations of other cations, total salt concentrations, and organic matter content (Foy, 1974).

Plants are most susceptible to Al injury in the early stages of growth, especially during the transition period, from dependence on seed reserves to external sources of nutrients (Rorison, 1958). In general, the toxic effects of Al injury are local rather than systemic and are induced most rapidly in the young meristematic tissues (Jackson, 1967; Fleming and Foy, 1968; Clarkson and Sanderson, 1969). Aluminum toxicity represents a combination of effects. The first visual effect of Al

injury is inhibition of root growth. Damage to tops occurs much later. Typical symptoms of Al-injured roots include stubby, stunted roots with collapsed or cracked tips and retarded branching (Foy and Brown, 1964; Clarkson, 1965; Jackson, 1967; Reid et al., 1971; Bartlett and Riego, 1972; Foy, 1974; Keser et al., 1975). This type of root system is lethal for seedlings since the failure of their roots to penetrate the soil to any depth can eventually lead to death by desiccation.

Aluminum toxicity symptoms in plant tops often resemble those of P deficiency. Symptoms include overall reduction in growth; small, abnormally dark green leaves; purpling of stems, leaves and veins; and chlorosis and dieback of leaf tips (Foy and Brown, 1964; MacLean and Chiasson, 1966; Foy, 1974). Aluminum toxicity symptoms can also resemble those of Ca deficiency. These include cupping or rolling of young leaves and collapse of plant apex or petioles (Jackson, 1967; Foy, 1974).

Evidence indicates that the relative degree of injury varies with the applied Al dosage (Jackson, 1967). In addition, different plant species are not equally sensitive to Al toxicity (Jackson, 1967; Black, 1968).

Plant varieties within species differ widely in tolerance to soluble aluminum. Varietal differences have been reported in snapbeans (Foy et al., 1972), rice (Ota, 1968), potatoes (Lee, 1971), barley (MacLean and Chiasson, 1966; MacLeod and Jackson, 1967; Foy et al., 1967b), soybean (Foy et al., 1969), wheat (Foy et al., 1965; Fleming and Foy, 1968; Kerridge et al., 1971; Foy et al., 1973, 1974), peanuts (Adams and Pearson, 1970) and perennial ryegrass (Vose and Randall, 1962).

Aluminum toxicity is associated with reduced yields. Significant reductions in top and root growth of five types of legume seedlings were obtained at Al concentrations above 1 ppm at pH 4.5 (MacLeod and Jackson, 1965). Similar results were obtained with barley at pH 4.3 (MacLeod and Jackson, 1967). Working with two barley varieties, MacLean and Chiasson (1966) found that top and root yields declined as the Al concentration was increased from 0 to 8 ppm at pH 4.8. In cotton, reductions in root weight were observed after 1 hour of exposure to 0.3 ppm Al (Lance and Pearson, 1969). Yields of roots and tops of eight potato varieties decreased when grown in nutrient solutions containing 20 ppm Al (Lee, 1971). Foy et al. (1972) reported that at 8 ppm Al, top and root yields of Al-sensitive Romano snapbeans were 53 and 59 percent respectively, of those with no Al at pH 4.8.

The effects of Al on plant growth at the cellular level have been investigated by a few workers. Microscopic examination of cotton plants grown in toxic concentrations of Al revealed an abnormally large number of binucleate cells in the meristematic region of the root tip. It was suggested that Al inhibited cell division (Rios and Pearson, 1964). Clarkson (1965) found that both 50 and 5 ppm Al-sulfate completely inhibited root elongation of onion after 6 to 8 hours. Examination of squashes of root apices showed that the cessation of root elongation was closely correlated with the disappearance of mitotic figures. It was concluded that the morphological abnormalities induced by Al were due to the effect of Al on cell division or cell extension. Clarkson and Sanderson (1969) hypothesized that Al probably blocked the mitotic cycle

during interphase. In wheat, Fleming and Foy (1968) observed binucleate cells in the meristematic regions of Al-treated root tips. They cited this as evidence supporting the conclusion of Clarkson (1965), that Al reduces cell division. Others (Kerridge et al., 1971) reported that Al treatments resulted in disorganized cellular tissue in the root tips of wheat plants. Aluminum treatments, at concentrations of 4, 8 and 12 ppm, resulted in detached root caps in sugar beet roots (Keser et al., 1975). In addition, the cortical cells adjacent to the root cap were abnormally large and divided irregularly.

Numerous workers have demonstrated that Al has inhibitory effects on plants, especially on the growth of roots. However, relatively few attempts have been made to relate these effects to specific physiological processes. Clarkson (1966a) reported that Al markedly decreased the rate of sugar phosphorylation in barley roots. Aluminum was believed to accomplish this by inhibiting the enzyme hexokinase or by combining with inorganic phosphate to render it unavailable. However, Wong and Chan (1973) found that pretreatment of sugar cane with about 27 ppm Al-sulfate had no effect on the extent of phosphorylation.

Aluminum has been reported to reduce DNA synthesis (Sampson et al., 1965; Clarkson, 1969). Sampson et al. (1965) found that Al-injured roots of barley synthesized a type of DNA that had an unusual base composition and was metabolically labile. Woolhouse (1969) and Klimashevskii and Bernatskoya (1973) reported that Al inhibited the activity of acid phosphatases and ATP-ase.

Various workers have shown that Al increases the viscosity of protoplasm in plant roots and decreases overall permeability (McLean and

Gilbert, 1927; Lance and Pearson, 1969; Clarkson and Sanderson, 1969). It was suggested that Al decreased overall permeability by the formation of cross linkages between adjacent protein molecules (Clarkson and Sanderson, 1969). Rorison (1958) reported that Al also decreased the extensibility of cell walls by the cross linking of pectins in the middle lamella.

The effect of Al on P nutrition has received more attention than any other nutritional effect (Black, 1968). Yet evidence bearing on the subject of P concentration in roots is subject to many ambiguities (Jackson, 1967). Hartwell and Pember (1918) first suggested that the observed P deficiency symptoms in the shoot of barley could be attributed to an internal precipitation of phosphate by Al. Since then numerous experiments have been conducted to determine the nature and location of the Al-phosphate interaction in plant roots. Wright (1943) and Wright and Donahue (1953), working with barley in culture solutions, reported that Al precipitated P within plant roots. However, Wallihan (1948) presented evidence which suggested that Al was not precipitated internally. He suggested that Al and perhaps P were held to root surfaces by ionic exchange.

Clarkson (1966a) suggested that the interaction between Al and phosphate in barley roots was an adsorption-precipitation reaction that occurred at the cell surface or in the free space of the root. These suggestions were supported by those of Rasmussen (1968), who used an electron microprobe X-ray analyzer to determine the mode of entry of Al and its distribution and localization in corn roots. He found that Al

was precipitated on the surface of the root epidermal cells. Aluminum did not penetrate the cortex as long as the root surface remained intact. On the other hand, the root cap was freely permeable to Al ions and contained the highest concentration of Al. The epidermis prevented the movement of Al into the cortex and stele. Aluminum penetrated the cortex and conducting tissue only at the site of emergence of lateral roots. The localization of phosphate was the same as that of Al, thus suggesting a precipitation of phosphate by Al.

Rasmussen's (1968) conclusions were later supported by the results of McCormick and Borden (1972), who used photomicrographic techniques to identify sites of phosphate fixation by Al in barley and poplar roots. The Al-phosphate interaction appeared to be associated with the cell wall and cytoplasmic membrane of the epidermal and cortical cells. Their results also indicated that Al adsorbed to the root surface or present in the intercellular free space, may be capable of binding phosphate. These same workers (McCormick and Borden, 1974) later presented further supporting data. Microprobe analysis of Al-treated sugar beet roots by Keser et al. (1975) also supported the results of Rasmussen (1968).

Results contrary to those discussed above were reported by Waisel et al. (1970). They investigated the localization of anionic Al in the cells of bean and barley roots by X-ray microanalysis. They found no correlation between the distribution of Al and phosphate. In addition, only minute amounts of Al were located in the cell walls. The authors suggested that Al-phosphate precipitates were formed either in the free space or in the osmotic space. Differences in the results of Waisel et

al. (1970) and Rasmussen (1968) could be attributed to differences in plant species or to differences in preparation of the tissue for microprobe analysis.

Numerous workers have found that Al reduces the P concentration in plant tops (Wright, 1943; Wright and Donahue, 1953; Randall and Vose, 1963; Foy and Brown, 1964; MacLeod and Jackson, 1965; Munns, 1965; Clarkson, 1966a; MacLean and Chiasson, 1966; Lance and Pearson, 1969; Andrew et al., 1973). Many of these workers have reported increased P in plant roots at the time of its reduction in the tops. MacLean and Chiasson (1966) and MacLeod and Jackson (1967) cited this as evidence supporting their contention that Al depressed the translocation of P rather than its uptake by the roots.

Although Al exerts large influences on calcium accumulation and distribution, the manner in which these effects come about simply is not known (Jackson, 1967). In Ca deficient plants root growth is seriously arrested, both at the apex, and in the development of lateral primordia (Tisdale and Nelson, 1966; Jackson, 1967). Various workers (Johnson and Jackson, 1964; Munns, 1965; Foy et al., 1969; Lance and Pearson, 1969; Clarkson and Sanderson, 1971; Foy et al., 1972; Andrew et al., 1973) have associated Al toxicity with decreases in the concentration of Ca in both tops and roots. Clarkson and Sanderson (1971) suggested that the mechanism of interference of Ca uptake by Al, in barley, involved surface reactions, rather than the disruption of metabolically dependent transport processes. Calcium accumulation in bean roots was reported to be insensitive to inhibitors and low temperature (Drew and Biddulph, 1969).

This suggested to Clarkson and Sanderson (1971) that Ca movement across the plasmalemma was not an active process. They concluded that the superficial location of Al and other polyvalent cations allowed them to control not only the entry of Ca, but also the entry of other cations of lower valency into the free space.

Foy et al. (1969) found that Al toxicity was increased in soybean by lowering the level of Ca in the nutrient solution. This supported the contention of Wallace et al. (1966) that Ca serves as a detoxifier of other ions that may be present in excess. Foy et al. (1972) reported that 4 ppm Al at pH 4.8 decreased the Ca concentration in root cell walls, mitochondria, supernatant, and total roots of Al-sensitive Romano snapbeans. These workers suggested that Al may injure sensitive varieties by reducing the Ca concentration at important membrane sites of subcellular structures. That Ca may have a protective action against Al was also suggested by Munns (1965).

Several workers have attempted to overcome the adverse effects of Al by increasing the Ca concentration of the medium. Slight alleviation of Al toxicity by a high Ca level in the medium was reported by Millikan (1949) and Munns (1965). Clymo (1962) found that the inhibiting effect of Al on root growth was greatest when the Ca supply was low. In cotton seedlings, Lance and Pearson (1969) reported that the inhibition of Ca uptake was overcome by increasing the Ca concentration. They suggested that Al altered the structural configuration of the plasmalemma by replacing calcium. However, Johnson and Jackson (1964) found that in wheat, the reduction in calcium uptake caused by Al could not be overcome by supplying additional calcium.

Some investigators have recorded decreased Ca in tops and increased Ca in the roots (Ouellette and Dessureaux, 1958; MacLeod and Jackson, 1965; MacLean and Chiasson, 1966). Ouellette and Dessureaux (1958) proposed that Ca reduced the toxic effects of Al by reducing Al uptake and by immobilizing part of the absorbed Al in the roots. However, no direct evidence for this causal effect was presented.

Calcium plays an important role in regulating the structural integrity and permeability characteristics of the membrane (Epstein, 1961; Jackson, 1967; Black, 1968). The high affinity of Al for pectin indicates that much Al could be attracted to cell wall material and Clarkson (1965) reported that 85 to 90 percent of the Al in barley roots was associated with the cell wall preparations.

The accumulation and distribution of many other mineral elements are often strongly affected by Al. Depressions in uptake of Mg (Harward et al., 1955; DeWard and Sutton, 1960; Ota, 1968; Lance and Pearson, 1969; Lee, 1971), K (Harward et al., 1955; MacLeod and Jackson, 1967; Ota, 1968; Lance and Pearson, 1969; Lee, 1971), Mn (Ota, 1968; Bartlett and Riego, 1972), Cu (Hiatt et al., 1963) and Zn (Lee, 1971) have been described. Others have reported that Al increased the concentration of Mg (MacLeod and Jackson, 1965; Andrew et al., 1973) and K (DeWard and Sutton, 1960; Andrew et al., 1973). Andrew et al. (1973) suggested that the increased Mg and K uptake may not be a direct effect, but may be brought about by the capacity of the plants to preserve cationic balance. In certain varieties of Irish potatoes, tolerance to Al was associated with the abilities of plant roots to absorb Mg and K (Lee, 1971). Other

workers (Clarkson and Sanderson, 1971) suggested that Al blocks, neutralizes, or reverses the negative charge on the pores of the cells in the free space and thereby reduces the ability of such pores to bind other cations.

The mechanism of Al toxicity is still debated and largely speculative. Researchers have associated various physiological and biochemical properties with Al tolerance. Generally, plants that are tolerant to Al are characterized by an ability to maintain normal growth under conditions of low P and Ca availability. The manner in which Al-tolerant plants accomplish this is not clear.

The results of several workers (Subramoney and Saukaranarayanan, 1964; Foy et al., 1965, 1967b; Otsuka, 1968) suggest that the differential tolerance of some plants to Al may be due to the ability of these plants to induce pH-changes around their roots. These workers have shown that some Al-sensitive plants lower the pH around their roots while tolerant plants raise the pH. Lower plant-induced pH around the roots presumably increases the solubility and potential toxicity of aluminum (Foy et al., 1965, 1967b). Plant-induced lowering of pH could be brought about by hydrogen ion release; release and hydrolysis of carbon dioxide; efflux of organic acids and other ions; and preferential uptake of anions over cations (Pitmann, 1970).

Adams and Pearson (1970) reported that cotton was more sensitive to Al than peanuts. They showed that peanuts absorbed ions at a lower cation/anion ratio than cotton, thereby creating a less acid condition resulting in a lower Al concentration in the ambient solution. In

addition, peanut roots showed a tendency to preferentially absorb ions of lower valency to the exclusion of higher valency ions. However, Al tolerance between Dade and Romano snapbean varieties (Foy et al., 1972) and Perry and Chief soybean varieties (Foy et al., 1969) was not associated with root-induced pH changes in the nutrient solutions.

At physiological pH values within root tissues Al would be expected to form insoluble hydroxides or phosphates (Jackson, 1967). The tendency to form insoluble inorganic precipitates may be prevented in tolerant species by the chelation of Al by organic acids (Jones, 1961). The formation of insoluble precipitates may also be hindered by the absorption of Al on macromolecules such as cell wall materials or membrane components (Jackson, 1967).

Several lines of evidence indicate that the chelating agent was the organic acid pool in the root. Small (1946) showed that acidophilous plants generally possess strong organic acid buffer systems in the cell sap. Alkaliphiles, on the other hand, have a predominantly phosphate buffer system. These differences in buffer systems may possibly explain the differential Al tolerance of plant species and varieties (Jones, 1961). Acid tolerance in several plant species was positively correlated with the citric acid content of roots (Chamura and Koike, 1960). This led to the suggestion that citric, malic and possibly tartaric and oxalic acids were the chelating agents (Jones, 1961). Furthermore, Clarkson (1966b) found that cationic Al inhibited cell division in roots of Agrostis stolonifera whereas chelated Al did not. In excised barley roots, Johnson and Jackson (1964) reported that Al, in the form Al-EDTA,

reduced Al uptake and increased Ca uptake. It was suggested that Al tolerant plants produced root exudates that chelated Al, prevented P fixation and thereby increased P availability (Drake and Steckel, 1955).

Aluminum is generally regarded as a nonessential element for plant growth. However, various claims have appeared in the literature which seem to indicate that low concentrations of Al benefit plant growth. In alfalfa and red clover seedlings MacLeod and Jackson (1965) reported that 0.1 and 0.2 ppm Al at pH 4.5 increased yields and P uptake. Dry matter production of several tolerant legume species increased when treated with 0.5 ppm Al (Andrew et al., 1973). Increased P uptake at low levels of Al was reported for perennial ryegrass (Randall and Vose, 1963), barley seedlings (Clarkson, 1966a) and for excised snapbean roots (Ragland and Coleman, 1962). In potatoes, Lee (1971) showed that 1 to 5 ppm Al at pH 3.7 stimulated vegetative growth and the uptake of K and Mg. The exact mechanism whereby low concentrations of Al stimulate plant growth is not known. The beneficial effects of Al are probably indirect and may be due to the prevention of toxic effects of other ions (Jackson, 1967). Foy (1974) suggested that stimulation of growth was probably due to increased Fe solubility and availability as a result of the hydrolysis of Al and a lower pH.

CHAPTER III

MATERIALS AND METHODS

I. GROWTH AND MINERAL NUTRITION

Cultural Conditions

Seeds of Phaseolus vulgaris L. varieties Dade and Romano were germinated in trays containing vermiculite in a greenhouse. The trays were watered with deionized water. When the seedlings were 7 days old they were transferred to 8-liter capacity plastic growth tanks and grown hydroponically. The sides of each tank were covered with a layer of black plastic to prevent light penetration and subsequent algal growth.

The seedlings were inserted through small holes in a styrofoam board, 2.5 cm thick, that completely covered and floated on the nutrient solution. The seedlings were supported in the holes with glass wool. The growth medium was one-fifth strength Hoagland's Number Two nutrient solution (Hoagland and Arnon, 1950). Iron was supplied as the ethylenediaminetetraacetic acid complex (Steiner and van Winden, 1970). Each growth tank was continuously aerated with humid air forced through a gas dispersion stone. Care was taken to ensure that the flow of air to each tank was regulated uniformly.

Aluminum Treatments

After 4 days in nutrient solutions the seedlings were selected for uniformity and transferred to tanks (four seedlings per tank) containing one-fifth strength Hoagland's Number Two nutrient solution (Hoagland and

Arnon, 1950) containing Al. One-fifth strength nutrient solution was used in order to reduce the degree of removal of the Al ion as insoluble Al-phosphates. Aluminum was supplied as Al-sulfate at concentrations of 0, 4, 8 and 12 ppm Al. The pH of the nutrient solution was initially adjusted to 4.8 with 0.1N H_2SO_4 or 0.1N NaOH to assure solubility of the added Al. The experimental design was a randomized complete block with three replications and four plants per replication. The nutrient solutions were renewed after 5 days.

Plant Analyses

Plants were harvested after 10 days in treatment solutions and divided into roots and tops. At harvest the pH of the nutrient solutions was determined. The roots were washed in deionized water and blotted dry. The plant parts were dried at 70°C for 24 hours, weighed, ground to pass a 2 mm screen in a Wiley mill and stored in plastic vials.

One gram of each sample was used for the determination of Al. The sample was dry ashed in a muffle furnace at 550°C and dissolved in 1.0N HCl. Aluminum in these samples was determined colorimetrically by a modification of the aluminon method (Yuan and Fiskell, 1958) on a Beckman Model B spectrophotometer.

Another 0.5 g of each sample was wet ashed for the determination of K, P, Mg and Ca. Calcium was determined on a Perkin-Elmer Model 303 atomic absorption spectrophotometer. Potassium, P and Mg were determined on a Technicon Model III AutoAnalyzer. Potassium was determined by flame emission photometry, Mg by the Mg blue method, and P by the ammonium vanadate colorimetric procedure using standard Technicon procedures.

II. pH AND TOTAL ACIDITY

Three week old plants, which were grown as described previously, were divided into roots and tops. The tissue was prepared utilizing essentially a technique modified from Sideris et al. (1948) by Ting and Dugger (1968). The plant tissue was cut up finely and 40 g weighed. The tissue was then pulped in a blender with 100 ml water for 2 minutes. The pulped tissue was boiled for 30 minutes and then filtered to remove the coarse sediment. To remove the fine sediment the filtrate was centrifuged at 5,000 × g for 30 minutes. The supernatant was transferred to a 100 ml volumetric flask and made to volume. The pH of the extract was determined with a Beckman pH meter. Titrimetric acidity was estimated as soon as possible after extraction by titration with 0.01N NaOH.

III. ALUMINUM FIXATION BY ROOT MACERATES

Forty grams root tissue from 4-week old plants were used for preparation of root macerates. The tissue was prepared utilizing a technique modified from Jones (1961). The tissue was macerated for 2 minutes with 100 ml acetic acid/sodium acetate buffer at pH 4.8 in a Waring Blendor. The macerate was centrifuged at 2,400 × g for 30 minutes. The supernatant was made up to 200 ml with deionized water. Fifteen ppm Al were added to the extract as Al-sulfate and the flasks shaken intermittently for 1 hour. Three ml aliquots of the extract were centrifuged at 2,400 × g for 10 minutes to separate the soluble from the fixed Al. Aluminum in the residue was determined by the aluminon method described previously.

IV. ORGANIC ACID DETERMINATION

Roots and tops of 20-day old Dade and Romano plants were harvested and freeze dried for the determination of organic acids. Organic acids were extracted according to the procedure of Prior et al. (1973). Two ml of the extract were used to determine citric acid colorimetrically, on a Perkin Elmer 202 spectrophotometer, using a method modified from Camp and Farmer (1967).

Malic acid was determined on a 0.2 ml aliquot of the extract and was determined fluorometrically as described by Hummel (1949). Fluorescence was measured on a Perkin Elmer 203 spectrofluorometer using an exciter wavelength of 366 nm and an analyzer wavelength of 444 nm.

V. LOCATION OF PHOSPHATE IN ROOTS BY STAINING

Molybdenum in the molybdate form is widely used in the colorimetric determination of phosphate. Molybdenum reacts with phosphate to form the phosphomolybdate anion. Upon reduction, the anion turns blue in color. Root tissue that contains phosphate can be stained with molybdate and subsequently reduced by an appropriate reducing agent such as stannous chloride to indicate sites of phosphate fixation by Al. Tests using Al oxide and Al phosphate precipitates indicated that the molybdate reagent would become attached and reduced to its blue color only if phosphate were present (McCormick and Borden, 1972).

Plant Growth

Dade and Romano seeds were germinated in trays between moist paper towels. The seeds in each tray were covered with moist paper towels for

3 to 4 days to ensure the necessary humidity for uniform germination. The seeds were watered as needed with deionized water. As soon as the radicles had emerged the seedlings were transferred to growth tanks containing deionized water.

The radicles were inserted through small holes in a 1 cm thick polyurethane foam disc, which had a diameter of 7.6 cm. Each disc supported four seedlings. Five discs were supported by a styrofoam lid, 2.5 cm thick, that fitted inside each growth tank. The foam discs were saturated with deionized water to keep the seedlings moist. The seedlings were grown in this manner until the radicles were about 4 cm long and became immersed in the deionized water. The growth tanks were continuously aerated.

Treatments

The seedlings were subjected to the treatments shown in Table 1.

Table 1. Treatments applied to snapbeans to locate phosphate in roots.

Treatment	Treatment Solutions	
	8 hours	16 hours
Control	Deionized water	Deionized water
P	Deionized water	12 ppm P Solution
Al	20 ppm Al Solution	Deionized water
Al + P	20 ppm Al Solution	12 ppm P Solution

Al was supplied as Al-sulfate and P as dibasic K-phosphate. The Al treatment solution contained no phosphate while the P treatment solution

contained no Al. Separate solutions were necessary to prevent the precipitation of Al-phosphate from the solution. The pH of all solutions was adjusted to 4.8 by the addition of 0.1N H_2SO_4 or 0.1N NaOH. All solutions were continuously aerated. At the end of the first 8 hours of treatment the roots of all seedlings were rinsed in deionized water prior to transfer to the second treatment solutions.

At the end of the 24-hour treatment period the roots were rinsed in deionized water and harvested. Root tips were prepared for microscopic examination according to a procedure modified from Jensen (1962).

Fixation and Dehydration

Root tips, 0.5 to 1 cm in length, were placed in glass vials containing formalin-acetic acid-alcohol (FAA) fixative (Jensen, 1962), and left for at least 4 hours. The fixed tissue was completely dehydrated using mixtures of water, tertiary butyl alcohol and ethyl alcohol (Jensen, 1962).

Paraffin Infiltration, Embedding and Sectioning

Many modifications of Jensen's (1962) procedure were used for paraffin infiltration and embedding. Immediately following dehydration the tissue was placed in toluene. After 4 hours the tissue was transferred to a 1:1 mixture of toluene and paraffin oil for 4 hours. This was followed by immersion in paraffin oil for 2 hours. The tissue was then infiltrated with Paraplast, a histological infiltration and embedding wax, at 62°C for 24 hours. This was followed by further infiltration in pure Paraplast at 62°C for 96 hours.

The infiltrated root tips were then mounted in a block by embedding with Paraplast. The embedded tissue was sectioned with a rotary microtome into 15 μ-thick sections.

Staining

The sections were placed onto glass slides coated with Haupt's adhesive by the water flotation method (Jensen, 1962), then dried overnight on a slide warmer at 40°C. The embedding medium was removed by dissolving it in xylene. Phosphate in the root sections was located by the Mo blue staining technique developed by McCormick and Borden (1972). One drop of molybdate solution (1 g of ammonium molybdate to 100 ml of 0.1N H_2SO_4) was placed on each section. After 1 minute the sections were rinsed with deionized water. One drop of stannous chloride reducing agent was then applied to each section. The reducing agent was freshly prepared each hour by mixing 1 ml stock $SnCl_2$ solution (2.5 g $SnCl_2 \cdot 2H_2O$ to 25 ml concentrated HCl) to 100 ml deionized water.

Color photomicrographs were taken with a Leitz microscope using Kodachrome II film.

VI. MORPHOLOGICAL EFFECTS OF ALUMINUM

Plant Growth and Treatments

Dade and Romano seedlings were grown hydroponically as described previously. Ten-day old plants of each variety were divided into two groups. The first group, the control, did not receive Al. The second group was administered 20 ppm Al as Al-sulfate. All solutions were adjusted daily to pH 4.6 with 0.1N NaOH or 0.1N H_2SO_4. The nutrient

solutions were renewed after 6 days. Root length and plant height were measured at 3-day intervals during the treatment period. Root tips 0.5 to 1 cm in length were harvested after 12 days of treatment.

Tissue Preparation

The root tips were placed in formalin-acetic acid-alcohol fixative. Subsequent dehydration, paraffin infiltration, embedding and sectioning have been described previously.

The 8 μ-thick sections were stained with safranin and fast green according to the procedure described by Jensen (1962). The procedure is briefly outlined below. The paraffin was removed from the sections by dissolving it in xylene for 5 minutes and then in a 1:1 mixture of xylene and absolute alcohol for another 5 minutes. The sections were partially rehydrated by passing them through absolute, 95 percent, 70 percent and 50 percent alcohols. The partially hydrated sections were stained in safranin for 18 hours; washed in water; and passed through acidified 70 percent ethanol, 95 percent and absolute ethanol. The sections were then counterstained in fast green for 2 minutes. The fast green was differentiated by placing the sections in two changes of a mixture of clove oil, absolute alcohol and xylene. The sections were finally passed through three changes of xylene, then mounted with Permount.

Color photomicrographs of the root sections were taken with a Leitz microscope using Kodachrome II film.

VII. ELEMENTAL LOCALIZATION IN ROOTS BY ENERGY DISPERSIVE ANALYSIS OF X-RAYS GENERATED IN THE SCANNING ELECTRON MICROSCOPE

Plant Growth and Aluminum Treatments

Dade and Romano snapbean seeds were germinated in vermiculite for 12 days and uniform seedlings selected for transfer to nutrient solution. Nutrient solutions were one-fifth strength Hoagland's solution containing 0 or 20 ppm added Al. The solutions were adjusted to pH 4.6 daily and renewed after 6 days. After 12 days the snapbeans were harvested and the primary and lateral roots separated for study.

Tissue Preparation

The root tips were dehydrated, infiltrated with paraffin and embedded according to the procedure described previously. Since the concentrations of mineral elements in the roots were in the lower limits of detection by energy dispersive X-ray analysis, thick sections (25 μ) were used. The sections were transferred to rectangular graphite blocks, which were 2.5 cm long, 1.8 cm wide and 0.5 cm thick, by the water flotation method. The graphite blocks were placed on a slide warmer at 40°C for 12 hours. The embedding medium was removed from the sections by immersing the blocks in xylene for 10 minutes. The blocks containing the sections were air-dried and stored in a desiccator over silica gel. The specimens were not coated with nonconducting materials.

Energy Dispersive Spectroscopy

Elemental localization in root sections was determined by an ORTEC Energy Dispersive X-ray Analyzer on an AMR 900 Scanning Electron

Microscope. The accelerating voltage used was 21 Kev. Two types of scans were performed on the specimens. With most sections a single, very slow X-ray line scan was performed across the section (approximately 200 channels with a dwell time of 0.8 sec per channel). Each line scan was repeated several times in order to increase the peak to background ratio. In a few cases spot scans were performed in areas of particular interest. In order to obtain good X-ray statistics the time for each spot scan exceeded 4 min. At the voltage employed charging of the uncoated specimen was a problem. Charging of the specimen surface resulted in low resolution in the secondary electron image. This was not considered to be important since localization of elements in root tissues was the primary objective of this study.

VIII. STATISTICAL ANALYSES

Analyses of variance and F tests were applied to data obtained. When significant differences were detected treatment means were compared by Duncan's Multiple Range Test. Correlation coefficients were calculated between selected variables. Differences between the two varieties for various treatments were compared by the use of an unpaired t-test.

CHAPTER IV

RESULTS

I. ROOT AND TOP GROWTH

Plants exposed to 8 and 12 ppm Al showed typical symptoms of Al toxicity. These included overall reduction in growth, stunting of roots with very little branching and root discoloration. Aluminum-treated roots were brown in color compared to the white color of untreated roots. Plants exposed to 4 ppm Al looked as healthy and as vigorous as the control plants.

The pH of the nutrient solutions, which was measured at the termination of the experiment, is shown in Table 2. For both varieties the pH increased slightly with increasing Al concentration. Analysis of the data by Duncan's Multiple Range Test did not reveal any significant differences, either within each variety, or between varieties.

The dry weights of plant roots exposed to various levels of Al in solution are shown in Table 2 and Fig. 1. Root weights decreased at higher levels of Al, the decrease being greater for Romano than for Dade. At 12 ppm Al the root weights of Dade and Romano were 93 percent and 73 percent, respectively, of those with no Al. Differences between varieties at 12 ppm Al were significant at the 5 percent level. In addition, there was a significant negative correlation between Al levels in nutrient solution and dry weight of roots at the 1 percent level of probability for both varieties (Table 3).

Table 2. Final pH of nutrient solutions and dry weight of roots and tops of snapbeans at various levels of Al.

Variety	Al in Nutrient Solution (ppm)	pH	Dry Weight (g) Roots	Tops
Dade	0	5.4 a[1]	4.1 a	15.1 a
	4	5.9 a	4.1 a	13.0 b
	8	6.0 a	3.8 a	11.0 b
	12	6.1 a	3.8 a	9.1 c
Romano	0	5.5 a	4.1 a	15.0 a
	4	5.7 a	3.7 a	12.3 b
	8	5.8 a	3.7 a	11.3 b
	12	5.9 a	3.0 b	7.3 c

[1]Means followed by the same letter in a column are not significantly different at the 0.05 level of probability.

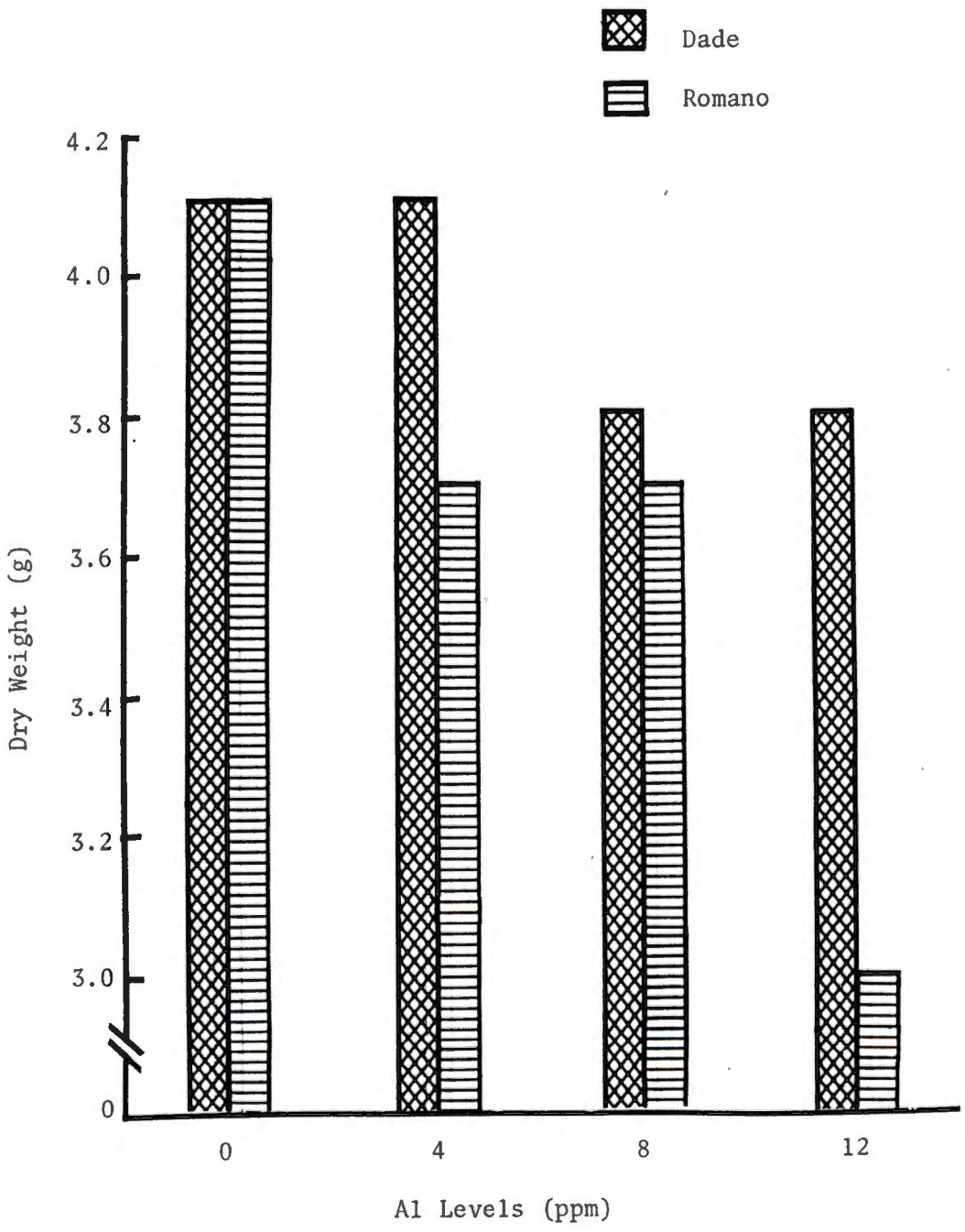

Fig. 1. Effect of different levels of Al on the dry weight of snapbean roots.

Table 3. Correlation coefficients between selected growth and physiological characters of snapbeans.

Characters Correlated		Correlation Coefficients Dade	Romano
Al in Nutrient Solution	—Root Dry Weight	-0.89**	-0.96**
	—Top Dry Weight	-0.99**	-0.97**
	—Al in Roots	+0.66	+0.68
	—Al in Tops	-0.10	-0.76*
	—Ca in Roots	+0.04	-0.91**
	—Ca in Tops	+0.47	-0.91**
	—P in Roots	-0.97**	-0.97**
	—P in Tops	-0.99**	-0.97**
	—K in Roots	+0.43	+0.78
	—K in Tops	+0.86**	+0.47
	—Mg in Roots	-0.85**	-0.72*
	—Mg in Tops	+0.76*	-0.96**
Al in Roots	—P in Roots	-0.65	-0.67
	—Ca in Roots	-0.59	-0.99**

*Significant at 0.05 level of probability.

**Significant at 0.01 level of probability.

The dry weight of tops of both Dade and Romano decreased with increasing Al concentration (Table 2 and Fig. 2). Top yields from Al treatments were significantly different at the 5 percent level from those with no Al. At 12 ppm Al top yields of Dade and Romano were 60 percent and 49 percent, respectively, of those with no Al. These values were significantly different from the 0, 4 and 8 ppm Al levels within each variety. There were no significant differences between varieties. Aluminum levels in nutrient solution and yield of tops for both varieties were negatively correlated (Table 3).

II. CHEMICAL COMPOSITION OF ROOTS AND TOPS

The Al concentration of roots and tops are shown in Table 4. The major part of the Al in the plants was present in the roots, while only a small amount was translocated to the tops. For example, at 12 ppm Al the roots of Dade and Romano retained 80 percent and 91 percent, respectively, of the Al present within the plants. There were no significant differences in Al concentration between treatments for either roots or tops. In addition, there was no correlation between Al levels in nutrient solution and Al concentration of roots for both varieties (Table 3).

In plants not treated with Al, the Ca concentration in both roots and tops was higher in Romano than in Dade (Table 5). Aluminum treatments reduced the Ca concentration in Romano roots, but had little effect on the Ca concentration of Dade roots. At 12 ppm Al the concentration of Ca in the roots of Dade and Romano were 97 percent and 75 percent,

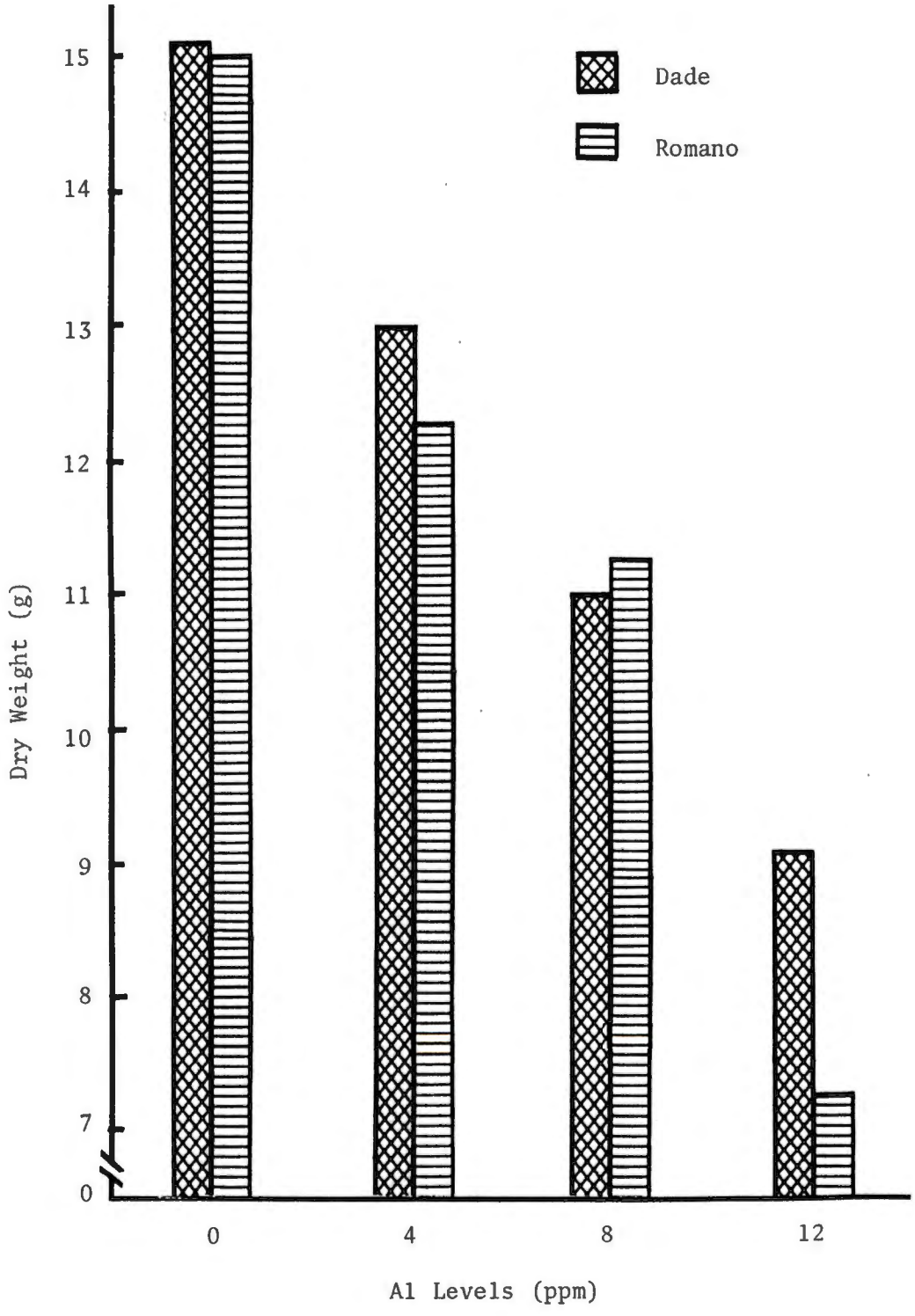

Fig. 2. Effect of different levels of Al on the dry weight of snapbean tops.

Table 4. Aluminum concentration of roots and tops of snapbeans at various levels of Al.

Variety	Al in Nutrient Solution (ppm)	Al Concentration (meq/100g) Roots	Tops
Dade	0	1.51[1]	2.62
	4	29.71	1.82
	8	23.72	1.52
	12	24.98	2.58
Romano	0	2.04	1.79
	4	27.86	2.29
	8	19.94	1.37
	12	25.05	1.02

[1]Values in each column are not significantly different at the 0.05 level of probability.

Table 5. Calcium concentration of roots and tops of snapbeans at various levels of Al.

Variety	Al in Nutrient Solution (ppm)	Calcium Concentration (meq/100g) Roots	Tops
Dade	0	38.3[1]	108.1
	4	34.4	110.3
	8	38.6	108.8
	12	37.1	109.8
Romano	0	48.2	116.5
	4	36.2	116.8
	8	38.7	94.8
	12	36.2	92.8

[1]Values for both roots and tops are not significantly different at the 0.05 level of probability.

respectively, of those with no Al. However, differences between treatments were not significant.

The Ca concentration in Romano tops decreased at 8 and 12 ppm Al (Table 5). The Ca concentration of Dade tops was not affected by Al. At 12 ppm Al the Ca concentration in the tops of Romano was 80 percent of that with no Al, while that for Dade was 102 percent. Although differences between treatments were not significant, there was a high negative correlation between Al added and the Ca content of Romano roots and tops. There was no correlation between Al added and the Ca content of Dade roots and tops (Table 3, page 29).

Aluminum treatments reduced the P concentration in the roots and tops of both varieties (Table 6 and Figs. 3 and 4). Differences between treatments for both varieties were significant at the 1 percent level of probability. Even at 4 ppm Al there were significant reductions in P content of roots (but not tops) for both varieties. There were drastic reductions in P concentrations of roots and tops of both varieties at 8 and 12 ppm Al. At 12 ppm Al the P concentration in the roots and tops of Romano was only 39 percent and 27 percent respectively, of those with no Al. Corresponding P levels in roots and tops of Dade were 34 percent and 43 percent respectively. In addition, P concentrations in roots and tops of both varieties were negatively correlated with Al added to nutrient solutions (Table 3).

The K concentrations of roots are shown in Table 7 and Fig. 5. The effects of Al on the K content of roots of both varieties were variable. At higher levels of Al the K content of roots increased. For example,

Table 6. Phosphorus concentration of roots and tops of snapbeans at various levels of Al.

Variety	Al in Nutrient Solution (ppm)	Phosphorus Concentration (meq P/100g) Roots	Tops
Dade	0	39.08 a[1]	21.54 b
	4	30.31 b	17.02 bc
	8	15.22 c	14.31 c
	12	13.41 c	9.28 d
Romano	0	37.02 a	28.50 a
	4	28.51 b	24.89 a
	8	16.64 c	12.12 cd
	12	14.44 c	7.61 d

[1]Means followed by the same letter in a column are not significantly different at the 0.05 level of probability.

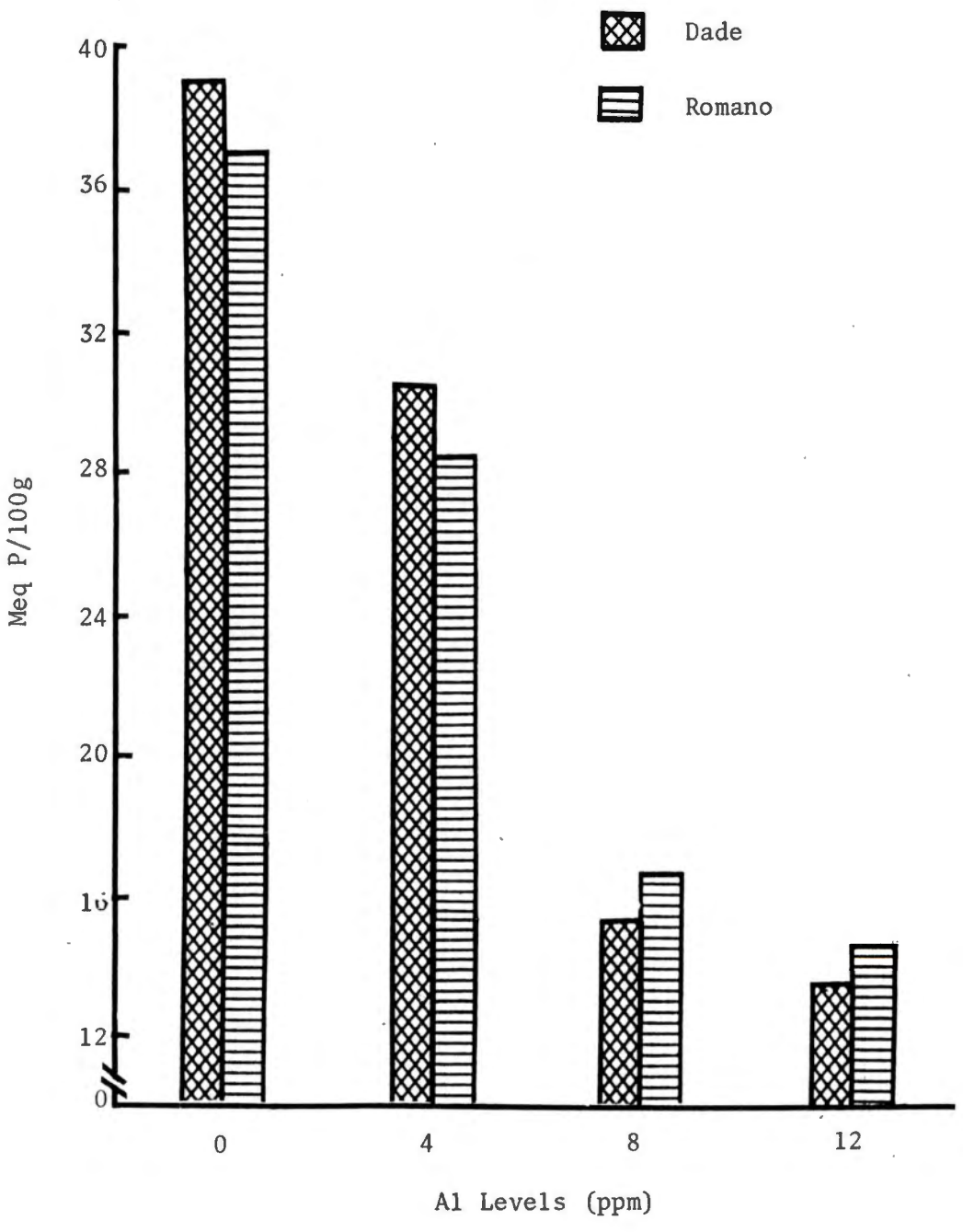

Fig. 3. Effect of different levels of Al on the P concentration of snapbean roots.

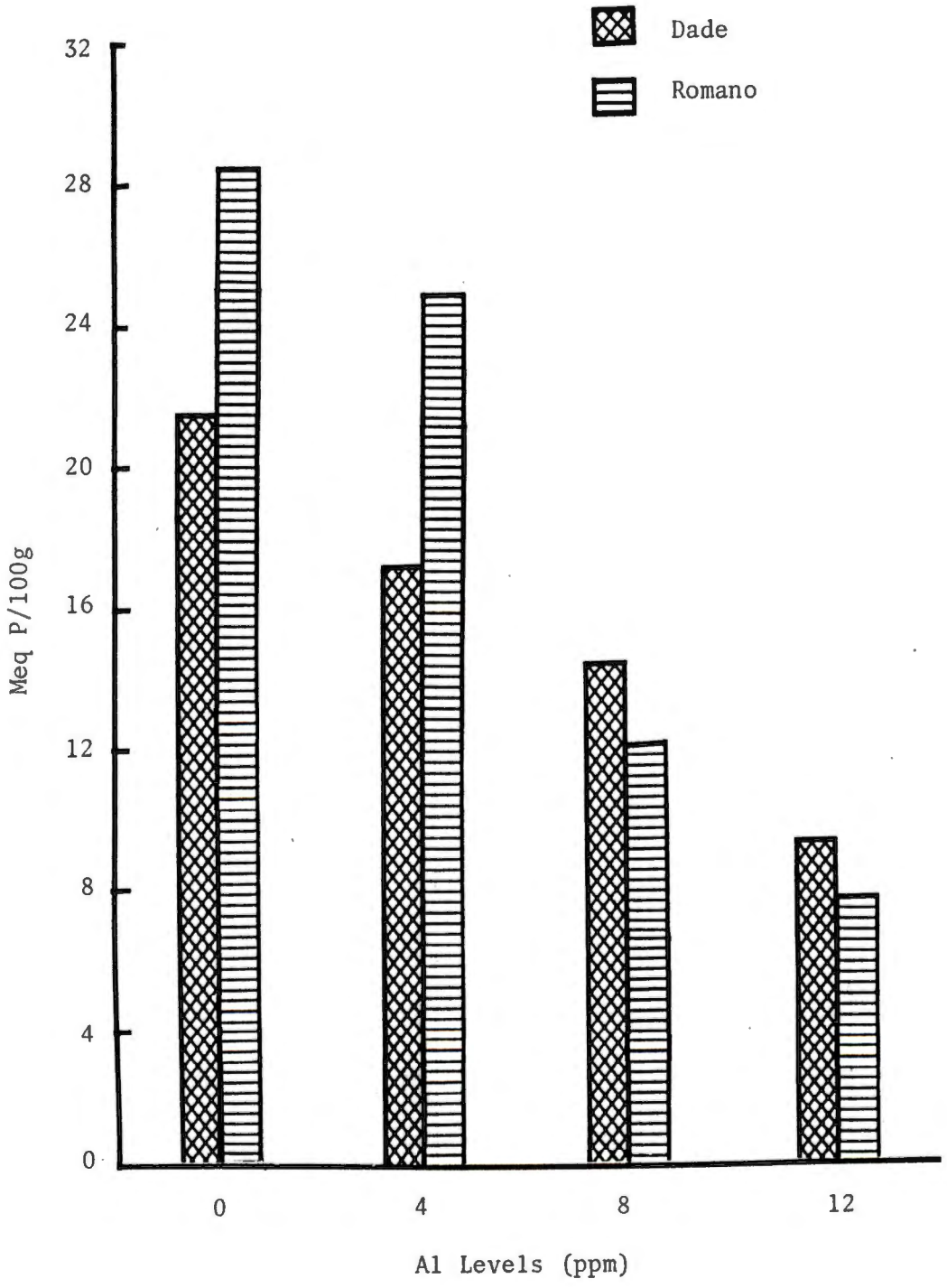

Fig. 4. Effect of different levels of Al on the P concentration of snapbean tops.

Table 7. Potassium concentration of roots and tops of snapbeans at various levels of Al.

Variety	Al in Nutrient Solution (ppm)	Potassium Concentration (meq/100g) Roots	Tops
Dade	0	239 ab[1]	135 c
	4	276 a	143 bc
	8	229 ab	178 ab
	12	286 a	169 abc
Romano	0	211 b	172 ab
	4	181 b	187 a
	8	238 ab	182 a
	12	263 ab	181 a

[1]Means followed by the same letter in a column are not significantly different at the 0.05 level of probability.

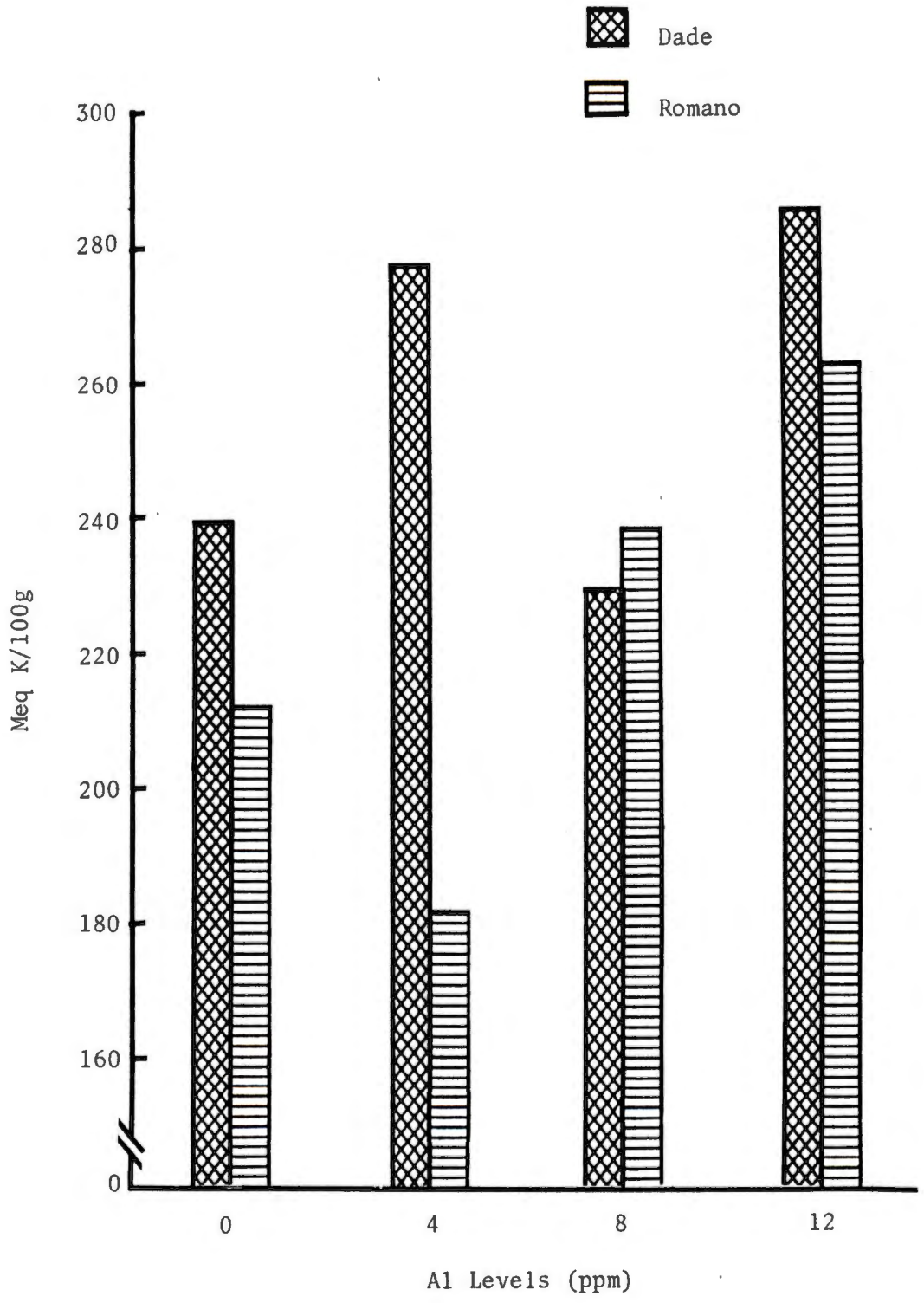

Fig. 5. Effect of different levels of Al on the K concentration of snapbean roots.

at 12 ppm Al the K concentration in the roots of Dade and Romano was 120 percent and 124 percent respectively, of those with no Al. However, these differences were not significant at the 5 percent level. Only at 4 ppm Al was there a significant difference in root K between varieties.

Aluminum treatments increased the concentration of K in the tops of both Dade and Romano (Table 7 and Fig. 6). At 12 ppm Al the concentration of K in the tops of Dade and Romano was 125 percent and 105 percent respectively of those with no Al. Within each variety differences in K content of tops between treatments were not significant, but there were significant differences between varieties at 4 ppm Al.

The trends in Mg concentration in both roots and tops were similar to those of Ca. At 4 ppm Al the concentration of Mg in the roots of both varieties was greater than that with no Al (Table 8 and Fig. 7), but these differences were not significant. Higher levels of Al decreased the concentration of Mg in roots of both varieties. For example, at 12 ppm Al the Mg concentration in the roots of Dade and Romano was 51 percent and 44 percent respectively, of those with no Al. These differences were significant at the 5 percent level of probability.

There were distinct differences in the Mg concentration of tops between the two varieties (Table 8 and Fig. 8). In Romano, the Mg concentration decreased progressively as the Al level was increased. In Dade, 4 and 8 ppm Al had little or no effect on the Mg concentration in the tops. At 12 ppm Al, however, the Mg concentration in the tops of Dade and Romano was 146 percent and 63 percent respectively of those with no Al. Differences in Mg concentration of tops between the two varieties

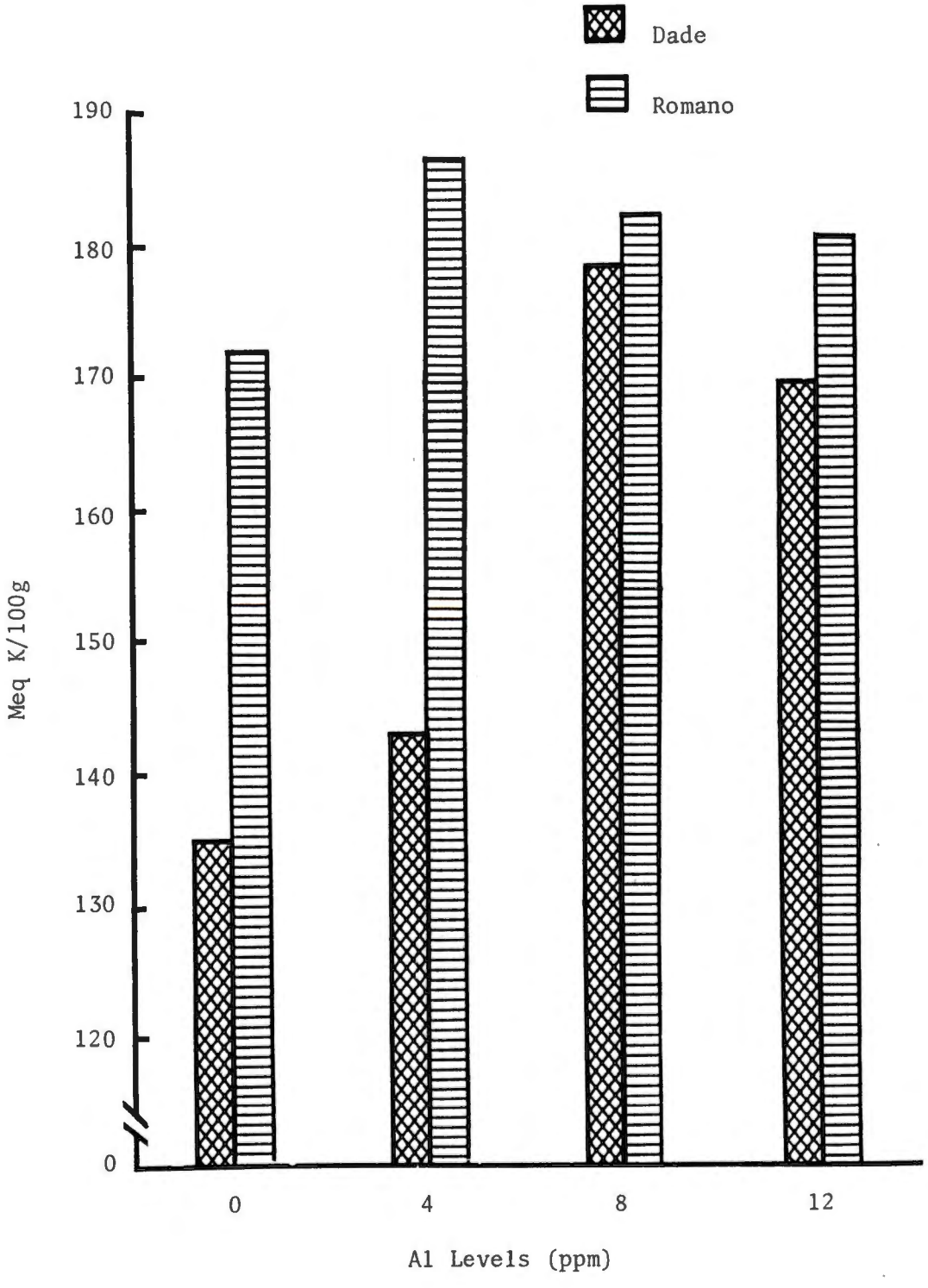

Fig. 6. Effect of different levels of Al on the K concentration of snapbean tops.

Table 8. Magnesium concentration of roots and tops of snapbeans at various levels of Al.

Variety	Al in Nutrient Solution (ppm)	Magnesium Concentration (meq/100g) Roots	Tops
Dade	0	78 b[1]	34 b
	4	91 ab	32 b
	8	62 bc	33 b
	12	40 c	49 a
Romano	0	86 ab	36 b
	4	116 a	33 b
	8	80 b	24 c
	12	38 c	23 c

[1]Means followed by the same letter in a column are not significantly different at the 0.05 level of probability.

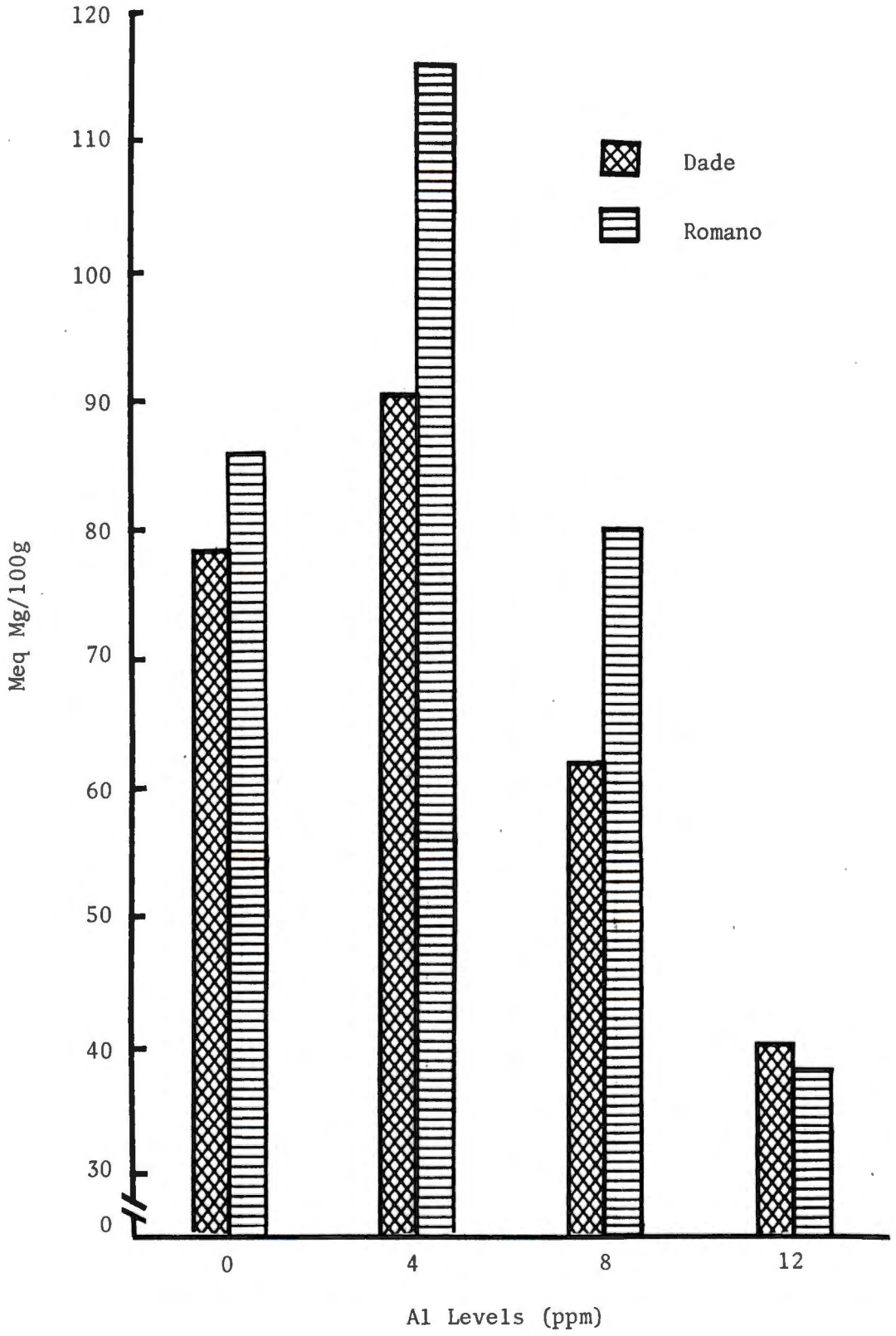

Fig. 7. Effect of different levels of Al on the Mg concentration of snapbean roots.

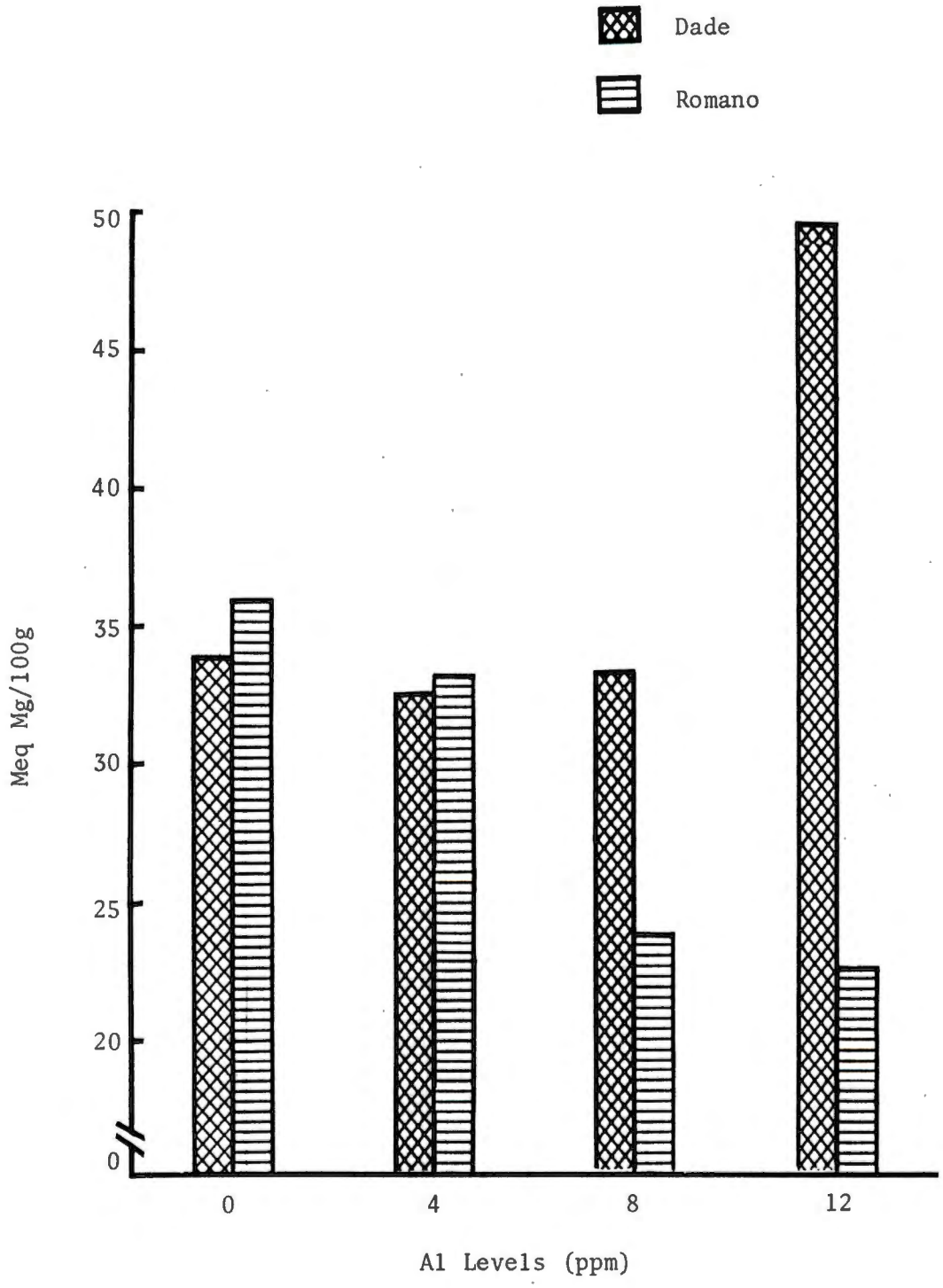

Fig. 8. Effect of different levels of Al on the Mg concentration of snapbean tops.

were significant at 8 and 12 ppm Al. In both varieties there was a significant correlation between Al levels in nutrient solution and the Mg concentration in both roots and tops (Table 3, page 29).

III. pH AND TOTAL ACIDITY

The pH and titratable acidity of roots and tops of Dade and Romano snapbeans are shown in Table 9. In both varieties root extracts had a slightly lower pH than the tops. There were no significant differences in pH between varieties for roots or tops.

Comparison of the total acidity of both roots and tops, as determined by titration (Table 9), did not reveal any significant differences between varieties.

IV. AL FIXATION BY ROOT MACERATES

The Al fixing capacity of root macerates of Dade and Romano is shown in Table 9. The results indicated that snapbean root macerates have the ability to fix soluble Al.

In all replications Dade root macerates fixed more Al than Romano. Dade root macerates fixed 55 percent of the added Al, whereas Romano macerates fixed 43 percent. Analysis of the data by an unpaired pooled variance t-test revealed that differences between varieties were not significant.

V. ORGANIC ACID CONCENTRATION

The results obtained for the determination of citric and malic acids are presented in Table 10. In both varieties citric and malic acid

Table 9. pH and titratable acidity of roots and tops and Al fixation by root macerates of snapbeans.

Variety	Plant Part	pH	Titratable Acidity (ml)[1]	Al Fixation[2]
Dade	Roots	5.53[3]	158	0.46
	Tops	5.67	333	--
Romano	Roots	5.50	147	0.36
	Tops	5.59	328	--

[1]Results reported as ml 0.01N NaOH per 100g fresh weight.

[2]Results reported as meq/100g fresh weight.

[3]Values for pH, titratable acidity and Al fixation are not significant between varieties at the 0.05 level of probability.

Table 10. Citric and malic acid concentration of roots and tops of snapbeans.

Variety	Plant Part	Citric Acid	Malic Acid
		ppm[1]	
Dade	Roots	102[2]	5
	Tops	172	37
Romano	Roots	82	9
	Tops	160	42

[1]Values are the means of 5 replicates.

[2]Values for both citric and malic acids are not significant between varieties at the 0.05 level of probability.

contents were higher in the tops than in the roots. The citric acid content of both roots and tops was higher in the more tolerant Dade variety. The reverse situation was true for malic acid content. The values obtained for citric and malic acids were not significant between varieties.

VI. LOCATION OF PHOSPHATE IN ROOTS BY STAINING

Roots of both Dade and Romano varieties from the control treatments, receiving no Al or P, are shown in Figs. 9 and 10 respectively. The photomicrographs of longitudinal root sections did not reveal any blue color development in the control treatments.

Cross and longitudinal sections of roots of both varieties from the P treatments, receiving no Al, revealed no blue color under low magnification (× 100). Under higher magnification (× 400) light traces of blue color were present throughout the entire root, including the vascular tissue. There was no localized concentration of blue color in any region. The color distribution was similar for both varieties. Results of the P treatments were not presented because the blue color was not clearly distinguishable in the photomicrographs.

Roots exposed to the Al treatment, receiving no P, are shown in Figs. 11 and 12. The photomicrographs of both varieties revealed a light blue coloration which was restricted mainly to the cells of the root cap and epidermis. Further back from the root tip the blue color was confined to the epidermis and one or two rows of cortical cells. The blue color was more distinct in the root cap of the Romano variety. In Dade the blue

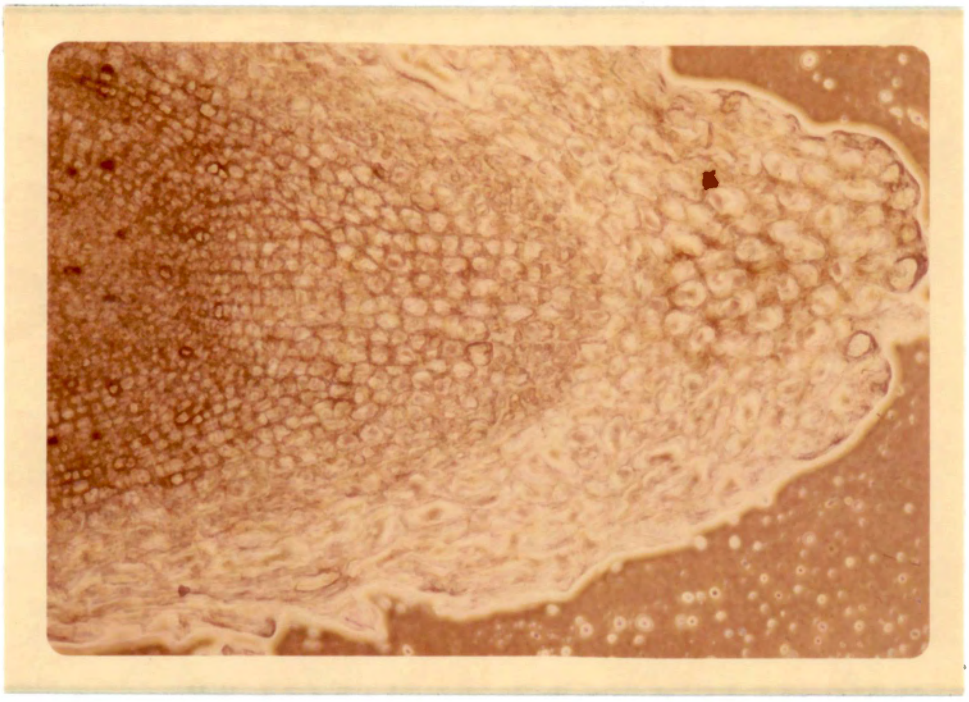

Fig. 9. Photomicrograph of a longitudinal section of a Dade root tip exposed to the control treatment (× 100).

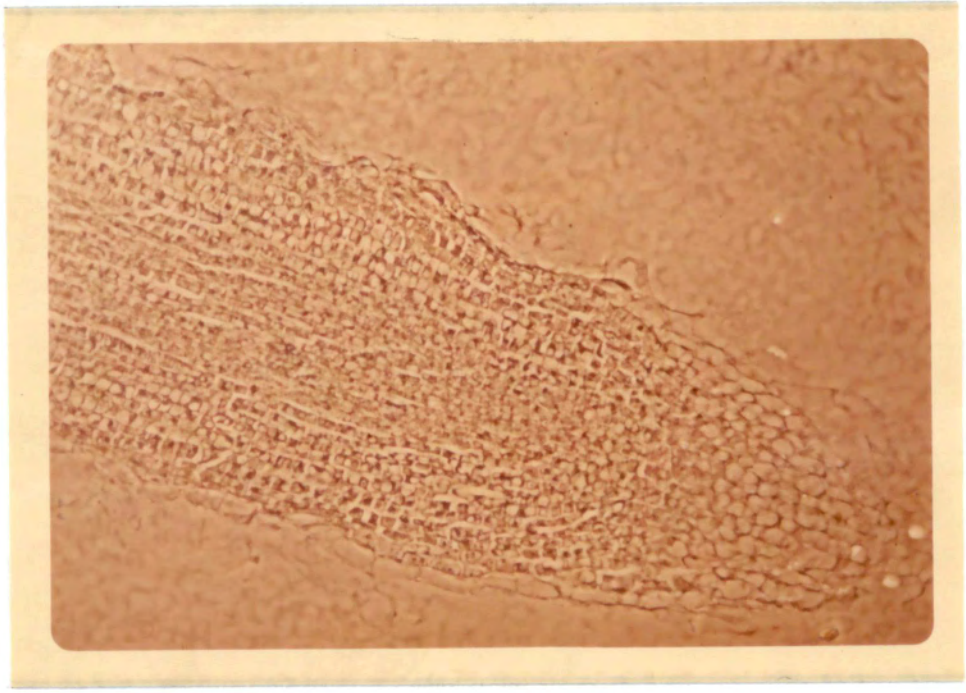

Fig. 10. Photomicrograph of a longitudinal section of a Romano root tip exposed to the control treatment (× 100).

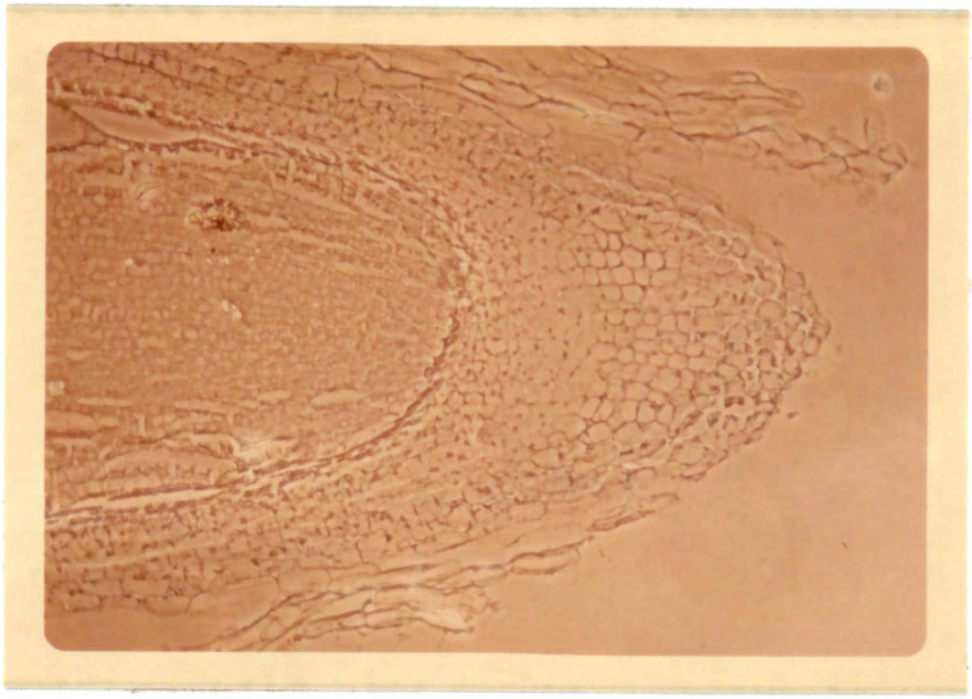

Fig. 11. Photomicrograph of a longitudinal section of a Dade root tip exposed to the Al treatment (× 100).

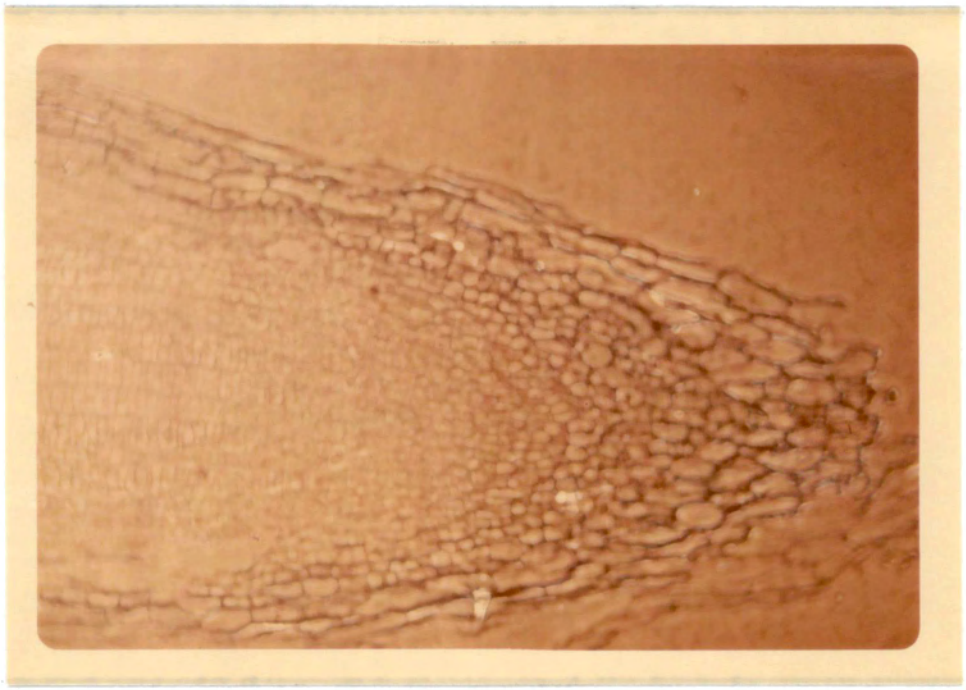

Fig. 12. Photomicrograph of a longitudinal section of a Romano root tip exposed to the Al treatment (× 100).

color was very light and was restricted mainly to the epidermal and root cap cells.

Photomicrographs of longitudinal sections of Dade roots which were subjected to the Al-P treatment (8 hours in 20 ppm Al followed by 16 hours in 12 ppm P) are presented in Figs. 13 and 14. Figure 13 clearly reveals the location of phosphate within the root tip. The intense blue color was located predominantly in all the cells of the root cap and epidermis. Further back from the tip the blue color was confined to the surface of the root, the cells of the epidermis and one to two rows of cortical cells adjacent to the epidermis. The blue color was restricted to about 2 to 3 mm of the root tip. Beyond this distance from the tip there was no blue color development. The photomicrograph at higher magnification (Fig. 14) shows the root meristem and part of the surrounding root cap cells. The blue color was confined to the cells of the root cap and epidermis. There was very little or no blue color behind the epidermis. The meristem of the root was clear.

Figure 15 shows a cross section of an Al-P treated Dade root about 2 mm from the root tip. The section shows two to four rows of root cap cells surrounding the epidermis. The blue color was present in the cells of the root cap, epidermis and outer cortical cells. At higher magnification (Fig. 16) the blue color can be seen very clearly, outlining the cells of the root cap and epidermis. Some blue color was present in the outer cortical cells. In addition, the blue color was present in cells adjacent to the stele. The stele was relatively clear.

Figure 17 shows a longitudinal section of a Romano root tip that was subjected to the Al-P treatment. The blue color was restricted mainly to

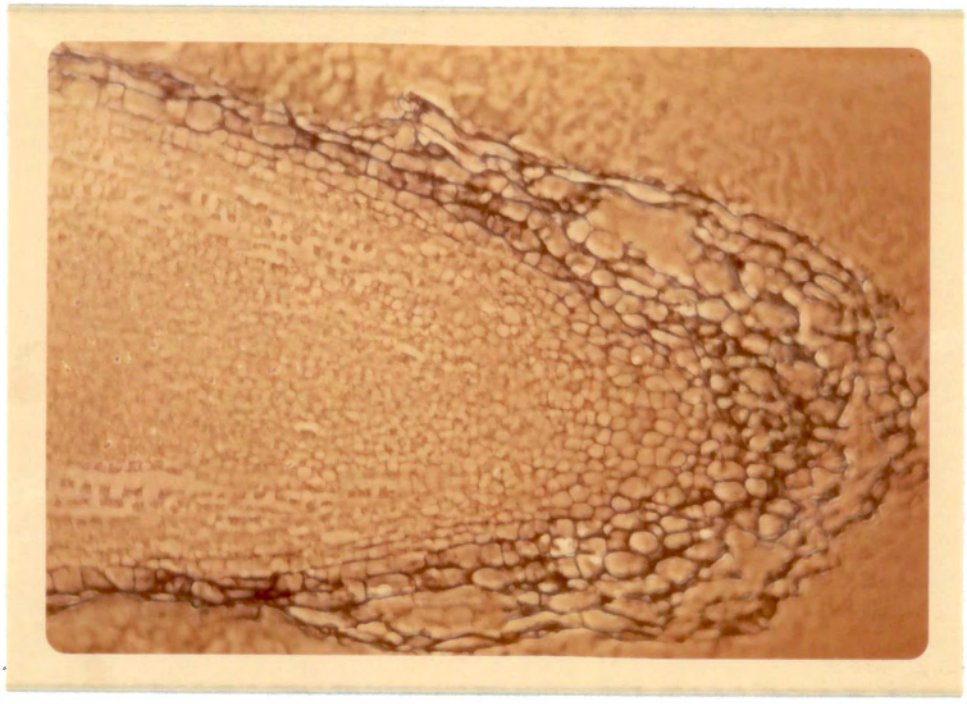

Fig. 13. Photomicrograph of a longitudinal section of a Dade root tip exposed to the Al-P treatment (× 100).

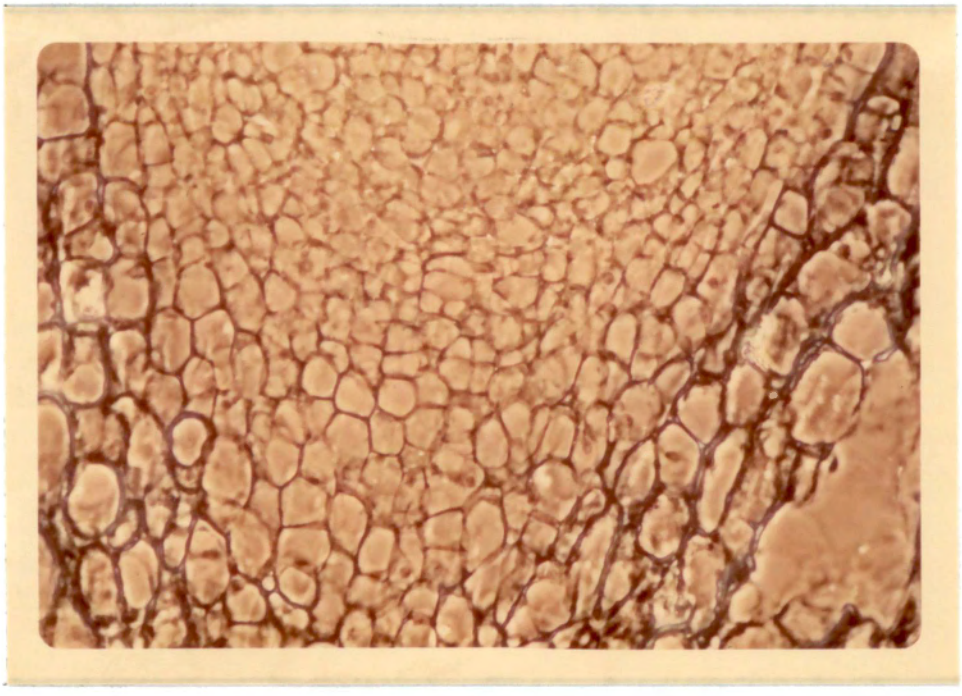

Fig. 14. Photomicrograph of a longitudinal section of a Dade root tip exposed to the Al-P treatment (× 250).

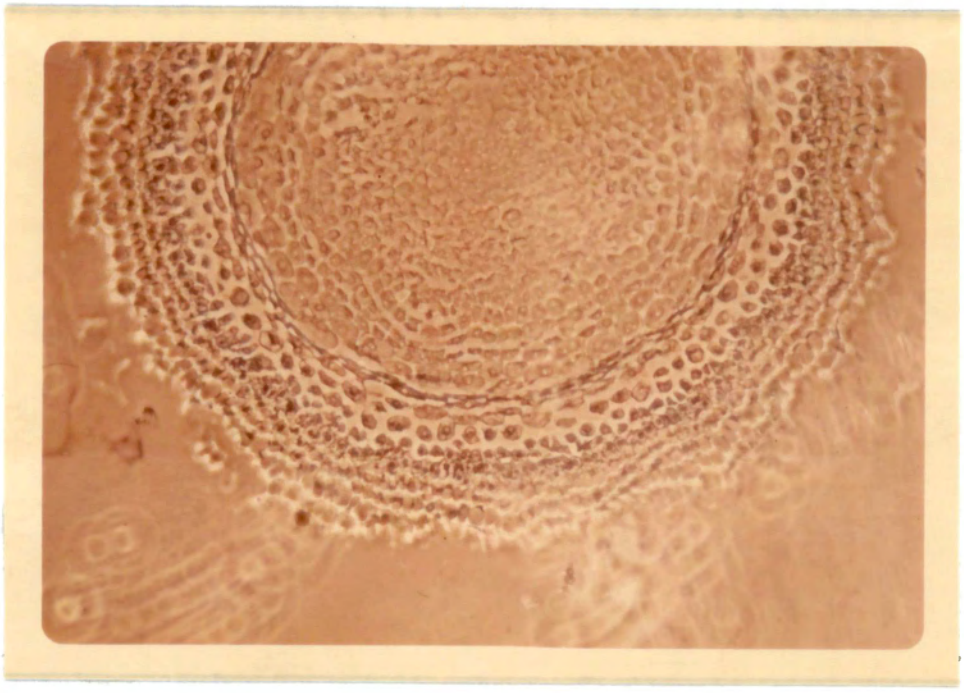

Fig. 15. Photomicrograph of a cross section of a Dade root tip exposed to the Al-P treatment (× 100).

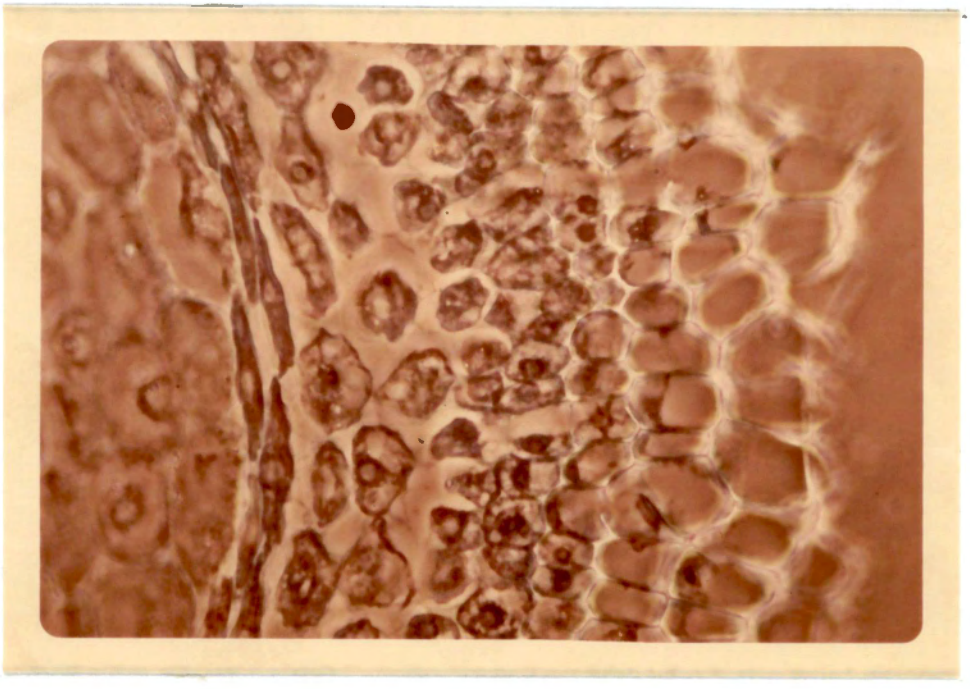

Fig. 16. Photomicrograph of a cross section of a Dade root tip exposed to the Al-P treatment (× 400).

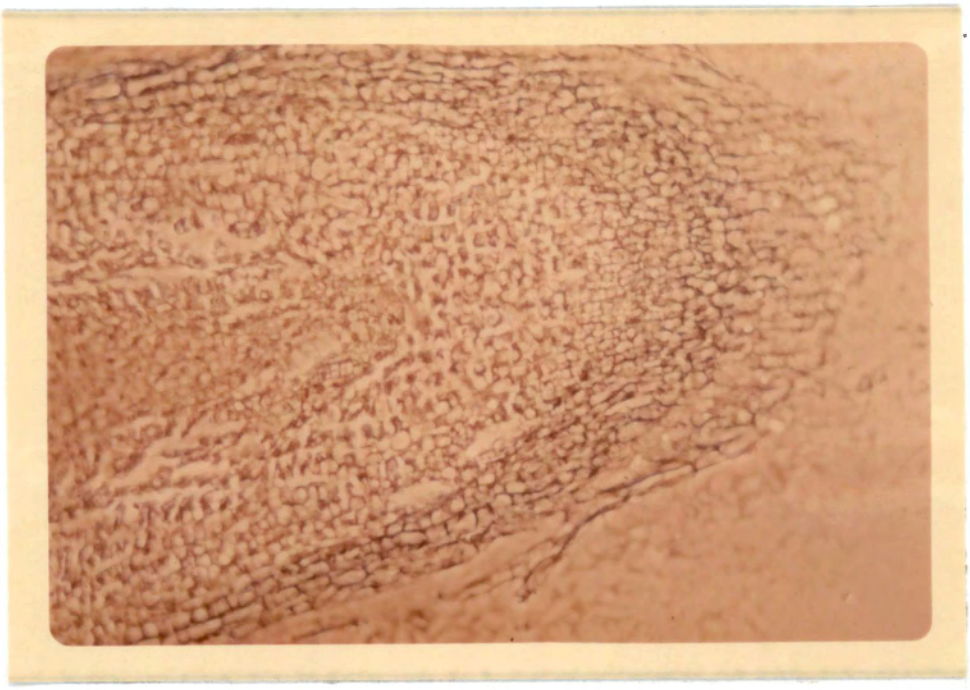

Fig. 17. Photomicrograph of a longitudinal section of a Romano root tip exposed to the Al-P treatment (× 100).

the outer regions of the root tip. The cells of the root cap, epidermis and many of the cortical cells were lightly stained. Further back from the tip the blue color was located in the cells of the epidermis and three or four rows of cortical cells. Traces of blue were present in the meristem and vascular tissues. These results are further substantiated by cross sections of mature Romano roots (Figs. 18 and 19). The blue color in these sections was located on the outer surface of the root, the epidermal and cortical cells. Occasionally, isolated blue patches were present in some cortical cells adjacent to the endodermis and in some of the cells of the vascular tissue (Fig. 19).

VII. MORPHOLOGICAL EFFECTS OF ALUMINUM

The roots of Dade and Romano plants exposed to solutions with no added Al had straight axes and were white in color. The primary roots produced numerous lateral roots which were several centimeters long (Figs. 20 and 21).

The first visual effect of the 20 ppm Al treatment, evident after 1 day, was inhibition of primary and lateral root growth. In addition, root tips in the Al treatment curled upward. This apparent reversal of the geotropic response of roots was more marked in the Romano variety.

After 2 to 3 days in 20 ppm Al, inhibition of root growth became more severe. Upward curling of the root tips was more pronounced. The primary root axes produced numerous lateral roots which ceased to grow upon emergence from the main axis. This gave the roots a stubby appearance. Many of the root tips were gelatinous in appearance. The

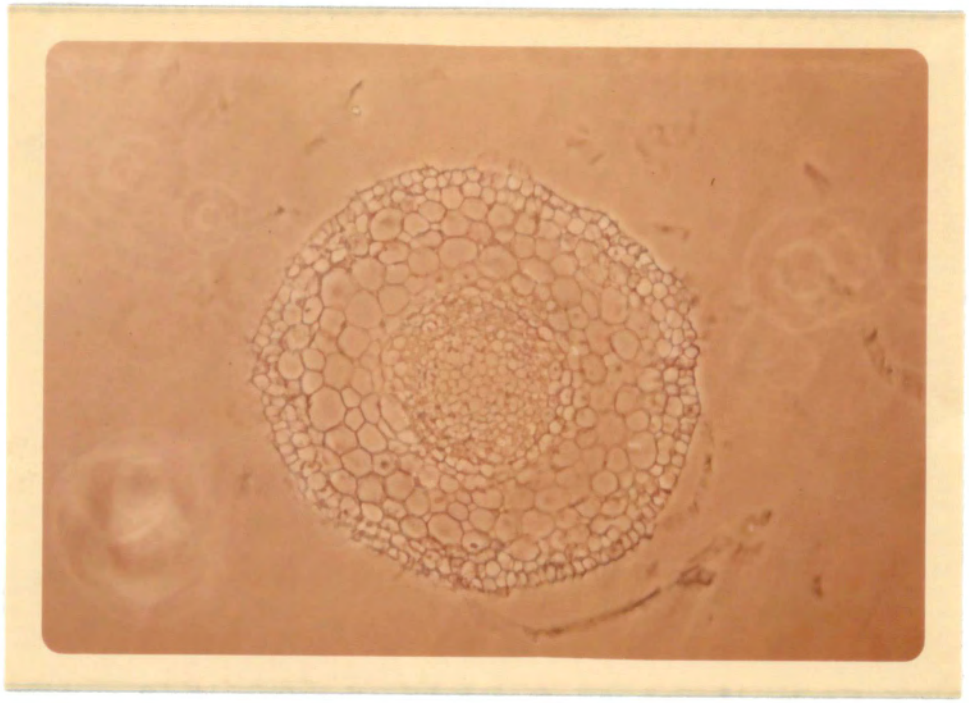

Fig. 18. Photomicrograph of a cross section of a Romano root tip exposed to the Al-P treatment (× 250).

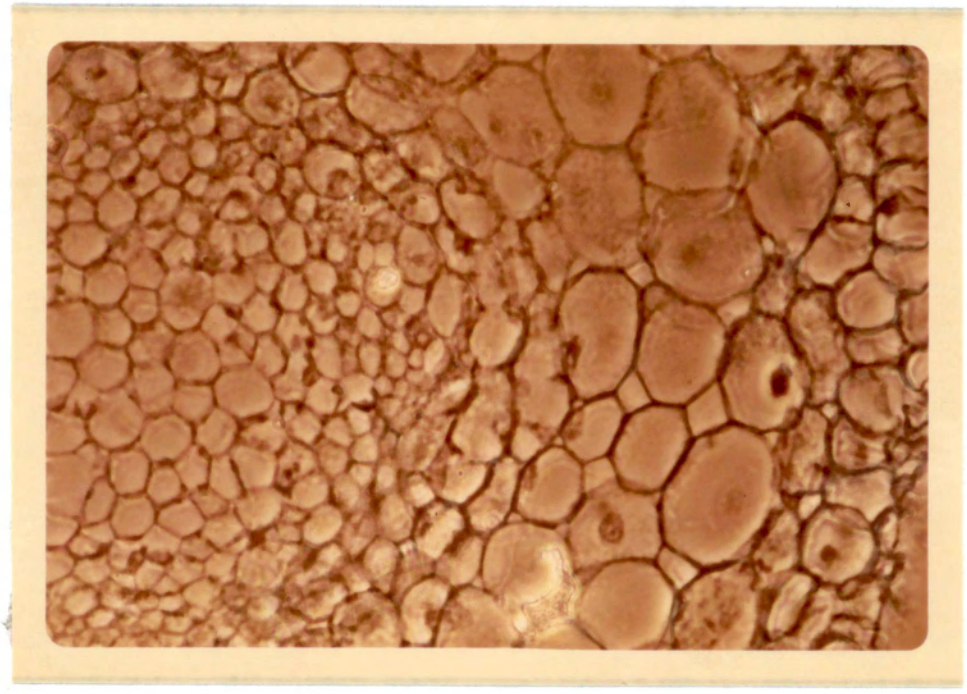

Fig. 19. Photomicrograph of a cross section of a Romano root exposed to the Al-P treatment (× 400).

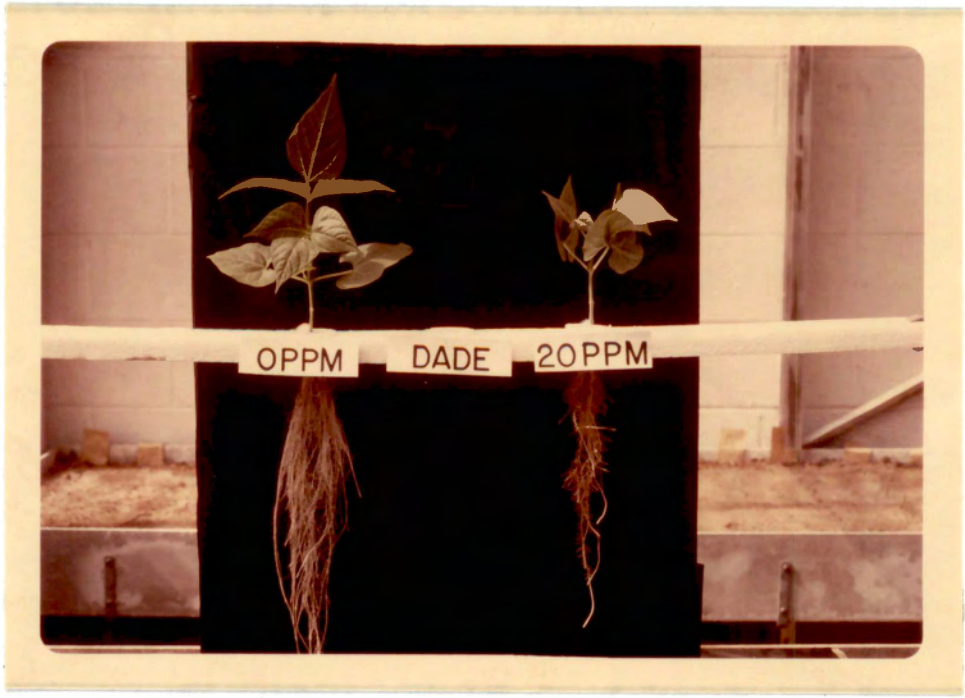

Fig. 20. Effect of 12 days of Al treatment on the growth of Dade snapbeans.

Fig. 21. Effect of 12 days of Al treatment on the growth of Romano snapbeans.

roots were brownish in color compared to the white color of the untreated roots (Figs. 22 and 23).

After 3 days and up to the end of the treatment period the above symptoms became more pronounced. Lateral roots in both Dade and Romano, in many cases, developed right up to the root tip. In some cases clusters of lateral roots were produced at the root tip (Figs. 22 and 23). In the 0 ppm Al treatment no lateral roots were produced within about 4 cm of the root tip. At the end of the treatment period the Al-treated roots of both varieties were completely brown in color, severely stunted and stubby in appearance (Figs. 20 and 21). Some of the Al-treated root tips lacked root caps. Root caps still intact on the root tips were swollen, curled backwards, and appeared to be gelatinous in nature. Root tips not treated with Al were white, pointed and were not gelatinous in appearance.

After a week in the 20 ppm Al treatment the leaves of both varieties developed an abnormally dark green color compared to those receiving no Al. Romano tops showed typical purpling symptoms of P deficiency, particularly at the base of the stem and petioles. No purpling of the tops was evident in the Dade variety. The leaves of the Al-treated plants were much smaller in size than those receiving no Al. In addition, top growth of Al-treated plants of both varieties was considerably reduced (Figs. 20 and 21). After about 10 days in the 20 ppm Al treatment, Dade and Romano plants showed peculiar leaf orientation. The leaflets assumed more or less vertical positions compared to the horizontal position of leaflets from the 0 ppm Al treatment (Figs. 20 and 21).

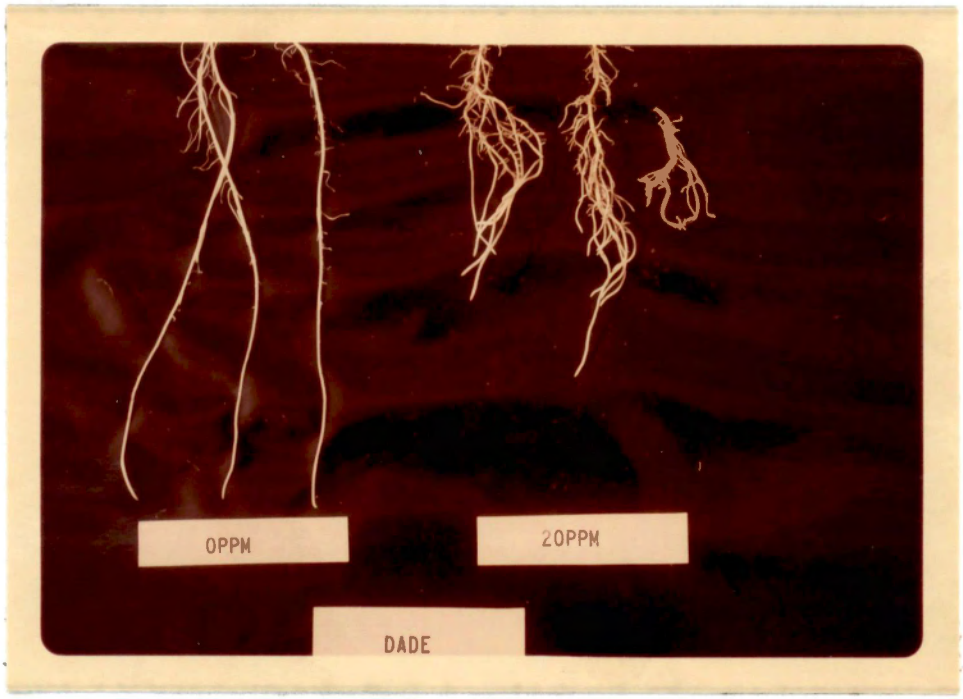

Fig. 22. Development of lateral roots at the root tips of Dade snapbeans treated with Al for 12 days.

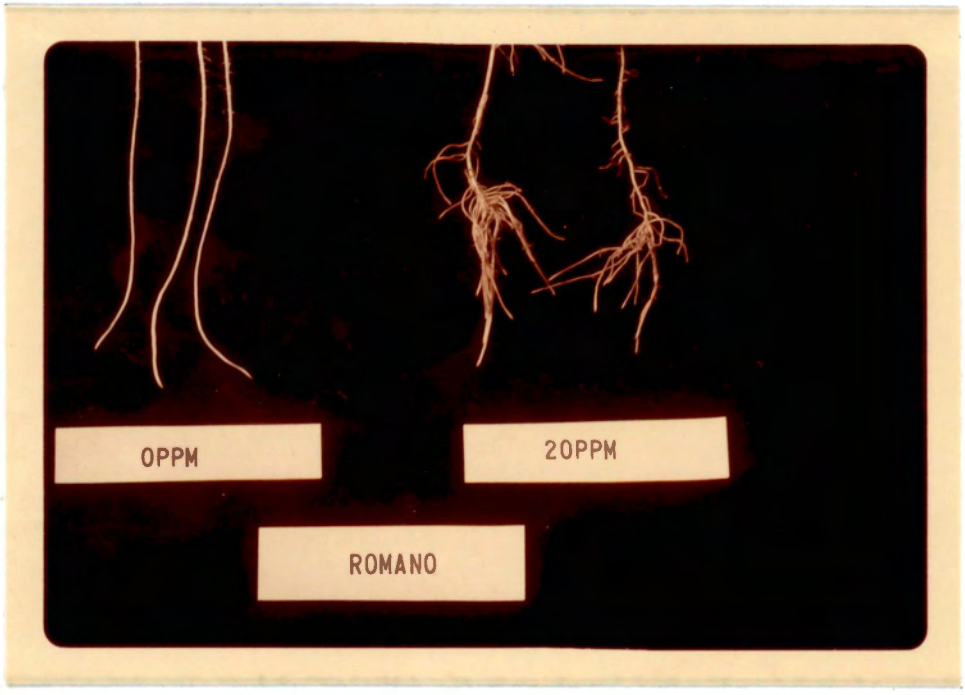

Fig. 23. Development of lateral roots at the root tips of Romano snapbeans treated with Al for 12 days.

Significant reductions in root elongation of Al-treated plants were observed after 3 days in the Al treatment (Table 11 and Figs. 24 and 25). After 3 days in the Al treatment the mean root lengths of Dade and Romano were 68 percent and 66 percent, respectively, of those with no Al. For the remainder of the treatment period the mean root length of the Al-treated plants was significantly different from those with no Al. With the exception of day nine there were no significant differences between varieties (Table 11, Figs. 24 and 25).

The mean height of plant tops at 3-day intervals during the treatments is shown in Table 12 and Figs. 26 and 27. Although Al-treated plants were generally smaller in size than the 0 ppm Al treatment, differences were only significant on day twelve. There were no significant differences between varieties for plant height.

Root tips of Dade and Romano not treated with Al had a typical dicotyledonous root cell organization (Figs. 28 and 29). All cells of the root arose from anticlinal and periclinal divisions of three distinct groups of initials. Each group of initials was composed of one tier of three to six cells (Fig. 30). The organization of cells in the apical meristem was similar for both varieties. For this reason a photomicrograph of the apical meristem of the Dade variety is not presented. All the cells of the root, especially the cells of the cortex and vascular tissue, were regularly arranged in vertical rows. This was due to the regular and orderly divisions of the initial cells. The root caps of both varieties were intact and pointed (Figs. 28 and 29). All root cap cells contained nuclei and cytoplasm. The root cap cells were regularly

Table 11. Mean root length of Dade and Romano snapbeans at 3-day intervals following exposure to 0 and 20 ppm Al.

Variety	Al in Nutrient Solution (ppm)	Mean Root Length (cm) 0	3	6	9	12 days
Dade	0	13.7[1]	24.0 a[2]	30.7 a	39.0 a	47.1 a
	20	13.5	16.2 b	19.2 b	22.0 c	27.4 b
Romano	0	14.1	27.3 a	34.8 a	41.9 a	49.5 a
	20	14.2	18.0 b	22.9 b	27.2 b	31.3 b

[1]Values for root length on day 0 are not significantly different at the 0.05 level of probability.

[2]Means in each column followed by the same letter are not significantly different at the 0.05 level of probability.

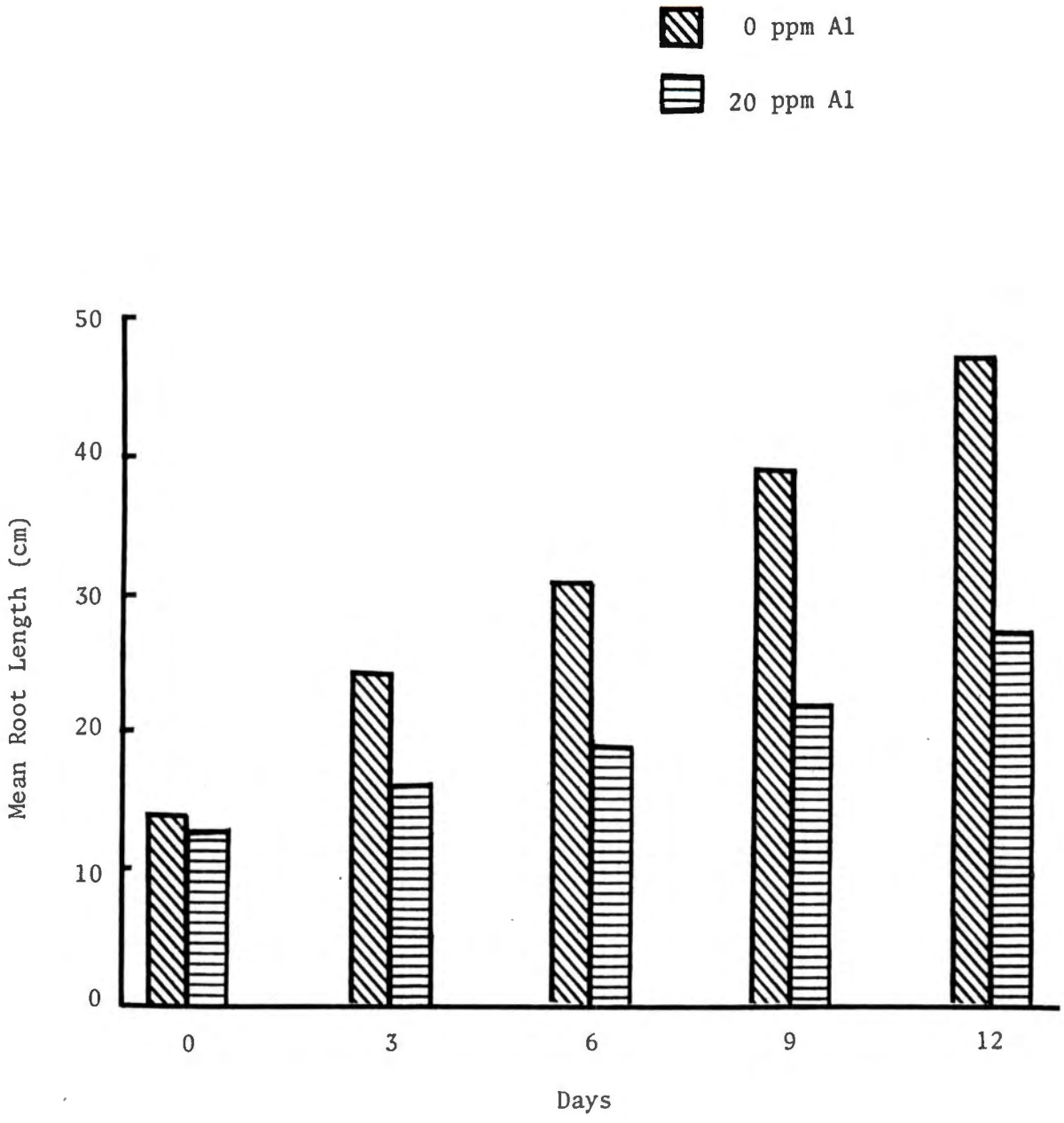

Fig. 24. Mean root length of Dade snapbeans at 3-day intervals following exposure to 0 and 20 ppm Al.

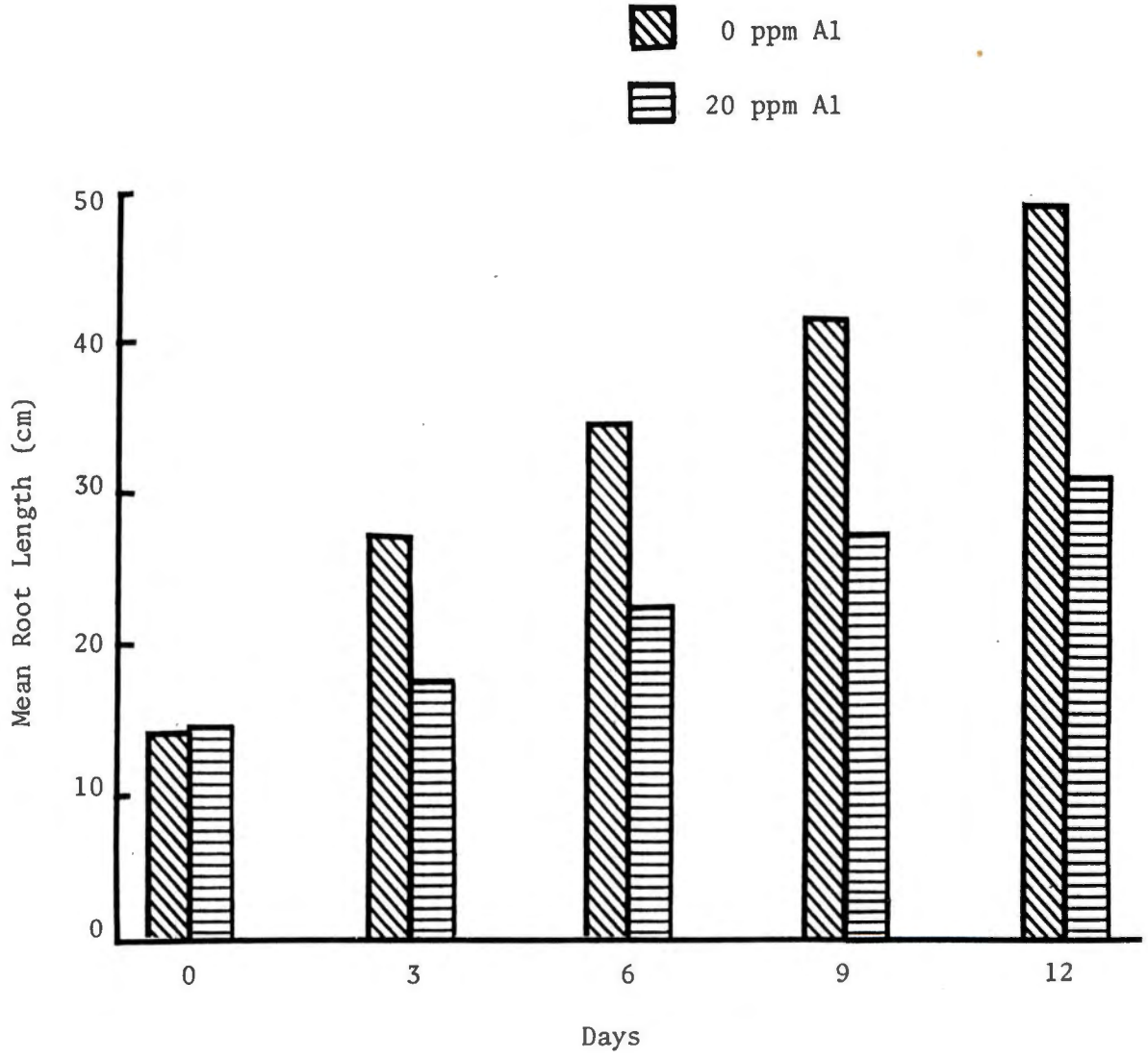

Fig. 25. Mean root length of Romano snapbeans at 3-day intervals following exposure to 0 and 20 ppm Al.

Table 12. Mean plant height of Dade and Romano snapbeans at 3-day intervals following exposure to 0 and 20 ppm Al.

Variety	Al in Nutrient Solution (ppm)	Mean Plant Height (cm) 0	3	6	9	12 days
Dade	0	7.0[1]	8.6	9.4 ab[2]	10.7 ab	16.3 a
	20	7.1	7.5	8.3 b	9.2 b	10.1 b
Romano	0	7.8	9.1	10.4 a	11.8 a	14.8 a
	20	7.6	8.2	9.2 ab	10.3 ab	11.5 b

[1]Values for plant height are not significantly different at the 0.05 level of probability at the 0 and 3 day intervals.

[2]Means in each column followed by the same letter are not significantly different at the 0.05 level of probability.

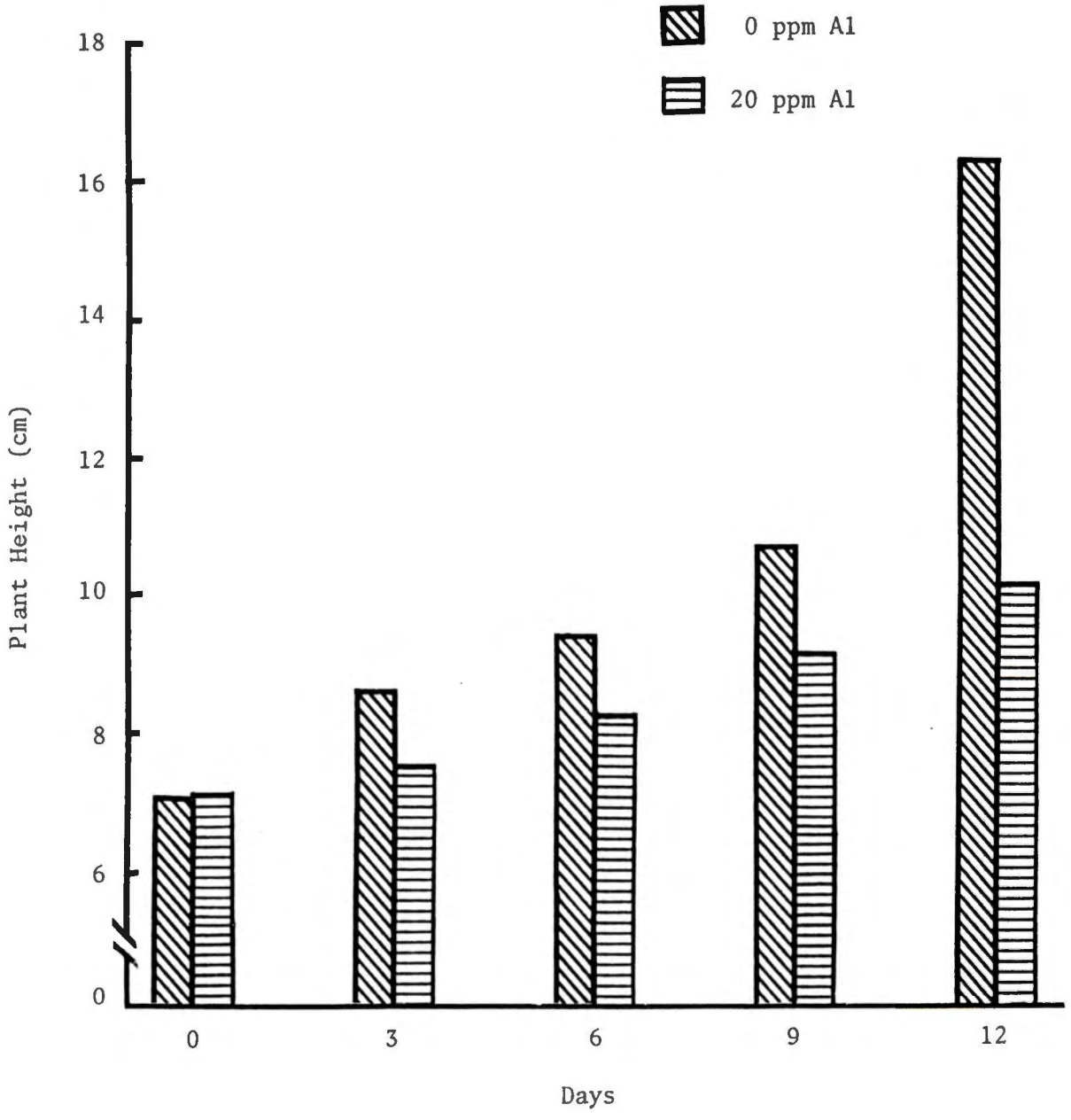

Fig. 26. Mean plant height of Dade snapbeans at 3-day intervals following exposure to 0 and 20 ppm Al.

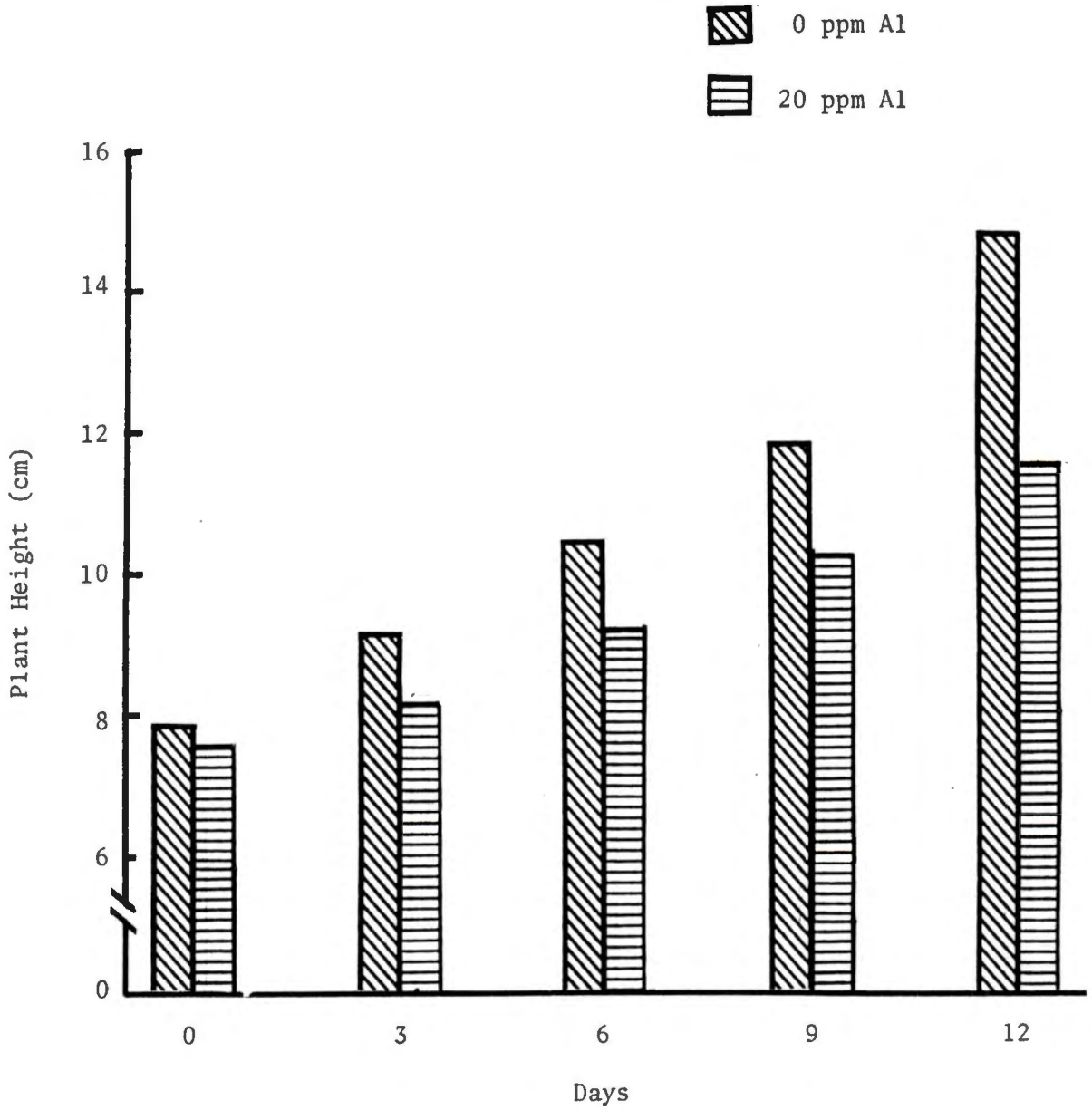

Fig. 27. Mean plant height of Romano snapbeans at 3-day intervals following exposure to 0 and 20 ppm Al.

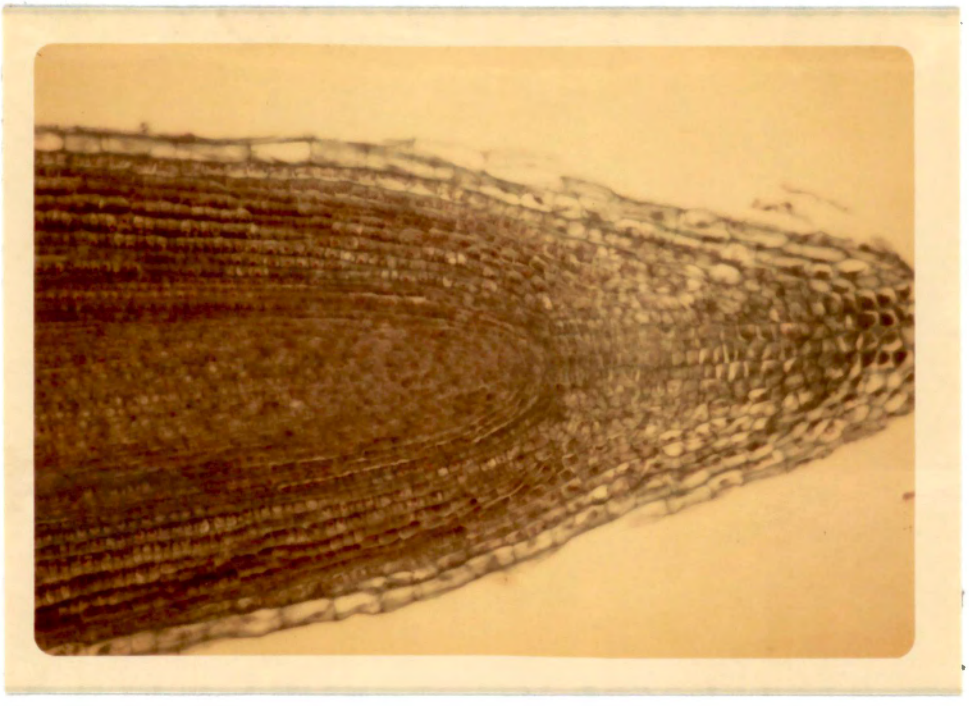

Fig. 28. Median longitudinal section of a primary root tip of Dade snapbean at 0 ppm Al (× 100).

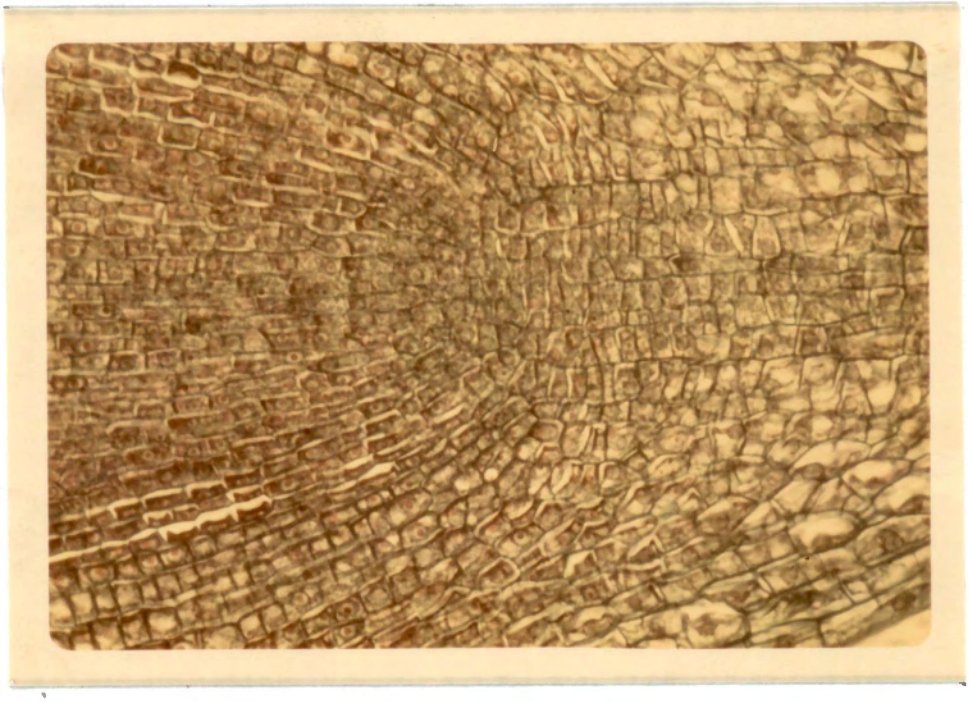

Fig. 29. Median longitudinal section of a primary root tip of Romano snapbean at 0 ppm Al (× 100).

Fig. 30. Median longitudinal section of a primary root tip of Romano snapbean at 0 ppm Al showing the meristem (× 250).

arranged in rows, being cut off by anticlinal divisions of the root cap initials (Figs. 28-30). The epidermis of both varieties was intact and continuous. All epidermal cells contained protoplasm. Almost all cells of the root possessed large nuclei which stained dark red. The large nuclei were especially prominent in the vascular and cortical cells (Figs. 28 and 29).

Primary root tips from Al-treated Dade plants are shown in Figs. 31-33. The root tips were swollen and possessed abnormally elongated root caps (Fig. 31). The sides of the root cap were curled back and many of the cells were sloughed off. The cells in the outer regions of the cap were irregularly arranged. Many of these cells lacked nuclei and other cell contents. The epidermal cells were not intact and continuous as in roots not treated with Al. Some of the cells had collapsed while others had sloughed off (Fig. 32).

Aluminum-treated Dade root tips showed no major differences in apical organization compared to the 0 ppm Al treatment (Fig. 33). The cells in the root cap region, immediately behind the meristem (Fig. 32), lacked the regular arrangement seen in the 0 ppm Al treatment. Many of the cells of the root cap seemed to contain some extraneous material which appeared black (Figs. 31 and 32). This was conspicuously lacking in roots not treated with Al (Fig. 28).

Median longitudinal sections of primary root tips from Al-treated Romano plants are shown in Figs. 34 and 35. The most conspicuous effect of the Al treatment was the curling backward and sloughing off of a major portion of the root cap (Fig. 34). Unlike Al-treated Dade roots

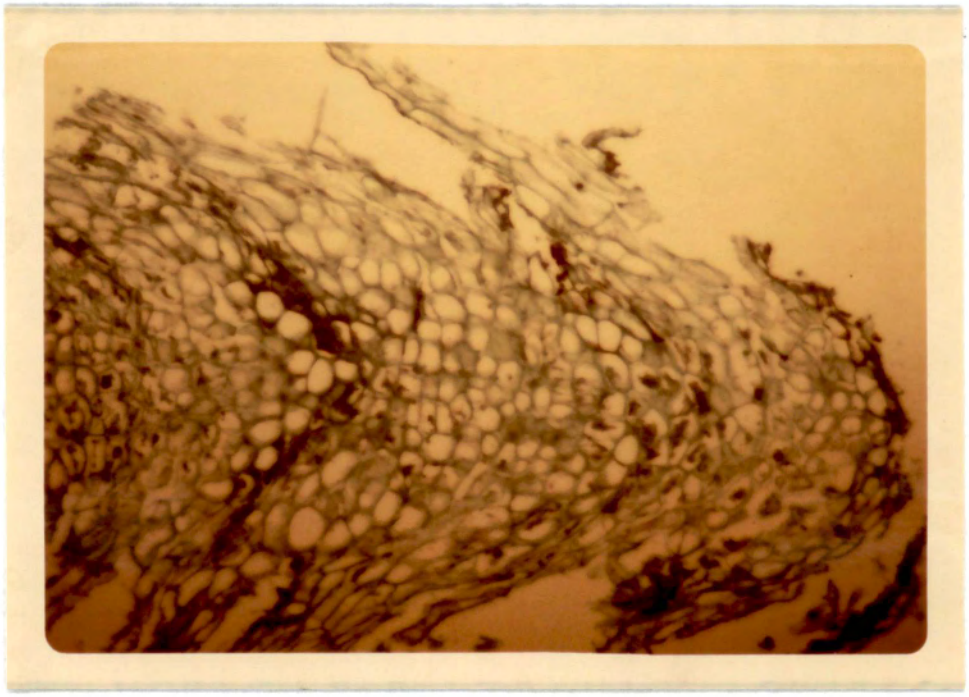

Fig. 31. Median longitudinal section of a primary root tip of Dade snapbean at 20 ppm Al showing part of the elongated root cap (× 100).

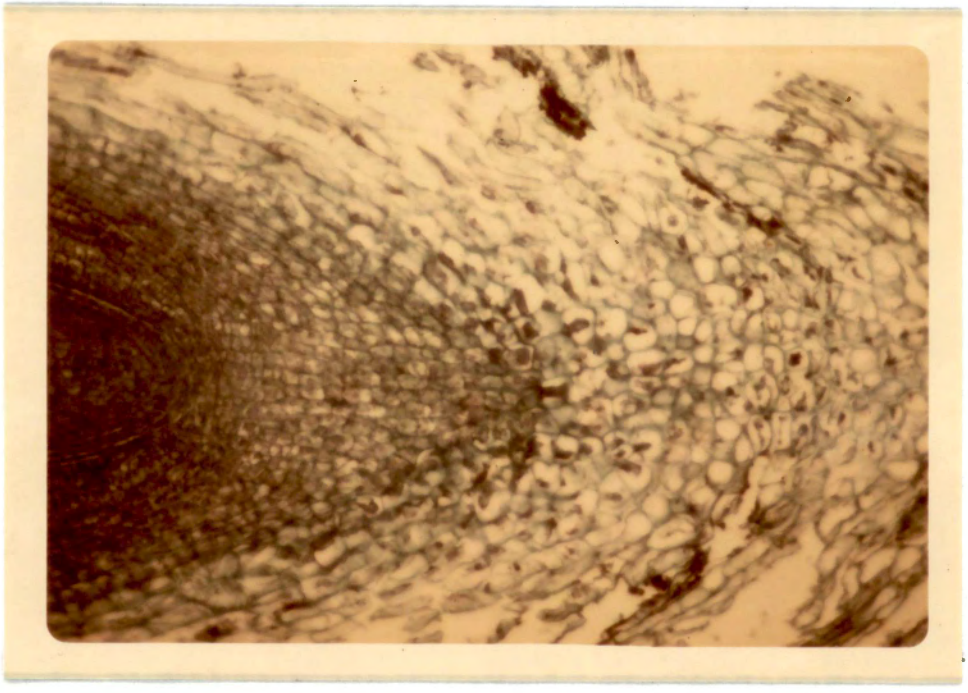

Fig. 32. Median longitudinal section of a primary root tip of Dade snapbean at 20 ppm Al showing the meristem and part of the root cap (× 100).

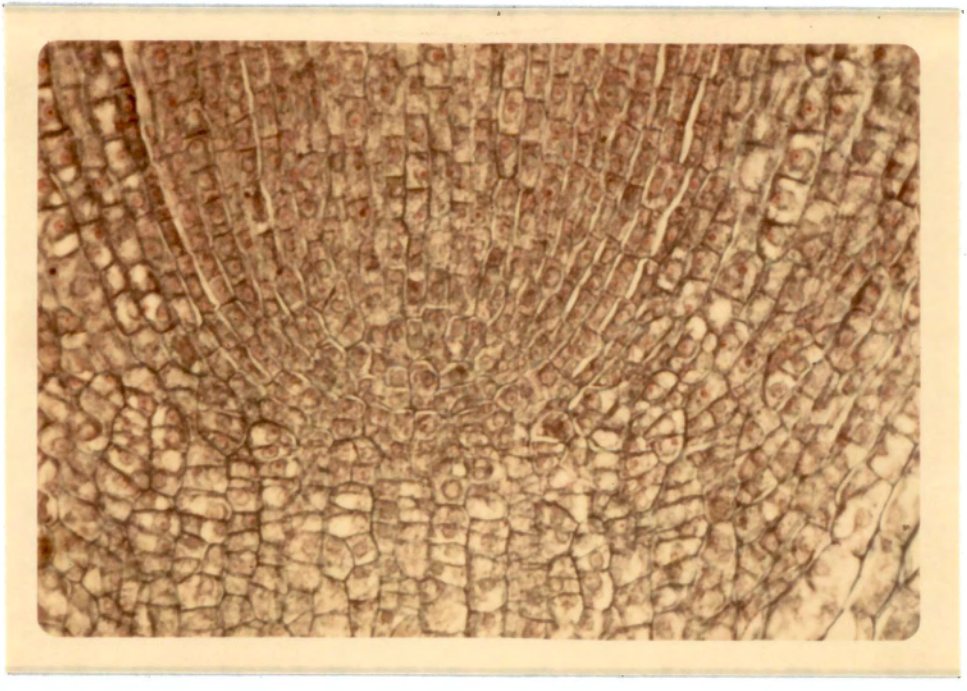

Fig. 33. Median longitudinal section of a primary root tip of Dade snapbean at 20 ppm Al showing the meristem (× 250).

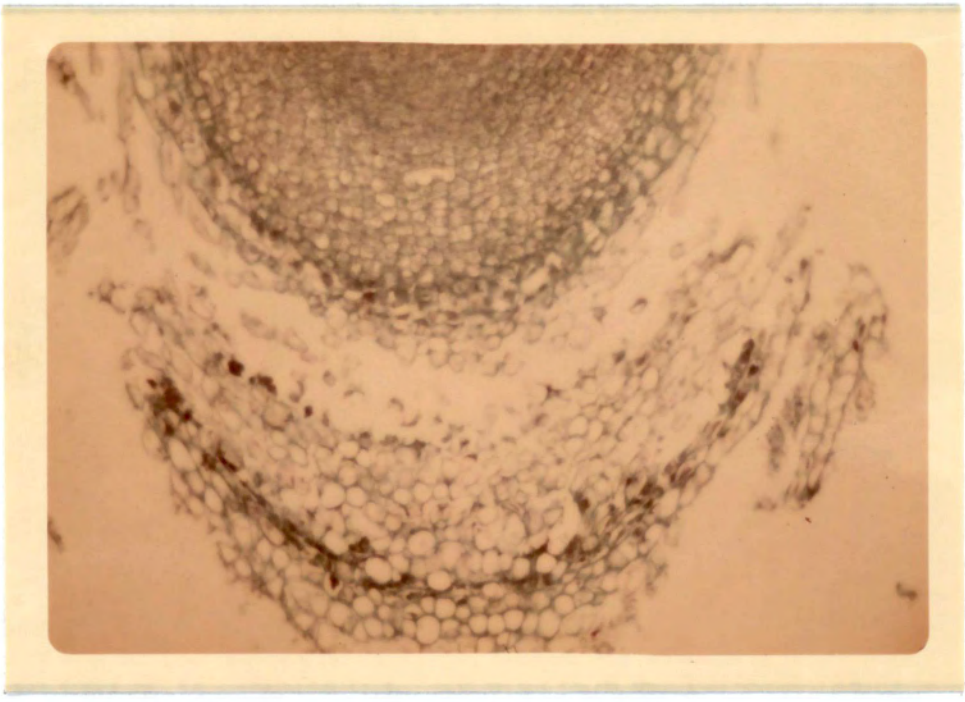

Fig. 34. Median longitudinal section of a primary root tip of Romano snapbean at 20 ppm Al showing the recurved root cap (× 100).

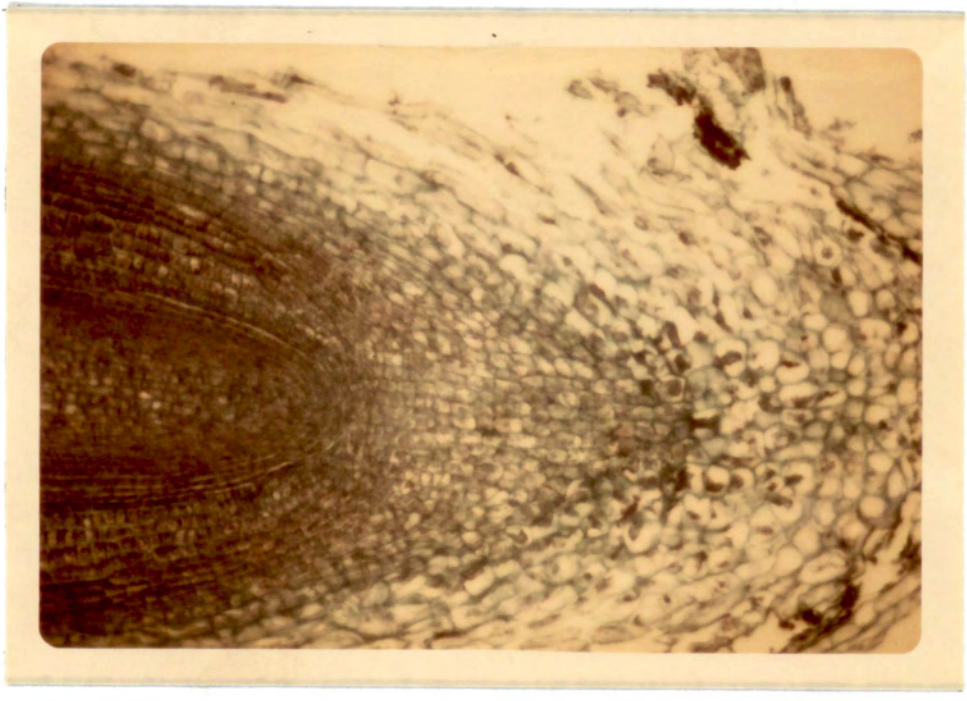

Fig. 35. Median longitudinal section of a primary root tip of Romano snapbean at 20 ppm Al showing the meristem and the root cap (× 100).

the root caps were not abnormally elongated. The root tip had a swollen appearance. The cells of the root cap were similar to those of Al-treated Dade roots. The outer cells of the cap were irregularly arranged. Many of the cap cells lacked nuclei and other cell contents. In comparison with the 0 ppm Al treatment, the root cap cells appeared to be disorganized. As in the Al-treated Dade variety some extraneous material, that appeared black, was present in the root cap (Fig. 34). The epidermal cells were either collapsed or sloughed off. In the apical meristem the root initials were not as clearly defined as in the 0 ppm Al treatment.

The apical organization in young lateral roots of Dade and Romano at 0 ppm Al were similar. Figure 36 shows a photomicrograph of a longitudinal section of a Romano lateral root at 0 ppm Al. The lateral root cells were regularly arranged as they were in the primary root tip of this variety. In Fig. 37 a young lateral root is shown emerging from the primary root axis at 20 ppm Al. The cortical cells were large, irregularly arranged and seemed to have reached maturity closer to the root tip than in those grown at 0 ppm Al.

Young lateral roots of Romano at 20 ppm Al were injured to a greater degree than those of Dade (Figs. 38 and 39). Lateral roots of both varieties from the Al treatment were swollen (Figs. 37-39) compared to those at 0 ppm Al (Fig. 36). In the Romano variety the cells of the apical meristem lacked their usual organization (Fig. 38). The meristematic cells were irregularly arranged. Cortical cells behind the root tip were rather large and disorganized. The cells lacked the regular arrangement seen in root tips treated with 0 ppm Al.

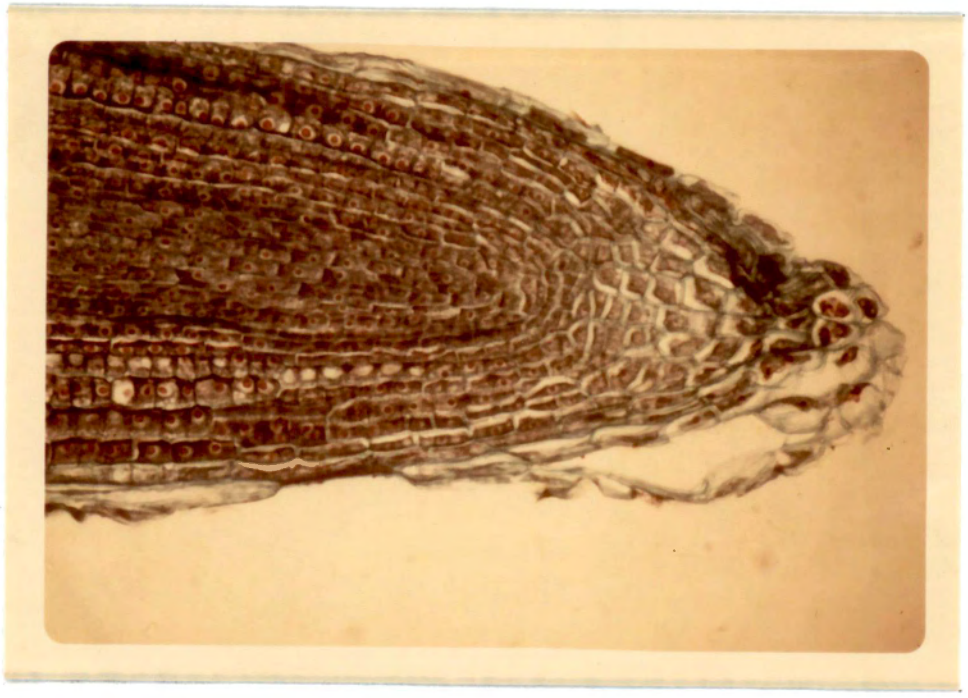

Fig. 36. Median longitudinal section of a lateral root tip of Romano snapbean at 0 ppm Al (× 250).

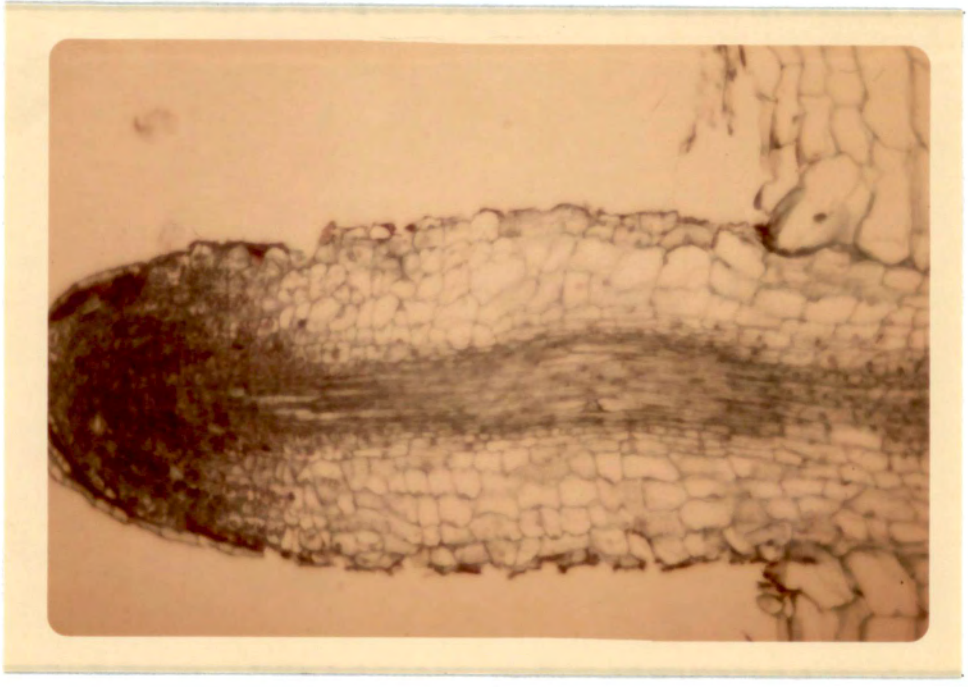

Fig. 37. Median longitudinal section of a lateral root of Romano snapbean, at 20 ppm Al, emerging from the primary root axis (× 100).

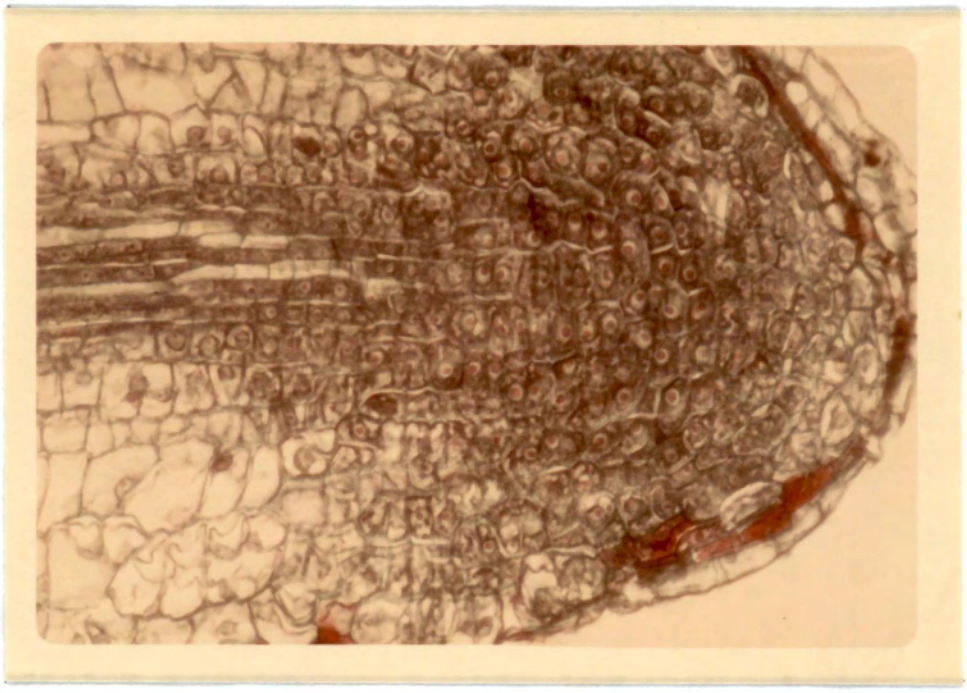

Fig. 38. Median longitudinal section of a lateral root tip of Romano snapbean at 20 ppm Al showing the disorganized meristem (× 250).

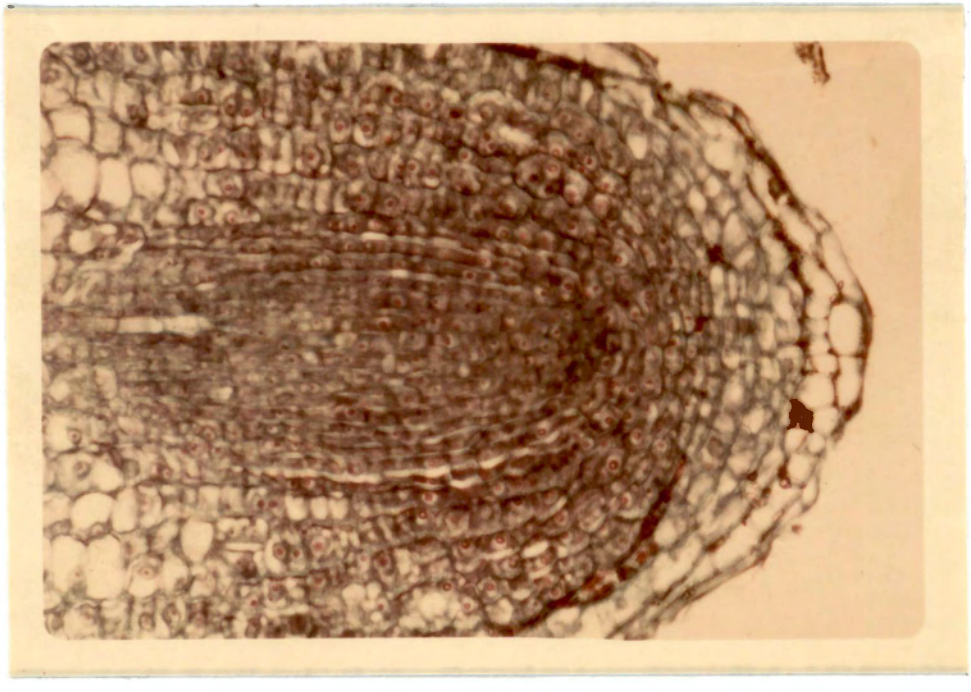

Fig. 39. Median longitudinal section of a lateral root tip of Dade snapbean at 20 ppm Al showing the meristem (× 250).

Figure 39 shows a median longitudinal section of a lateral root tip of the Dade variety at 20 ppm Al. Disorganization of cells was evident in the cortical region behind the meristem. As in the Romano variety many of the cortical cells were unusually large. Aluminum did not seem to have had much adverse effect on the organization of cells in the apical meristem of the Dade variety.

VIII. ENERGY DISPERSIVE ANALYSIS OF X-RAYS

X-ray scans for P, Ca and Al, and a secondary electron image of a Romano root tip at 0 ppm Al are presented in Fig. 40. The white line indicates the area scanned. High concentrations of P were present throughout the area scanned. There was no accumulation of P in any particular area of the root. The distribution of Ca was similar to that of P, except that the levels present were much lower. The X-ray scan for Al indicated that no detectable amounts of Al were present in the root tissues. Similar results were obtained for root tips of the Dade variety, not treated with Al.

X-ray scans across the meristem of a Romano root tip, grown in 20 ppm Al, are shown in Fig. 41. The X-ray scans indicated that the site of highest P was located in the same region of the root as the site of highest Al. The highest concentrations were located in the outer region of the root cap where the cap curled backward. Low levels of Al were present throughout the scan area.

Linear scans through the meristem of a Dade root tip treated with 20 ppm Al are presented in Fig. 42. The location of Al coincided with

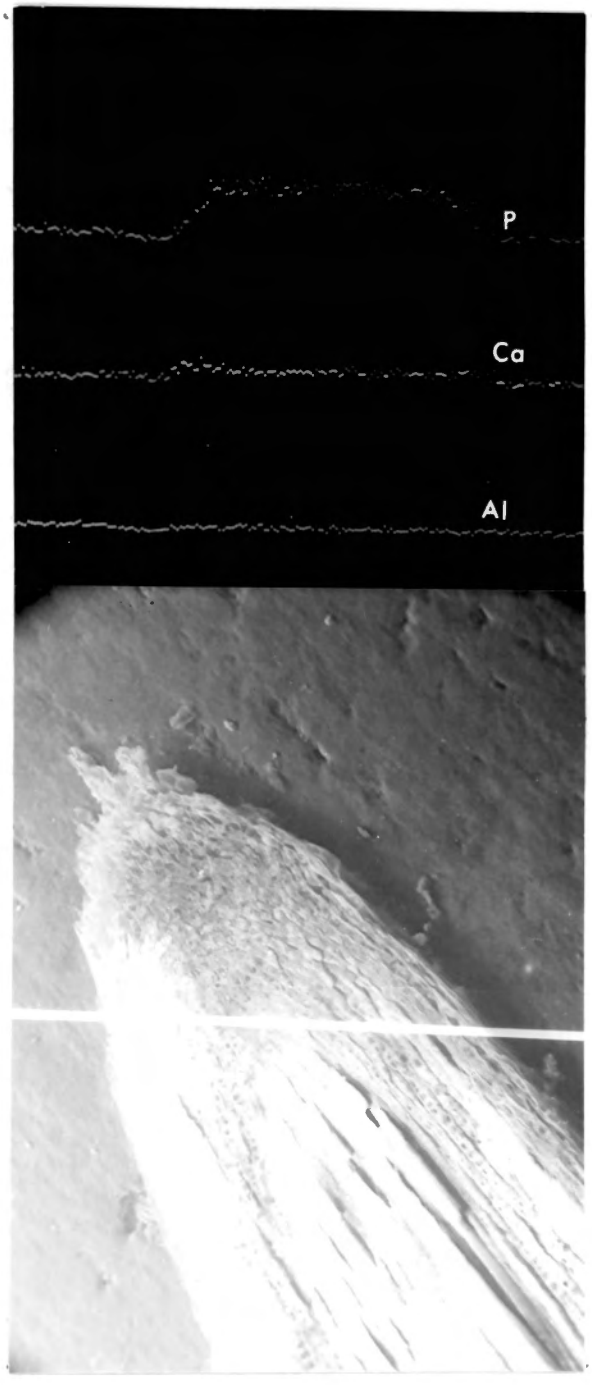

Fig. 40. X-ray scans for P, Ca and Al (top) and secondary electron image (bottom) of a longitudinal section of a Romano root tip at 0 ppm Al.

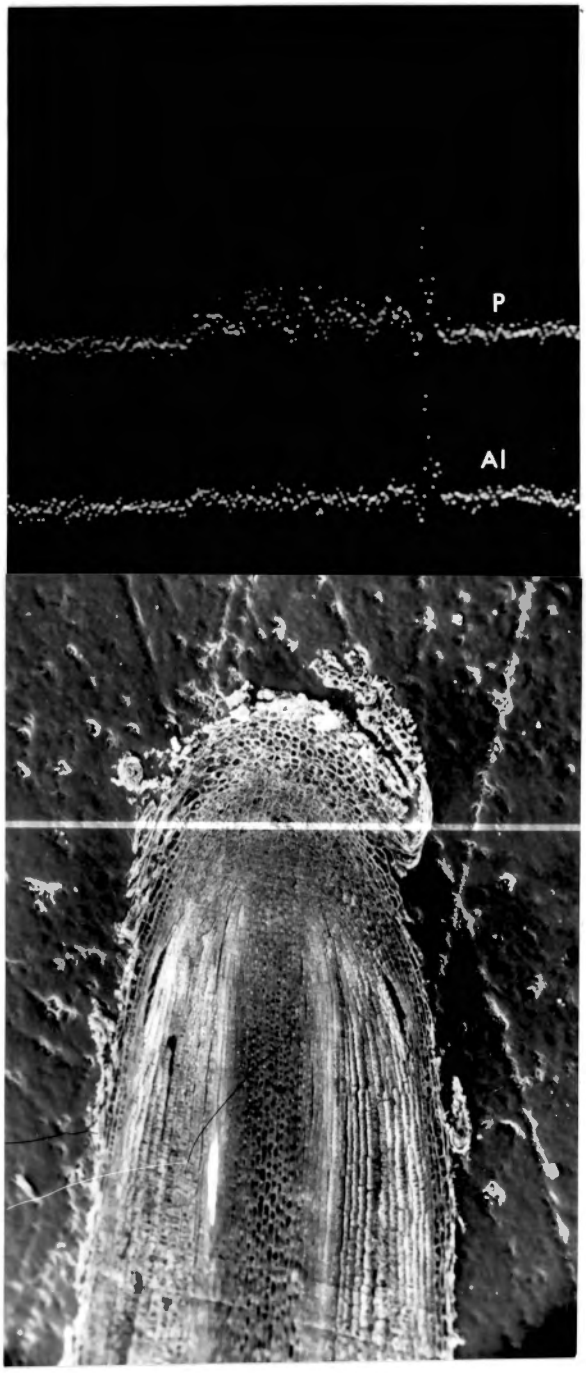

Fig. 41. X-ray scans for P and Al (top) and secondary electron image (bottom) of a longitudinal section of a Romano root tip at 20 ppm Al.

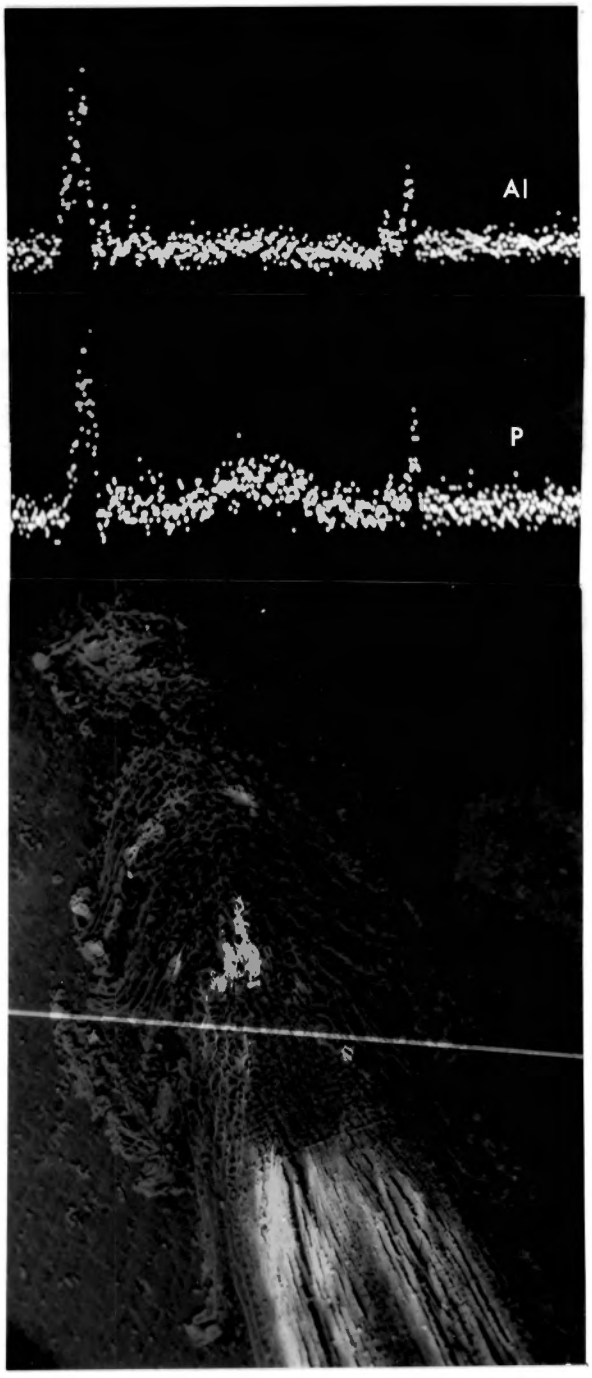

Fig. 42. X-ray scans for Al and P (top) and secondary electron image (bottom) of a longitudinal section of a Dade root tip at 20 ppm Al.

that of P. As in the Romano variety, the Al and P concentrations were highest in the outer region of the root cap. Appreciable amounts of P were found in the meristem of the Dade variety.

X-ray scans through the root cap of a Romano root tip treated with 20 ppm Al are shown in Fig. 43. The location of P coincided with that of Al. The greatest concentration of Al and P occurred in the outer regions of the root cap. The concentration of Al and P decreased towards the interior of the root cap. There was no relationship between the location of Al and Ca. The location of Al and P in Al-treated Dade root tips scanned through the root cap region was similar to that for the Romano variety. These results are not presented.

In Fig. 44 the surface cells of the root cap of Romano, at 20 ppm Al, were scanned for Al and P. The X-ray scan shows a number of Al peaks which corresponded to a similar number of P peaks. High concentrations of Al and P in surface cells of the root cap were also found in the Dade variety, treated with 20 ppm Al.

X-ray spectra of an Al-treated Romano root tip showing the major elemental composition of cell components of a root cap cell and a meristematic cell are shown in Figs. 45 and 46 respectively. Although Al was present in the cell wall and cell interior, the highest concentration of Al was found in the nucleus of both the root cap cell and the meristematic cell. Similar results were obtained for the Al-treated Dade variety.

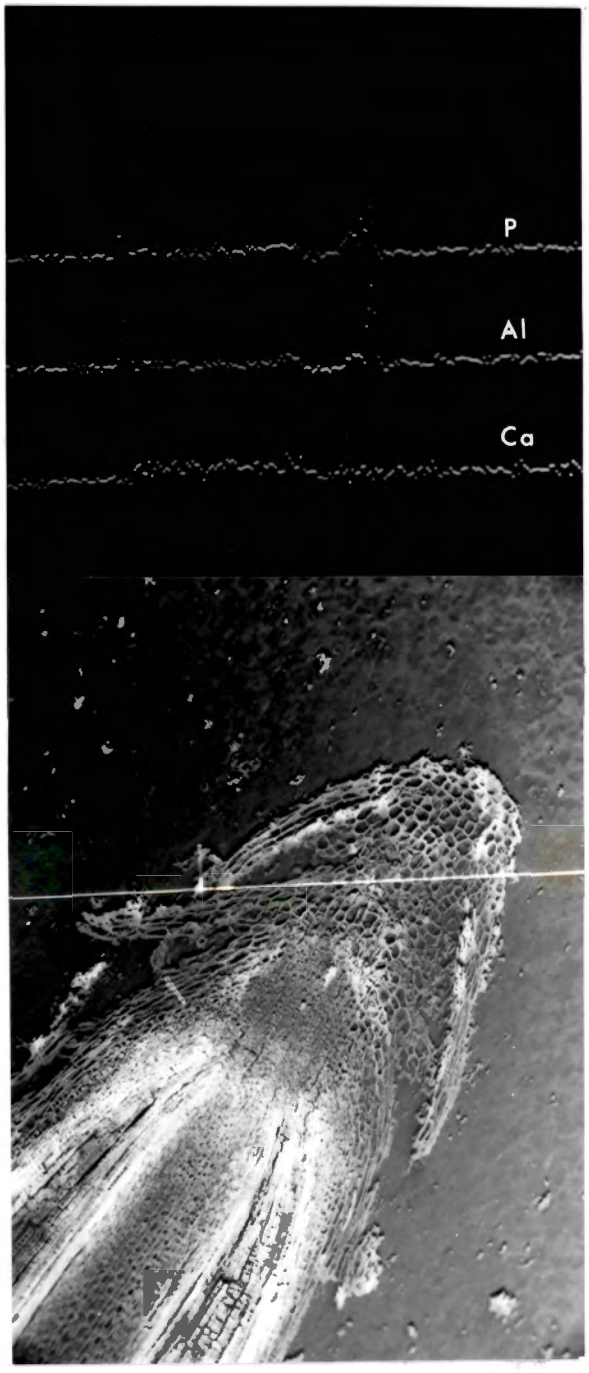

Fig. 43. X-ray scans for P, Al and Ca (top) and secondary electron image (bottom) of a longitudinal section of a Romano root tip at 20 ppm Al.

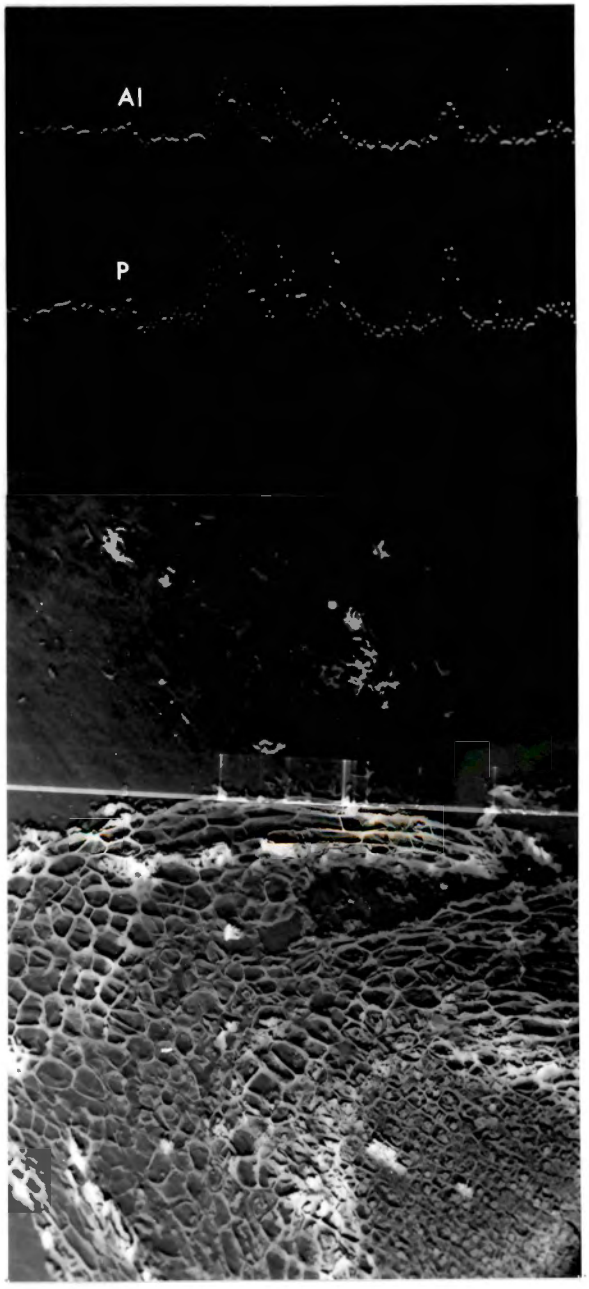

Fig. 44. X-ray scans for Al and P (top) and secondary electron image (bottom) of a longitudinal section of a Romano root tip at 20 ppm Al.

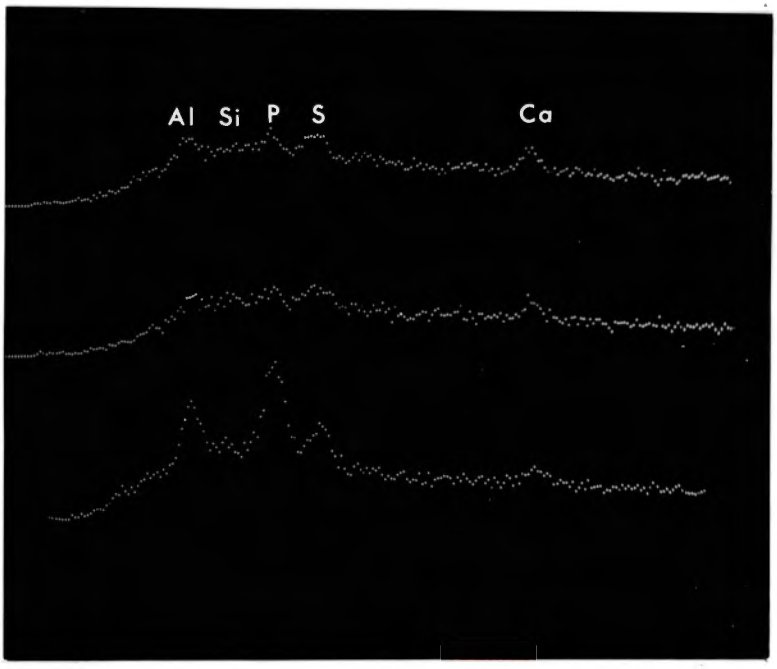

Fig. 45. X-ray spectra of the elemental composition of the cell wall (top), cell interior (middle) and nucleus (bottom) of a root cap cell from a Romano root tip at 20 ppm Al.

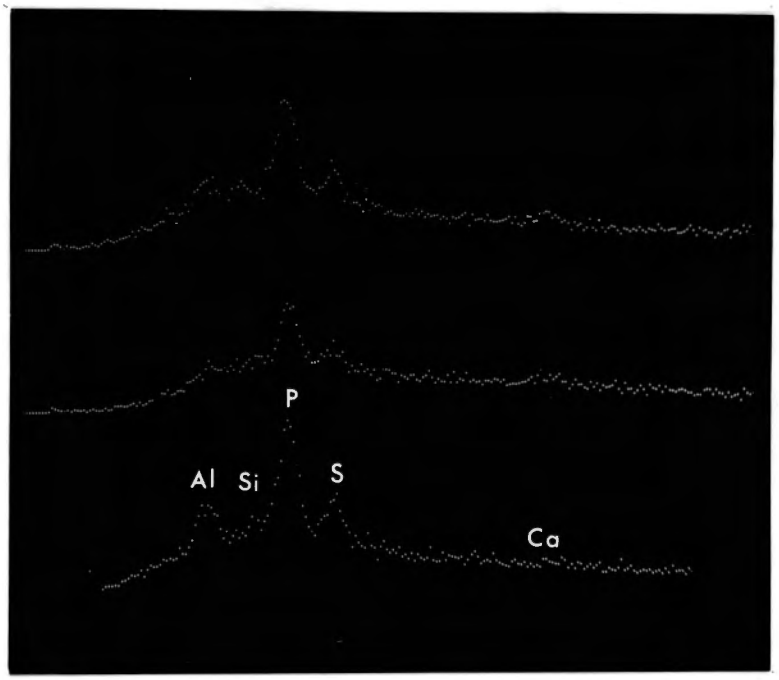

Fig. 46. X-ray spectra of the elemental composition of the cell wall (top), cell interior (middle) and nucleus (bottom) of a meristematic cell from a Romano root tip at 20 ppm Al.

CHAPTER V

DISCUSSION

The differential tolerance of some plants to Al has been suggested to be due to plant-induced pH changes around the roots (Foy et al., 1965, 1967; Otsuka, 1968). In this study there were no significant differences in pH of nutrient solutions between varieties. These results are in agreement with those reported previously for Dade and Romano snapbeans (Foy et al., 1972). The slight increase in pH with increasing Al concentration was probably due to the preferential uptake of anions over cations.

Aluminum treatments decreased yields of roots and tops. Although reductions in dry weight of roots were greater in Romano than in Dade, statistical analyses revealed a significant difference only between varieties at the 12 ppm Al treatment level. Yield of tops for both varieties at 4, 8 and 12 ppm added Al was significantly different from those with no Al. There were no differences between varieties for dry matter yield of tops. This was probably due to inadequate control of pH. Daily monitoring of the pH revealed that the pH increased slowly with time. Although solutions were initially adjusted to pH 4.8 and renewed every 5 days, rapid absorption of anions probably increased the pH and caused precipitation of the Al ions. In this manner the toxic effect of Al, especially at 4 and 8 ppm, was probably reduced. Decrease in root weight was due to lack of branching and proliferation of roots. The data obtained for dry matter production confirmed earlier contentions

(Foy et al., 1967a, 1972) that the Dade variety was more tolerant to Al in solution than was the Romano variety.

The chemical composition of snapbean plants indicated that considerable accumulation of Al occurred in the roots and that relatively little was transported to the tops. The relatively low concentration of Al in plant tops compared to that in the roots is well documented (Foy and Brown, 1964; Munns, 1965; Foy et al., 1972; Andrew et al., 1973). Aluminum present in the roots probably included that absorbed by the roots and that adsorbed to root surfaces. It has been demonstrated that root surfaces bind Al strongly enough to retain it during washing, prior to harvest (Rasmussen et al., 1968). There were no significant differences in Al concentration among treatments for roots or tops of both varieties.

Analysis of variance revealed that differences in Ca concentration between treatments for both roots and tops were not significant. There was a negative correlation between Al levels in nutrient solutions and the Ca content of Romano roots and tops, but no correlation in Dade. Aluminum tolerance among varieties of snapbeans (Foy et al., 1972), soybeans (Foy et al., 1969), wheat and barley (Foy et al., 1967b) has been attributed to the ability of plants to absorb and transport Ca in the presence of excess Al. These results imply that Al in some manner reduces or interferes with Ca uptake. The manner in which Al reduces Ca uptake is still speculative. The results obtained in this study suggested that, in snapbeans, Al had little or no effect on Ca nutrition. Foy et al. (1972) reported that reduced Ca uptake appeared to be a good indicator of Al sensitivity in Dade and Romano snapbean varieties. Their results were not subjected to statistical analyses.

The most drastic effect of the Al treatments was on P concentration. The effects of Al in significantly reducing P uptake were similar for both varieties. Decrease in P uptake with increase in Al concentration has been well documented (Wright, 1943; Randall and Vose, 1963; Munns, 1965; Lance and Pearson, 1969; Andrew et al., 1973). In this study P concentrations in roots and tops decreased progressively with increasing Al concentration. These results are contrary to those reported for Dade and Romano snapbeans by Foy et al. (1972). They found that at 2, 4, 6 and 8 ppm Al in nutrient solutions, Romano roots and tops contained higher P concentrations than those of Dade. They suggested that the Al-sensitive Romano variety may be less efficient in utilizing P already present in plant roots and tops. The results reported in the present study are supported by the appearance of typical P deficiency symptoms in Romano.

Various mechanisms have been proposed for the reduction of P uptake by Al. These include precipitation of P by Al in the external medium; precipitation of P at the root surface and in the cell free space of the root, and blocking of acceptor sites by the binding of Al to protein complexes (Rorison, 1958). Others (Klimashevskii and Bernatskaya, 1973) have proposed that Al reduces the activity of ATP-ase and acid phosphatases in roots. Results obtained in the present study by staining and X-ray spectroscopy support the hypothesis that P is precipitated by Al at the root surface and within roots.

The concentration of K in roots and tops of both varieties was unusually high. Although differences were not significant, the tops of

Al-treated plants had higher levels of K at all levels of Al than the untreated plants. Dade tops at 8 and 12 ppm Al treatments had significantly higher concentrations of Mg than those of Romano. There were no differences between varieties in the Mg content of roots. The literature on the effect of Al on K and Mg uptake is contradictory and confusing. Some workers (Lee, 1971) have suggested that Al tolerance in some plants may be related to the ability of plant roots to absorb K and Mg. While Al may have important effects on K and Mg uptake, these effects are probably indirect and are most likely brought about by the inherent capacity of the plants to preserve cationic balance.

The pH and total acidity of roots and tops of Dade and Romano did not reveal any significant differences between varieties. Reported pH values for roots and tops ranged from pH 5.5 to 5.7. At these physiological pH values Al would be expected to form insoluble precipitates as hydroxides or phosphates within the roots. Translocation of Al within the plant at these pH values would require the formation of Al chelates or complexes. That Al is relatively immobile within the roots is supported by the extremely low values found in plant tops.

The results on Al fixation by root macerates indicated that snapbean root macerates possess the ability to fix soluble Al. No significant differences were found in the Al fixing capacity of Dade and Romano varieties. Some authors (Jones, 1961) reported that a correlation existed between Al tolerance and the capacity of root macerates to immobilize Al. However, Jones (1961) used three groups of plants differing widely from one another in their tolerance to Al. The plants used were

barley (sensitive), pea (semitolerant) and mangold (tolerant). While it may be relatively simple to show differences in Al fixation between diverse groups of plants, the problem becomes more difficult when working with varieties within a single species.

Several workers have suggested that the tendency to form complexes with soluble organic molecules may be responsible for the ability of some species to tolerate high amounts of Al and to maintain adequate P contents in the foliage. Thoday and Evans (1931) showed that stable Al-organic acid complexes occur in some plants. Other workers (Jones, 1961; Clarkson, 1966a) suggested that this mechanism might provide one detoxification system in tolerant species. The mobile form of Al in plants was assumed to be an organic acid complex. Maintenance of this form was believed to prevent the formation of Al-phosphate precipitates. Some workers (Jones, 1961) have suggested that citric and malic acids were the chelating agents. In the present investigation there were no significant differences between Dade and Romano varieties in citric and malic acid contents. Furthermore, analysis of plant roots and tops for Al indicated that a major portion of the Al was located in the roots and that negligible amounts were present in plant tops.

The roots of Dade and Romano from the control treatment, which received no added Al or P, showed little or no blue color development. This indicated that there was little phosphate within the roots. If phosphate was present the concentration was insufficient to be detected by the staining technique used.

There was no concentration of blue color in any particular region of the root in the P treatments which received no Al. The even

distribution of the blue color indicated that phosphate was readily absorbed and subsequently translocated to all regions of the root, including the vascular tissue.

Roots of Dade and Romano varieties from the Al treatment, which received no added P, showed a light blue coloration in the cells of the root cap and epidermis. Since these roots were not supplied with external phosphate it must be concluded that the blue reaction was due to the withdrawal and precipitation of phosphate, initially present within the seeds, by Al present in the cells of the root cap and epidermis. Similar results were reported for barley and poplar roots by McCormick and Borden (1972).

The blue color in all Al-P treatments was located in the cells of the root cap. This suggested that the cells of the root cap were freely permeable to Al. In both varieties the blue color seemed to be confined mainly to the cell walls. This probably indicates that the Al-phosphate interaction occurred mainly on the cell walls and in the free space of the root. Some workers (Clarkson, 1965; McCormick and Borden, 1972, 1974) have presented evidence which supports the contention that Al is bound to the cell wall. In barley roots, Clarkson (1965) reported that 85 to 90 percent of the Al was found in the cell wall preparation. The Al-phosphate interaction was assumed to be an adsorption precipitation phenomenon. It has been suggested that Al may be impeded from penetrating the cell membrane because of its hydrated size and trivalent charge (McCormick and Borden, 1974). The low resolution of the Mo staining technique prevents any definite conclusions from being made about the specific location of the Al-phosphate interaction in the root cap region.

In the Dade variety the blue color in the Al-P treatment was restricted mainly to the cells of the root cap. In the Romano variety traces of blue color were present in the cortical and meristematic areas of the root. These results suggest that the Dade variety is able to exclude Al from the meristematic region to a greater degree than the Romano variety. The endodermis seemed to be a fairly effective barrier in preventing Al from entering the vascular tissues. The results indicate that the P deficiency symptoms commonly observed in Al-treated plants are primarily due to the precipitation of phosphate by Al at the root surface and in the freely diffusible space of the root.

In both varieties the blue color was restricted to about 2 to 4 mm of the root tip. There was no blue color development beyond this distance from the tip. Aluminum seems to be excluded from older regions of the root. Young meristematic tissues are the sites of Al-phosphate interaction. Similar results were reported for wheat roots by the aluminon staining technique (Fleming and Foy, 1968).

The results obtained in this study are in agreement with those reported for roots of corn (Rasmussen, 1968), barley (Clarkson, 1966a; McCormick and Borden, 1972) and wheat (Fleming and Foy, 1968). These results support the view that Al precipitates P within the roots and thereby interferes with the normal P metabolism of the plant. The staining patterns seem to suggest that roots of the Dade variety are capable of excluding Al from the meristematic tissues to a greater degree than the Romano variety.

Roots treated with Al were brown in color compared to the white color of the untreated roots. Aluminum is known to cause disintegration

and collapse of cells of the root cap and epidermis. Upon disintegration of the cells, the cell contents become exposed and tend to give the roots a gelatinous appearance. The brown color of the Al-treated roots was probably due to the oxidation of phenolic compounds present in the cell contents.

Within 24 hours of being exposed to 20 ppm Al, primary roots of Dade and Romano varieties curled upward. The cause of this apparent loss of the geotropic response is not known. Upward curling of roots in response to Al has been reported for sugarbeet (Keser et al., 1975). However, sugarbeet roots only curled following loss of the root cap. Juniper et al. (1966) demonstrated that mechanical removal of the root caps of corn roots resulted in the roots being completely insensitive to gravity. They postulated that, in corn, the root cap was the sole center of gravity perception. Many root tips of Dade and Romano curled even though they possessed root caps. Curling was probably due either to partial detachment of the cap or to some effect of Al on the geotropic mechanism within the root.

The first measurable effect of Al was on root growth. Growth of primary roots was considerably reduced. Lateral roots were particularly susceptible to Al injury. Growth of lateral roots was completely inhibited upon their emergence from the primary root axis. This gave the roots a stunted appearance with little or no side branching. In the field such severely stunted root systems would be lethal to seedlings. Tolerant varieties are desirable for deeper rooting and for more efficient water utilization in acid soils. Greater susceptibility of lateral roots

to Al is probably due to the lack of complete development of the exodermis. In addition, the casparian strips in the endodermis are not well developed in young lateral roots. In emerging lateral roots therefore, Al is probably able to penetrate the root meristem easily and in some way inhibit or disrupt the normal cell division process.

Some of the primary roots, grown at 20 ppm Al, produced numerous lateral roots at the root tip. This was probably due either to the disruption, by Al, of the normal sequence of lateral root development or to the decrease in Al concentration with time.

Plants of both varieties grown at 20 ppm Al displayed typical symptoms of P deficiency. These included overall reduction in growth and small abnormally dark green leaves. The tops of the Romano variety also showed purpling of stems and petioles. Purpling of plant tops was absent in the Dade variety.

The Mo blue stain used in this study revealed that the root cap is freely permeable to Al and that Al-phosphate precipitate accumulates in the root cap. Probably as a result of the accumulation of precipitate, the root cap begins to curl back and eventually becomes detached. In the primary root tips of Dade, grown at 20 ppm Al, abnormally large root caps were observed. This was probably a response by the root that resulted in Al exclusion from root tissues by continuous regeneration of the root cap. Abnormally large root caps were not produced by root tips of the Al-treated Romano variety. In the Dade variety, as cells were sloughed off or detached, more were produced by the root cap initials. By producing more root cap cells than Romano, Dade root tips were

probably able to exclude Al from the meristematic region to a greater degree than Romano. In Dade and Romano varietal differences in response to Al, therefore, seem to arise from a series of events that are initiated at the cellular level. The presence of Al in the cell free space of the root cap resulted in the disintegration and disorganization of cells in the root apex.

In the primary roots the meristem of Romano was also more susceptible to Al injury than Dade. The cells in the apical meristem of Romano were generally smaller and irregularly arranged, probably as a result of the effect of Al on the cell division process.

The young lateral roots of Romano were injured by Al to a greater degree than those of Dade. In both varieties large cells were observed close to the root apex. Since root growth had ceased, maturation of the cortical and vascular cells occurred closer to the root apex than in those roots not treated with Al. The disorganization of cells in the root tip was probably due to the disruption of the cell division process by Al.

Energy dispersive X-ray analysis of Al-treated roots revealed that Al and P were located in high concentrations at the root surface and within the root cap. In the Al-treated roots of both varieties a high level of Al was always accompanied by a high level of P. Linear scans for Ca, K and Mg indicated that these elements were distributed throughout the root. These results support the hypothesis that P is precipitated by Al at the root surface and within the root.

The high concentration of Al at the root surface suggests that Al is strongly bound to root surfaces since it was retained during washing,

dehydration and paraffin infiltration. The high levels of Al in the outer cells of the cap indicates that the root cap cells are permeable to Al. Linear scans across the root, about 5 mm from the tip (results not presented) revealed no local concentration of either Al or P. The root epidermis, as long as it remained intact, seemed to be effective in excluding Al from the root.

The major part of the Al that penetrates the root tip is precipitated from solution in the root cap as Al-phosphate. That Al which reaches the meristem of the root causes injury to the meristematic cells. Growth of roots is thereby decreased or completely inhibited.

Aluminum is probably able to enter the meristem in two ways. Aluminum causes the curling backward and detachment of the root cap. This exposes the susceptible cells of the meristem to the harmful effect of Al. Secondly, emergence of young lateral roots from the primary root axis provides easy access for Al entry into root tissues (Rasmussen, 1968). Linear scans of root sections with emerging lateral roots did not reveal high concentrations of Al in any localized region. The concentration of Al in these tissues was probably too low to be detected by linear scans. As Al diffused through the root tissues it precipitated P and thereby disrupted normal cellular metabolism. Aluminum was probably also bound to P containing structures such as membranes or DNA.

The results reported here are in agreement with those obtained for corn roots by electron microprobe analysis (Rasmussen, 1968; Rasmussen et al., 1968). In all cases the localization of P was the same as that for Al. The data obtained by energy dispersive spectroscopy are also in agreement with the results obtained in this study by the Mo blue stain.

Results contrary to those reported above were put forward by Waisel et al. (1970). These authors investigated the localization and distribution of anionic Al in bean and barley roots. Using electron microprobe analysis they reported that there was no correlation between the distribution of Al and P. They found that the distribution of Al overlapped that of N. These authors suggested that differences between their results and those of Rasmussen (1968) were due to differences in plant material and differences in experimental procedure. The findings of Waisel et al. (1970) are difficult to reconcile with the results obtained in the present study and those reported by Rasmussen (1968). Anionic Al may behave differently than cationic Al.

In both varieties elemental composition of cell components revealed that Al was present in highest concentration in the nuclei of both internal root cap cells and meristematic cells. As far as the author is aware this is the first reported instance of Al being physically detected in nuclei. The presence of Al in the nuclei could explain the reduction or inhibition of cell division that is so characteristic of Al toxicity.

Some workers (Henning, 1975; McLean and Gilbert, 1927) have used the haematoxylin stain to detect Al in root tissues. In corn and cabbage roots, McLean and Gilbert (1927) reported that Al absorbed by the roots accumulated in the cortex. The haematoxylin stain appeared to be localized especially in the protoplasm and nuclei of cortical cells. The meristem of the roots was clear. Henning (1975) also reported that Al, as detected by the haematoxylin stain, accumulated in the cortex of wheat roots. No stain was observed in the meristem of wheat roots.

These data should be interpreted with caution since it is possible to obtain a positive haematoxylin reaction with other polyvalent cations (Rasmussen, 1968).

Many reports have indicated that Al is bound to cell walls (Clarkson, 1965, 1966a; Rasmussen, 1968; McCormick and Borden, 1972, 1974). In the present investigation, the detection of Al in nuclei would explain the finding of Sampson et al. (1965) that Al-treated plants synthesized a metabolically unstable DNA that had an unusual base composition.

Energy dispersive analysis of X-rays with the scanning electron microscope was attempted by Henning (1975) to locate Al and P in root tips of wheat. He reported that the results were very disappointing. Failure to detect Al and P in the roots was probably due to the short scan time used.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Greenhouse experiments were conducted to investigate Al tolerance in Dade and Romano varieties of snapbeans (Phaseolus vulgaris L.). Seedlings were grown hydroponically at various levels of Al. Aluminum was supplied as Al-sulfate at concentrations of 0, 4, 8 and 12 ppm Al at pH 4.8. After 10 days of treatment plant parts were weighed and analyzed for Al, Ca, P, K and Mg. The final pH of the nutrient solutions was measured at harvest.

There were no significant differences in pH of nutrient solutions either within each variety or between varieties. Dry weights of roots and tops decreased at higher levels of Al. At 12 ppm added Al the Al-tolerant Dade variety had significantly higher root weights than the Romano variety. Yield of tops from the Al treatments of both varieties was significantly lower than those not treated with Al. There were high negative correlations between Al levels in nutrient solution and yield of roots and tops for both varieties.

Chemical composition of roots and tops of Al-treated Dade and Romano snapbean varieties revealed that a major part of the Al was located in the roots. There were no significant differences in Al content between treatments for either roots or tops. The Al treatments reduced the Ca concentration in the roots and tops of the Romano variety. Negative correlations between added Al and the Ca content of Romano roots and tops were highly significant. The Al treatments had little or no effect

on the Ca concentration of roots and tops of the Dade variety. Aluminum treatments significantly reduced the P concentrations in the roots of both varieties. There were significantly lower concentrations of P in the tops of both varieties, at 8 and 12 ppm, than in those not treated with Al. Phosphorus concentrations in roots and tops of both varieties were negatively correlated with added Al. Aluminum treatments generally increased the K concentration in the roots and tops of both varieties. High levels of Al decreased the concentration of Mg in the roots of both varieties. At 12 ppm added Al, the Mg concentration in roots of both varieties were significantly different from those with no Al. At 8 and 12 ppm added Al, Romano tops had significantly lower concentrations of Mg than those of the Dade variety.

Dade and Romano snapbean varieties were compared with respect to pH, total acidity, Al fixation by root macerates, and to citric and malic acid content. No significant differences were detected between varieties. In order to identify morphological changes induced by Al, Dade and Romano snapbeans were grown hydroponically at two levels of Al, 0 and 20 ppm. Plants were grown for 12 days at pH 4.6. Both varieties exhibited several symptoms of Al toxicity. These included upward curling of primary root axes; root discoloration; significant reduction in root elongation; inhibition of lateral root growth; significant reduction in top growth; small, abnormally dark green leaves and gelatinous root tips. Unlike the Dade variety, the Romano variety also exhibited purpling of stems and petioles. Many of the above symptoms are typical of those for P deficiency.

The anatomical study revealed that the primary root tips of both varieties, at 20 ppm Al, were swollen. The root cap curled backward and eventually became detached. Many of the root cap cells were disorganized and lacked nuclei and cell contents. In the Romano variety, the Al treatment also caused the disorganization of cells in the meristem of the root. The curling backward and subsequent detachment of the root cap was probably due to the accumulation of Al-phosphate precipitate in the root cap. The primary root tips of the Dade variety, at 20 ppm Al, produced abnormally elongated root caps. Continuous regeneration of the root cap was probably a response by the root that resulted in exclusion of Al from sensitive root tissues. Regeneration of the root cap was one of the factors that conferred on Dade a greater degree of tolerance to Al than the Romano variety.

Lateral roots of Romano were injured to a greater degree, at 20 ppm Al, than were those of Dade. The cells of the apical meristem in the lateral roots of Romano were disorganized. In both varieties, cortical cells of lateral root tips were disorganized and reached an unusually large size closer to the root tip than in those roots not treated with Al.

The location of Al in the root and the site of its effects were investigated by staining with Mo and by energy dispersive analysis of X-rays generated in the scanning electron microscope. The results of both techniques revealed that young meristematic tissues are the sites of Al action. Aluminum seems to be excluded from older regions of the root. The root cap was freely permeable to Al ions. X-ray spectroscopy revealed that the location of Al was identical to that for P. This

suggests that Al precipitates P within roots. There was no relationship between the distribution of Al and that of Ca, Mg or K. Most of the Al-phosphate interaction occurred on the root surface and in the outer cells of the root cap. As Al diffused into the root tip it was precipitated out of solution as Al-phosphate. That Al which reached the meristem of the root caused injury to the meristematic cells. X-ray spectroscopy revealed that Al is able to penetrate the cell membrane and reach the nucleus. In the nucleus Al probably disrupts the cell division process and thereby reduces root growth.

This study supported the hypothesis that Al precipitates P as Al-phosphate on root surfaces and within roots. This explains the characteristic P deficiency symptoms frequently observed in the tops of Al-treated plants. The effects of Al on the Ca, K and Mg concentrations within plants are probably indirect and are most likely brought about either by the capacity of plants to preserve cationic balance or through disruption of the permeability characteristics of the membrane by Al.

The Al which reaches the nuclei of the meristematic cells reduces or inhibits the cell division process, resulting in stunting of roots. This may be due to the direct effect of Al on the cell division process or to some effect of Al on P nutrition. Disruption of the normal process of cell division results in the disorganization of cells in the apical meristem.

The greater tolerance of the Dade variety to Al was probably due to the ability to exclude Al from the meristem to a greater degree than the Romano variety. This was accomplished by the continuous regeneration of

the root cap cells as the outer cells became sloughed off. In addition, the absence of purpling symptoms of P deficiency in Dade tops, compared to those of Romano, suggests that the Dade variety was able to maintain adequate levels of P in tops, in the face of precipitation by Al in the roots.

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VITA

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