THE PHYSIOLOGICAL ROLE OF BORON IN THE ROOTING OF HYPOCOTYLS OF *PHASEOLUS VULGARIS* L.

by

CONRAD JOHN WEISER

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APPROVED:

Associate Professor of Horticulture

In Charge of Major

Head of Department of Horticulture

Chairman of School Graduate Committee

Dean of Graduate School

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DEDICATION

This thesis is dedicated to my wife and family.

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INTRODUCTION

Horticulture is dependent upon the increase and perpetuation of superior plants as genetically uniform clonal lines. Cuttage is the most important single means of vegetative propagation. Although the use of this technique dates to antiquity, our basic knowledge of the factors involved in rooting is limited. Temperature, light, moisture, aeration, hormones, and numerous other factors are known to influence the rooting capacity of a cutting, but cuttings of some plants do not root under the most favorable conditions our present knowledge allows us to provide. Because of this, the emphasis in propagation research is gradually and logically shifting from artisan practice to investigations of the underlying physiological processes involved in rooting. The objective of this dissertation is to study one such process, namely, the physiological role of boron in rooting of cuttings. French bean, Phaseolus vulgaris L., was used as a test plant because it is genetically homozygous and easy to grow.

The ability of boron to promote rooting on bean hypocotyls has been recognized for some time, but this element has not been used on a practical scale as a root-inducing agent. Evidence of its potential for propagating English holly and clematis cuttings has been reported. No one knows how boron promotes rooting,

however; and its use as a rooting treatment would be enhanced by a knowledge of its basic action in the rooting process.

A study of the stimulatory properties of boron should also contribute to our understanding of root development and, perhaps, to the much studied, but little understood, role of boron in plants.

REVIEW OF LITERATURE

Boron and Rooting of Cuttings

In experiments with bean cuttings in 1951, Hemberg (15, vol. 4, p. 358-369) reported that, "plants placed in tap water form roots exceedingly well, while plants placed in nutrient solution or distilled water form few if any roots." The ensuing investigations revealed that the rooting stimulus in tap water was boron. The major plant nutrients and other trace elements had no effect on rooting. The effects of boron and auxin seemed to be unrelated in that boron apparently affected the growth of roots while auxin affected their initiation. An earlier non-specific report of boron influence on rooting appeared in a U. S. patent, issued in 1942 to Sylvia Colla (5), which stated that boron combined with auxin can greatly improve the type of roots and the nature of the callus growth.

Murray et al. (25, vol. 69, p. 498-501) studied the effects of various levels of boron, in complete nutrient solution, on the rooting of cuttings of geranium and currant. In separate experiments cuttings were rooted in sand and water culture and the rooting was expressed as dry weight of roots produced. Boron significantly increased rooting on cuttings of both plants. They concluded from their data that boron had no effect on root initiation but was essential for healthy growth. The data, however, expressed as dry weight of roots, and not root number, cannot substantiate this conclusion. They suggested further that the data support the view of

Gauch and Dugger (13, vol. 28, p. 457-466) that boron is involved in the translocation of sugars in plants, but no data are presented to support this contention.

Gorter's study (14, vol. 11, p. 1-9) with bean cuttings verified the work of Hemberg. She concluded that boron and indole-3-acetic acid (IAA) influenced different phases of the rooting process because boron increased root production by a constant factor at various levels of IAA. Although boron treatment increased the number of roots produced, she suggested that this apparent increase was due to stimulated growth of preformed root initials and not to initiation of new roots. Boric acid was used to supply boron at two concentrations, 10^{-6}M and 10^{-3}M, the latter concentration was supra-optimal.

Clematis cuttings, rooted in sand under intermittent mist, were found by Weiser (44, vol. 183, p. 559-560) to root in a shorter time and in greater numbers when boron was used in combination with the root initiating hormone, indole butyric acid (IBA). Treatments were applied by soaking the bases of cuttings for 12 hours in 50 ppm aqueous solutions of the rooting chemicals, IBA or boric acid. Weiser and Blaney (45, vol. 75, p. 704-710) reported a similar response with English holly cuttings. Neither holly nor clematis responded when boron was used alone as a rooting agent. When applied with IBA to holly cuttings, boron increased the percentage of cuttings rooted, increased the numbers of roots and to a lesser extent their length, and accelerated rooting. Boron appeared to enhance rooting

through a synergistic interaction with IBA. Miroshnichenko (23) reported a boron-growth regulator interaction with seedlings. The growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D) caused a marked decrease in the volume of roots on treated seedlings. Boron, on the other hand, caused an increase in volume, but maximum root volume was obtained by combining 2,4-D and boron. Sen, et al. (33) found that 50 ppm boron beneficially affected both initiation and development of roots on semi-hardwood cuttings of Justicia gendarussa L. The fact that boron was most effective as a rooting agent on some plants when synthetic auxins IBA, NAA, or 2,4-D were present, does not necessarily contradict the work on bean by Hemberg and Gorter which indicated that boron and auxin acted independently in rooting. The apparent contradiction can be reconciled if it is assumed that auxin is necessary for root initiation before boron is capable of exerting its stimulatory effect on growth of roots from initials. It is possible, for example, that endogenous auxin is limited, or that inhibitors are present in clematis and holly, while in bean, ample free endogenous auxin is available for root initiation.

In summary, published reports show that boron enhances the rooting of cuttings of bean, geranium, currant, clematis, English holly, and Justicia gendarussa L. The author has observed that cuttings of some other plants are not stimulated by boron treatment. Boron reportedly affects root growth and not root initiation, although results with holly appear contradictory. Auxin and boron

seem to exert their influence on separate phases of the rooting process in bean, while clematis and holly respond to boron only when auxin is added.

The Postulated Roles of Boron in Plants

It is logical that the role of boron in the rooting of cuttings is related to, if not identical with, its role or roles in intact plants. Present knowledge of the physiological roles of boron in plant growth, however, is inconclusive and contradictory, to quote Neales, (27, vol. 10, p. 435), "despite much experimental work no unequivocal evidence has been produced indicating the basic reaction (or reactions) in the plant which are dependent upon the presence of boron." Gauch and Dugger (12, p. 1-43) and Skok (37, p. 227-243) have written extensive reviews on this subject, and it would be unrewarding to consider all of the postulated roles here because many of them obviously could not be involved in the boron stimulus to rooting. The several physiological roles which have been proposed for boron and which could also logically be related to its stimulatory effects on rooting include the proposed roles of boron in auxin metabolism, sugar translocation, cell wall development, and oxidative processes. These four topics have been studied in the ensuing experiments to determine if they are singly or collectively responsible for the boron stimulus to rooting. A review of literature on these topics follows.

Interrelations of Boron and Auxin

If boron were linked with auxin metabolism it would be attractive to assume that this was the way in which boron stimulated rooting of cuttings. Reports of boron-auxin relations do indeed exist and generally fall into two categories. First, there is evidence suggesting that boron may be directly involved in auxin metabolism; second, that boron may exert an indirect effect on the translocation of auxin. Eaton (9, vol. 101, p. 700-705) reported in 1940 that applications of IAA to boron-deficient cotton seedlings partially cured boron deficiency. These results imply a direct effect of boron on IAA production by plants. Other workers have been unable to duplicate these results, and it has been shown (32, vol. 173, p. 957) that sufficient boron could have been liberated from the glass bottles used by Eaton to account for the apparent IAA response.

A less direct effect of boron on IAA is suggested by the work of Mitchell, Dugger, and Gauch (24, vol. 118, p. 354-355) who reported that IAA translocation from leaves to stems of bean was enhanced by additions of boron. Boron was thought to accelerate the movement of sugars and thus indirectly to accelerate the translocation of IAA. This interpretation agrees with work by Weintraub and Brown (43, vol. 25, p. 140-149) who found that movement of applied IAA did not occur unless sugar (sucrose, maltose, lactose, glucose, fructose, or galactose) was also provided. Chandler's

(4, p. 367-372) observation, that boron-deficient broccoli plants were unable to respond to the geotropic stimulus when placed horizontally, indicates that plants deficient in boron are unable to respond to auxin. Alexander (1, vol. 103, p. 479) reported similar findings with boron-deficient squash plants. These lagged in geotropic response after only two days in boron-free solutions. After five days in such solutions the plants failed to bend even after five hours in a horizontal position. These various reports indirectly imply a relation between boron and auxin, and the rooting studies with clematis, holly, and Justica gendarussa L. suggest a tie-in between boron, auxin, and rooting.

Boron and Sugar Translocation

The postulated role of boron in sugar translocation as proposed by Gauch and Dugger (13, vol. 28, p. 457-466) has generated more interest than any of the other varied roles that have been suggested for this element. The chemical basis for this proposed role of boron is its ability to react in vitro with the hydroxyl groups of sugars to form an ionizable sugar-borate complex. Such complexes are assumed to facilitate sugar movement through the plasma membrane, which is theoretically quite impervious to movement of highly polar sugar molecules. This indirect chemical evidence has been supported by in vivo studies with bean and tomato plants in which the movement of C^{14}-sucrose was found to be enhanced by boron. The method used

to establish this phenomenon involved the immersion of a single leaf of an intact plant in an unspecified concentration of uniformly-labelled sucrose with or without the addition of 10 ppm boron. After 4 to 24 hours the plants were fractionated, oven dried, and the various fractions were assayed for radioactivity. The results showed that the uptake and directional translocation of sucrose, or its hydrolytic products, to the stem tip and young leaves, was much greater in the plants treated with sugar plus boron than in those treated with sugar alone. The results led them to propose that sugar does not move readily through the cellular membranes unless the sugar is borated. If this theory were true it would mean that photosynthate produced in the leaves of bean cuttings would not move to the base or hypocotyl of the cutting unless ample boron were present. The intense metabolic activity in this section of the cutting during initiation and growth of new roots would be dependent on a ready supply of photosynthate and hence on boron.

More recently Dugger, et al. (8, vol. 32, p. 369) reported that boron inhibited starch synthesis in leaves of bean plants infiltrated with glucose. In vitro studies showed that boron slowed down the shift of the glucose-1-phosphate $\rightleftharpoons$ starch reaction to the right by combining with the glucose-1-phosphate at low concentrations. The final equilibrium was unaffected. The authors state that at any one time the quantity of soluble carbohydrate moving in the translocating tissue is a function, in part, of the quantity of soluble carbohydrate

in the cells of the leaves. Therefore, they suggest that the presence of boron, which slows down the condensation of soluble carbohydrates to starch, would tend to promote the movement of more carbohydrate out of the leaf cells to other parts of the plant. This hypothesis could account for the observed stimulatory effect of boron on sugar translocation.

The relation of these proposed sugar translocation mechanisms to rooting is uncertain. However, Humphries (18, p. 79) (17, p. 84) reported results of preliminary studies dealing with the interaction of boron and sugar in rooting of bean cuttings. He painted solutions of sodium borate, sucrose, or both, on leaves of cuttings, and comparisons of rooting revealed no sugar-boron connection.

The picture of a boron-sugar-rooting interdependence is further marred by numerous reports criticizing the postulated role of boron in sugar translocation. O'Kelley (30, vol. 44, p. 239-244), in work with pollen, found a much smaller increase in sugar absorption rate with boron than reported by Gauch and Dugger (13, vol. 28, p. 457-466). McIlrath and Palser (22, vol. 118, p. 43-52) found that carbohydrates were translocated in boron-deficient cotton plants as long as the phloem tissues were alive. Odhnoff (29, vol. 10, p. 997-998) reported that roots show the first sign of boron deficiency, and stated that the general rise in carbohydrate and cell wall material in boron-deficient plants contradicts Gauch and Dugger's assumption that lack of boron causes sugar deficiency. Skok

(38, vol. 32, p. 308-312) is more specific in his criticism. Fifty microliters of 10 per cent uniformly labelled sucrose was applied inside a lanolin ring on each cotyledon of nine-day-old sunflower seedlings. Boron was applied in sugar solution to some of the treatments at a concentration of 40 micrograms boron in each 100 microliters of sugar solution. After 92 hours the plants were divided into epicotyl and hypocotyl portions and assayed for activity. The amount of sugar translocated from the site of application to other parts of the plant was found to be extremely variable within treatments and there were no significant differences between treatments. The elements strontium, germanium, and aluminum, which are known to form sugar complexes similar to those formed by boron, were tested and found to alleviate boron-deficiency symptoms temporarily and to increase the growth of sunflower seedlings in the absence of boron. It appeared, therefore, that the physiological role of boron is related to its complexing ability. Germanium was the most efficient complexing substance tested with regard to alleviation of boron-deficiency symptoms, but it was equally as ineffective as boron in enhancing sucrose translocation. These results imply that boron is not functional in sugar translocation and directly contradict the work of Gauch and Dugger. A possible objection to Skok's work is that the concentration of boron used, 40 micrograms per 100 microliters of solution (400 ppm), is an excessive and possibly toxic concentration. Nevertheless, these and other studies, Neales, (26, vol. 183, p. 483) (27, vol. 10, p. 426-436), Nelson and

Gorham (28, vol. 35, p. 339-347) and Whittington (46, vol. 8, p. 353-367) raise serious doubts about the validity of Gauch and Dugger's original hypothesis.

Boron and Cell Wall Development

Much attention has recently been focused on the possible relation of boron to cell wall development and especially to elongation. The observed effects of boron on growth of roots (14, vol. 11, p. 1-9), but not on initiation (15, vol. 4, p. 358-369), could be related to its proposed action in cell elongation. Smith (39, vol. 22, p. 257) found 55 per cent of the boron in normal plants in the cell wall, while in boron-deficient plants 70 per cent of the boron was found there.

Odhnoff (29, vol. 10, p. 996) has described the changes that take place during growth of cell walls. She separated elongation into two phases: first, a loosening and plastic stretching of the cell wall; second, an active wall formation by intussusception of new microfibrils. The loosening and stretching phase is promoted by auxin and thought to be the most important in shoot elongation. The intussusception phase is inhibited by auxin and is dominant in root elongation. This latter phase is strongly influenced by carbohydrate supply and hence may be dependent upon boron. In Odhnoff's experiments, the earliest and most severe boron deficiency symptoms occured in roots, where growth by intussusception dominates. Sugars

accumulated in roots of boron-deficient plants after growth stopped, suggesting that boron was instrumental in the synthesis of cell wall materials and not in sugar translocation.

Torssell (42, vol. 9, p. 652-664) tested the effect of various forms of boron on the growth of excised wheat roots. Boric acid did not stimulate the growth of these roots, but various organic boron compounds, such as phenylboric acid, caused pronounced stretching and elongation of the root cells. Phenylboric acid, which was capable of causing this root elongation, forms sugar complexes more readily than boric acid as evidenced by their comparative abilities to delay the rates of retrogradation of amylose in vitro. The compound 2-nitro-4-carbomethoxyphenyl boric acid did not stimulate root elongation and was also incapable of forming sugar complexes. These facts have led Torssell to hypothesize that complexing ability determines the capacity of a compound to affect elongation. The ability of a plant cell to elongate is thought to be limited by the "crystallization" of cell wall materials which occurs with aging as a result of an increasing orientation of the polysaccharide chains. At points where boron complexes are formed in the cell wall, the bonding of chains by van der Waal's forces or hydrogen bonds is inhibited by steric hindrance and repulsion between the ionized complexes. This delays stiffening of cell walls and allows cell elongation to continue for a longer period. If the action of boron in rooting of bean cuttings were connected with Torssell's proposed system, phenylboric acid would be more efficient

than boric acid in causing the rooting stimulus.

Spurr's (40, vol. 126, p. 78-80) observations on the profound alterations in cell wall morphogenesis in boron-deficient celery plants also suggest the importance of boron in wall metabolism.

Boron and Oxidative Processes

It was observed in the course of this study that cuttings supplied with boron characteristically formed roots along the entire length of the immersed hypocotyl while those in water without boron formed roots only near the surface of the solution. One could hypothesize from this that boron is involved in oxidative processes which enable the cuttings to root in an environment deficient in oxygen, i.e. the aqueous rooting solutions. Shkol'nik and Steklova (34) postulated that boron somehow supplies oxygen to plants, because flax plants supplied with dilute hydrogen peroxide in the substrate grew almost as well with boron as without it. Aeration alone was not as effective as H_2O_2 and gave subnormal plants. They suggested that perhaps boron is involved in the production of organic peroxides in plants. This work has not been corroborated. MacVicar and Burris (21, vol. 17, p. 31-39) reported that uptake of oxygen by ground leaf tissue from boron-deficient leaves was higher than from normal leaves. Boron-deficient tissues were also shown to have a more active polyphenoloxidase than normal tissue. Additions of boric acid to the

leaf homogenates only slightly reduced the rate of oxygen uptake by the boron-deficient plants, but the oxidation of dihydroxyphenyl-L-alanine by tobacco, tomato, and soybean polyphenoloxidase was shown to be inhibited by 0.01 M borate. The enhanced rate of oxidation and polyphenoloxidase activity in boron-deficient plants and their subsequent reduction when boron was added suggested to the authors that one role of boron in plants is to inhibit the polyphenoloxidase system. Conversely, O'Kelley (30, vol. 44, p. 239-244) reported that boron increased oxygen uptake by germinating pollen grains. Bouillenne[1] has proposed a scheme for the rooting of cuttings that involves an oxidizing enzyme. He suggests that auxin and compound X (probably a diphenol) are produced in the leaves and move down the stem in a polar manner to the base of the cutting. There, compound X is oxidized by a phenolase enzyme to quinone, which then reacts with auxin, and in turn, acts in the protein synthesis necessary for root initiation.

The postulated roles of boron discussed in this chapter have provided four basic working hypotheses for this dissertation. The hypotheses are stated as follows:

(1) Boron stimulates rooting of cuttings through effects on auxin metabolism.

(2) Boron stimulates rooting of cuttings by enhancing sugar translocation.

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[1] Professor Raymond Bouillenne, Director of the Institute of Botany. Universite de Liege. Interview 1960.

(3) Boron stimulates rooting of cuttings through effects on cell wall development.

(4) Boron stimulates rooting of cuttings through effects on oxidative processes.

These hypotheses were studied in the subsequent experiments and rejected or accepted on the basis of the experimental results.

METHODS

Seedlings of French bean, Phaseolus vulgaris L., cultivar Black Valentine, were grown under controlled conditions and used as test plants to evaluate the effect of various treatments on rooting. Seeds of uniform size were selected and sown in plastic flats in soil or other media, as indicated.

The average boron content of the uniform Chehalis silty clay loam soil used throughout the study was .0066 per cent. Most normal soils contain from .003-.008 per cent with .005 per cent being considered average (20, p. 25). Test plants were grown in soil in most of the experiments to avoid possible boron-deficiency and a possible involvement of root stimulation with the correction of a deficiency.

Plants were grown on a 14-hour photoperiod at a light intensity of approximately 1000 foot-candles. Light was provided by a bank of 18 mixed Champion and Ken Rad 96 T8 standard warm white flourescent lamps spaced about one inch apart, plus four 40 watt incandescent lamps. The ballasts were GE Tulamp Ballasts, (300 MA and 69 watts). Air temperature was 23°C during the photoperiod and 20°C during the dark period. Humidity was high but not controlled.

The Cutting

Ten- to twenty-day-old bean plants were selected for uniformity and cuttings were taken by severing the hypocotyls near the surface of the soil, four inches below the cotyledons. After cuttage

the cotyledons were removed and the cuttings were washed gently for about three minutes in a dilute sodium hypochlorite (2% Chlorox) solution to remove soil particles and reduce surface bacteria and fungi. (Captan and $CuSO_4$ (0.1 ppm) were also tested as control measures, but the latter was not too effective and Captan was found to be unsatisfactory because it stimulated rooting slightly.) If control measures were not taken the microorganisms would invade some of the treatments and ruin the experiment. The cuttings generally consisted of the first trifoliolate leaf just beginning to expand, two fully expanded cordate leaves, about two and three-fourths inches of epicotyl between the cordate leaves and the point of cotyledon attachment, and four inches of hypocotyl.

Rooting

Rooting was carried out in wide-mouth, soft-glass pint jars. The jars were lined before each experiment with new pint plastic bags to preclude organism carry-over in jars and to reduce the possibility of boron contamination from the glass. Many types of glass contain boron, and some, such as Pyrex, contain as much as 13 per cent B_2O_3 (35, p. 389-390 and 561-566). After the rooting solutions were poured into the jars the mouths were covered with aluminum foil through which holes were punched to support the cuttings. Usually four to five cuttings were placed in each jar and arranged so there was essentially no overlap and shading of the cordate leaves. The basal 4½

inches of the cutting, including about 4 inches of hypocotyl and ½ inch of epicotyl, were placed in the solution. When rooting was evaluated the epicotyl portion was ignored. The original level of the solutions was maintained by adding fresh solution every third day during the 9- to 15-day rooting period.

The water used in the studies was prepared by running metal distilled water through a mixed cation-anion exchange resin (Amberlite MB-1). The mixed-bed resin reduced the level of boron in the distilled water from about 0.0022 ppm to about 0.0017 ppm. Boron was supplied to solutions as the very weak ($K = 6 \times 10^{-10}$) boric or orthoboric acid, H_3BO_3. This compound contains 17.5 per cent boron which is available to plants in the form of the trivalent anion $BO_3^{=}$. The level of boric acid most commonly used in these studies (0.5 ppm) had no measurable effect on the pH of the water. (See preliminary study 6.)

The cuttings were rooted in the control room under the same conditions of temperature, light intensity, and photoperiod previously described for growing the seedlings. Bases of the cuttings were not shaded.

Evaluation of Rooting

Rooting was evaluated by counting the roots and estimating or measuring their length. The average number and average length of roots per cutting were calculated from these measurements. In some cases the distance from the base of the cutting to the nearest root

was measured to point out differences in the distribution of roots on the hypocotyl. Where the results of several studies are in agreement, the results of just one of the studies are reported. L.S.D.'s $_{.05}$ were calculated for the rooting trials.

Specific Techniques

1. Boron Analysis.

Boron analyses were run on the foliage (cordate and first trifoliolate leaf) of some cuttings at the end of the rooting period. The colorimetric, simplified curcumin, procedure of Dible, Truog and Berger (6, vol. 26, p. 418-421) was employed for the analysis. Lingle and Carolus (19, vol. 71, p. 507-515) found 52.7 ppm boron, on a dry weight basis, in the foliage of field-grown Black Valentine beans in Michigan. This level was probably sufficient or supra-optimal in that additions of boron fertilizer depressed yield.

2. Warburg Study.

The effect of boric acid on the respiration (apparent O_2 uptake) of bean hypocotyl sections was studied with a Warburg apparatus. Uniform one centimeter sections of untreated bean hypocotyls were placed in Warburg flasks in water and either water or five ppm boric acid was added from a sidearm after a 3½-hour stabilization period. Temperature was maintained at 30°C and CO_2 was absorbed with KOH. Readings were taken at least every half hour during the 7½-hour experimental period.

3. C^{14}-Sucrose Study.

Uptake and translocation of sucrose were studied in cuttings rooted in water, boric acid, and aluminum chloride (to supply aluminum). Aluminum was used in order to tell if the effects of boron on sugar translocation and rooting resulted from the complex-forming ability of boron, since aluminum, like boron, forms complexes with polyhydroxy compounds. The cuttings used in this study were taken from 14-day-old bean plants which had been grown in vermiculite. They were rooted for seven days in either water, 10^{-5}M (0.62 ppm) boric acid, or 10^{-5}M (1.33 ppm) aluminum chloride. At the end of this period one cutting from each treatment was treated with U-C^{14}-sucrose. The remaining nine cuttings in each treatment were allowed to root three more days before roots were counted and measured, and boron analyses were run on the foliage. Those cuttings treated with labelled sugar received 100 microliters of 0.83 per cent sucrose (in 0.2% Dreft) on prescribed areas of both cordate leaves. The areas treated in this manner consisted of the leaf surface enclosed by a lanolin ring seven millimeters in diameter on each side of the midrib of each cordate leaf. Thus 25 microliters of sucrose were applied to each of four such areas on a cutting. The activity of the sucrose applied to each cutting amounted to five microcuries. Areas to which the sucrose was applied were re-wet with water five times in the first five hours after treatment.

Of interest in this study were the comparative rates of translocation of sucrose or its hydrolytic products in the three cuttings,

and also comparisons of the quantity of material translocated in each case. The translocation rate from treated leaves to the base of the cutting was tested by removing single roots at timed intervals from the base of each cutting and checking them for radioactivity. The root samples, taken at a measured distance from the leaves, were killed in boiling carbon tetrachloride, pressed, and radioautographed. These samples, starting 26 minutes after sucrose was applied, were taken at progressively longer intervals until 72 hours had elapsed. After 72 hours the three cuttings were also killed in carbon tetrachloride, pressed, and radioautographed to determine the overall distribution of the radioactivity. The light intensity in the control room during the first 26 minutes was 1000 foot-candles and half a foot-candle for the duration of the sampling period. Radioautographs of root samples and whole cuttings were made by 14 days of exposure to X-ray film.

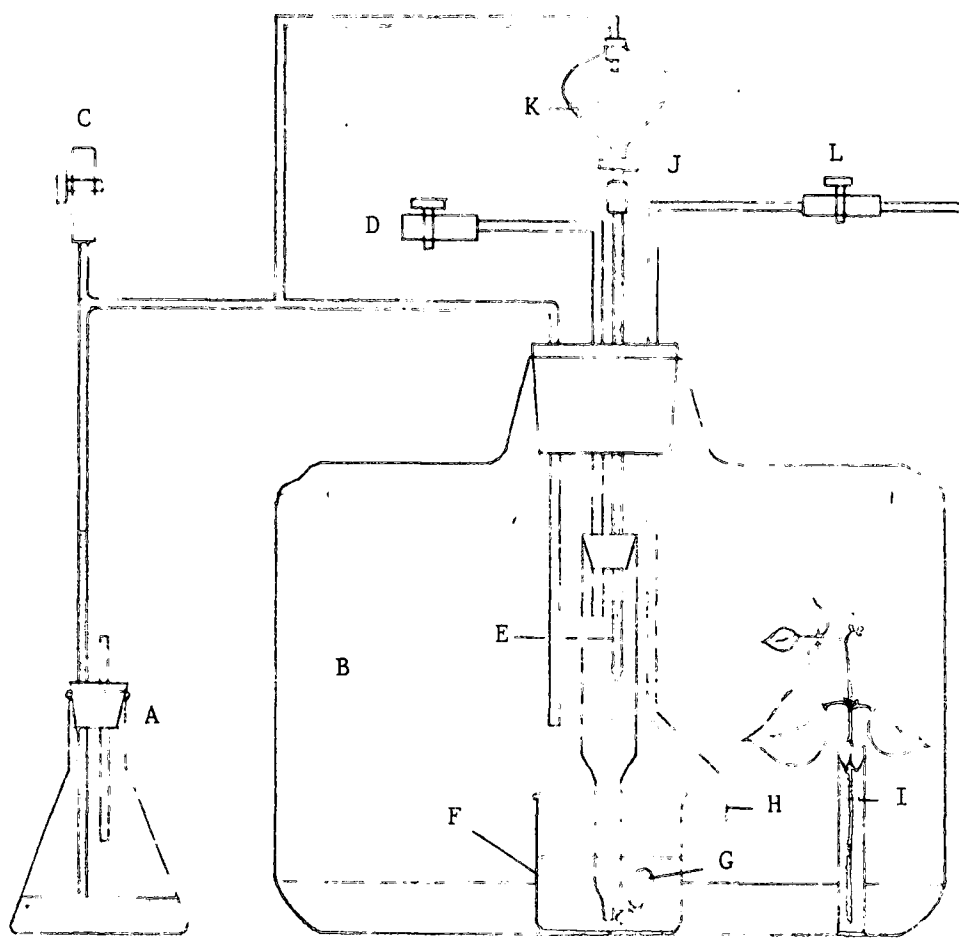
Just as in the C^{14}-sugar studies of Skok and Gauch and Dugger, this study was limited by the impossibility of telling whether differences in distribution of radioactivity in the cuttings were actually due to boron effects on translocation or merely to some other factor, such as enhanced cuticular absorption of sugar by leaf cells of boron treated plants. In this study the treatment of plants with boron in the rooting solutions, rather than in the sugar solutions applied to the leaves, eliminated the possibility of *in vitro* sugar-boron reactions. The fact remains, however, that photosynthate

may react quite differently to boron than does sugar applied to leaf surfaces.

4. $C^{14}O_2$ Study.

To circumvent the inherent experimental weakness in the U-C^{14}-sucrose study, an experiment was performed which more nearly assessed the effects of boron on cuttings under normal conditions. Conditions were provided so that cuttings fixed $C^{14}O_2$ photosynthetically and the effects of different rooting solutions on the quantitative and qualitative disposition of photosynthate were evaluated by liquid scintillation counting, chromatography, and radioautography. This study eliminated the nonphysiological situation created by applications of relatively high concentrations of sucrose to the leaf surfaces. The $C^{14}O_2$ was fed to the cuttings over a four-hour period at near atmospheric concentration. The advantages of this type of feeding over the traditional, short-time, high concentration method are that abnormally high or low CO_2 concentrations were avoided and small localized differences in CO_2 concentration due to poor mixing of the atmosphere were minimized or eliminated by extending the feeding period. Figure 1, page 24, shows a schematic diagram of the apparatus in which the cuttings were exposed to $C^{14}O_2$.

Uniform 10-day-old, soil-grown, bean cuttings were rooted in either water, 10^{-5}M (0.62 ppm) boric acid, or 10^{-5}M (1.33 ppm) aluminum chloride. After three days, three cuttings from each treatment were transferred, with appropriate rooting solution, into



A) MANOMETER (at 260 mm Hg)
B) GLASS CO_2 CHAMBER
C) VACUUM AND AIR INTAKE FOR SWEEPING
D) AIR INLET FOR MIXING
E) ACID DROP TUBE
F) $NaC^{14}O_3$ + $NaC^{12}O_3$ SOLUTION
G) AIR AND CO_2 BUBBLES
H) SWEEPING LINE
I) ROOTING SOLUTION
J) ACID DROP RATE CONTROL
K) LACTIC ACID (under vacuum)
L) VALVE TO SWEEPING SYSTEM
M) SAFETY TRAP
N) 2M NaOH, CO_2 TRAP
O) VALVE CONTROLLING SWEEPING RATE
P) SUCTION PUMP

Figure 1. SCHEMATIC DRAWING OF THE APPARATUS USED TO FEED $C^{14}O_2$ TO CUTTINGS

individual soft glass vials and placed in the $C^{14}O_2$ feeding apparatus. Cuttings at this stage were showing the first signs of rooting in the form of four longitudinal rows of root initials along the length of the submerged hypocotyl. The four cuttings remaining in each treatment were allowed to root for a total of nine days in the normal manner before roots were counted and measured. The light intensity in the CO_2 chamber was 1000 foot-candles, the temperature 26°C, and the relative humidity 100 per cent.

A vacuum was pulled on the system reducing the pressure to 500 millimeters of mercury. A mixture of $C^{12}O_2$ and one millicurie of $C^{14}O_2$ was liberated over a three-hour period from a mixed "cold" and "hot" sodium carbonate solution by the calibrated dropwise addition of 60 milliliters of 0.5N lactic acid. Cold Na_2CO_3 was added to the $Na_2C^{14}O_3$ to make a total of 15 millimoles, which was the estimated amount of CO_2 the plants could fix in three hours at the prevailing light intensity. The rate of acid addition was calculated to supply CO_2 at approximately the rate the plants would be able to fix the CO_2 photosynthetically. The estimate of the CO_2 fixation rate was based on an average of known CO_2 fixation rates for several plants. The fixation figure arrived at was 100 milligrams of CO_2 per hour per eight grams of fresh leaves. Liberation of CO_2 from N_2CO_3 can be expressed by the equation:

$$\underset{(15mM)}{Na_2CO_3} + \underset{(30mM)}{2C_3H_6O_3} \longrightarrow \underset{(15mM)}{CO_2} + \underset{(15mM)}{H_2O} + \underset{(30mM)}{2C_3H_5ONa}.$$

The vacuum on the system provided room for the 336 milliliters of CO_2 evolved and also for the outside air which was allowed to flow into the chamber during the fixation period. This air stream created currents which mixed the atmosphere in the chamber and reduced the tendency of the CO_2 to accumulate in the bottom of the chamber.

One hour after the last of the CO_2 was evolved, the atmosphere in the chamber was swept for two hours by pulling fresh air through the system with a suction pump. Carbon dioxide was trapped in 2M NaOH. One cutting was removed from each treatment, killed in boiling carbon tetrachloride, and pressed for radioautography. The lights were turned off and when 10 more hours had elapsed (16 hours from the start of the experiment) another cutting from each treatment was killed and pressed for radioautography. The cutting remaining in each treatment was cut into sections including hypocotyl, epicotyl, petioles, cordate leaves, and the trifoliolate leaf. The hypocotyl and cordate leaf sections were weighed, killed in boiling carbon tetrachloride, blended in 90 per cent ethanol, and the residue was extracted in a Sohxlet apparatus in 80 per cent ethanol until no radioactivity could be detected in the extracting solution. The killing solution and successive Sohxlet extracts of each section were combined and reduced to near-dryness in a rotary-vacuum evaporator. After drying, the residue was redissolved in successive 25 milliliter aliquots of absolute ethanol, petroleum ether, and water. Two-tenth milliliter

aliquots from each 25 milliliters of extract were added to five milliliters of 95 per cent ethanol and 10 milliliters of phosphor solution (three grams of terphenyl and 30 milligrams of POPOP in a liter of toluene) and counted in a Tri-Carb liquid scintillation spectrometer. The relative amounts of radioactivity in leaves and hypocotyls of cuttings from the three treatments (water, boron, and aluminum) were calculated from this data. Qualitative assay of the extracts was accomplished by two-dimensional chromotography using the phenol: ammonia: water and n-butanol: proprionic acid: water systems described by Bassham and Calvin (2, p. 19). Radioautographs were made of the chromatograms and the major spots were identified by their position on the paper, co-chromatography with standards in several one-dimensional systems, and by specific staining reactions. The organic acids were further separated and identified by the gradient elution method of Hulme and Wooltorton (16, vol. 9, p. 150-158) wherein the acids in the trifoliolate leaf extract of the water-rooted cutting were trapped on a Dowex 50W-X4 column and eluted with acid. The eluate was collected as 80 four milliliter fractions and titrated. The acids in the leaf extract were identified by comparison of the position of their peaks with the peaks of known acids.

5. Anatomical Study.

A limited anatomical examination was made of the hypocotyls of cuttings rooted in water or boric acid solutions. The purposes

of the study were to observe the chronological sequence of rooting in the two treatments, to check for differences in the anatomical makeup of the roots, and to compare the visual counts of roots and root initials with the actual numbers revealed microscopically. Doutt (7, p. 1-32) has done a complete anatomical study of Black Valentine bean.

Cuttings of 13-day-old, soil-grown bean plants were rooted in water or 0.5 ppm boric acid in the normal manner. Sections of hypocotyl from six cuttings in each treatment were killed and fixed in Randolph's Solution each day during the rooting period. These samples were sectioned and examined to study the chronological sequence of rooting. The sampling unit was a $\frac{1}{2}$-inch section of hypocotyl between 2 and $2\frac{1}{2}$ inches from the base of the hypocotyl.

After $8\frac{1}{2}$ days, four cuttings from each treatment were cut into half-inch sections starting from the base of the cutting and including the four inches of hypocotyl and epicotyl normally submerged in the rooting solution. The roots on comparable sections from the four cuttings in each treatment were counted and measured. This provided a picture of the root distribution along the length of the rooting zone. A count was also made of small "bumps" which commonly appear along the hypocotyl and which were revealed microscopically to be root initials. The sections were then killed and embedded as before. The visual counts of roots and root initials were compared with the microscopic counts after sectioning to determine whether

visual observations gave an accurate count of root initials. This information was necessary to determine whether boron was involved only in growth of preformed initials or in root initiation as well.

RESULTS AND DISCUSSION

Preliminary Studies

Before it was possible to proceed with an evaluation of the four hypothetical roles of boron in rooting, it was necessary to consider some of the more general aspects of the boron rooting stimulus. Studies which provide this general background information are discussed in the Preliminary Studies section because they deal with a diversity of subjects, the discussion of which would detract from the continuity of the more important experiments.

Topics considered in this section in the order of presentation include: 1) the optimum concentration of boron for rooting, 2) root distribution on the hypocotyl, 3) the chronological sequence of rooting, 4) the effect of shading the rooting zone on the boron response, 5) the effect of different forms of boron on rooting, 6) the pH of different concentrations of boric acid, 7) the response of cuttings of different bean cultivars to boron, 8) the effect of boron fertilization of plants on the subsequent response of cuttings to boron, 9) response of cuttings to boron from plants grown in different media, 10) the influence of complexing elements other than boron on rooting, 11) the influence of aluminum on rooting, and 12) the effect of boric acid on the respiration of bean hypocotyl sections.

1. Boric acid concentration and rooting.

A rooting trial was run to find the optimum boron concentration for rooting. Cuttings in the trial were taken from 11½-day-old, soil-grown bean plants which were rooted for 10 days in one of eight levels of boric acid. There were 20 cuttings in each treatment. The $LSD_{.05}$ between the treatment means was 15.7 for average root number, 327.3 millimeters for average root length, and 0.43 centimeters for the distance from the base of the cutting to the nearest root.

Figure 2 shows the influence of the eight levels of boric acid, between 0 and 20 ppm, on the average number and on average length of roots per cutting. Figure 3 shows the level of boron in the foliage of the cuttings at the end of the rooting period and the distance from the base of the cutting to the nearest root, which illustrates differences in root distribution on the hypocotyls in different treatments. Representative cuttings from each of the treatments are pictured in Appendix Plate 1, page 109.

The data show that boron enhanced rooting over a wide range of concentrations. Very low concentrations (0.039 ppm) were stimulatory but best rooting occurred at 0.156 and 0.625 ppm. The cuttings rooted in 10 and 20 ppm boric acid showed toxicity in the form of marginal necrosis, but still rooted well. In most later experiments boric acid was used at a concentration of about 0.5 to 0.625 ppm, (approximately 10^{-5}M) because this level provided good overall rooting.

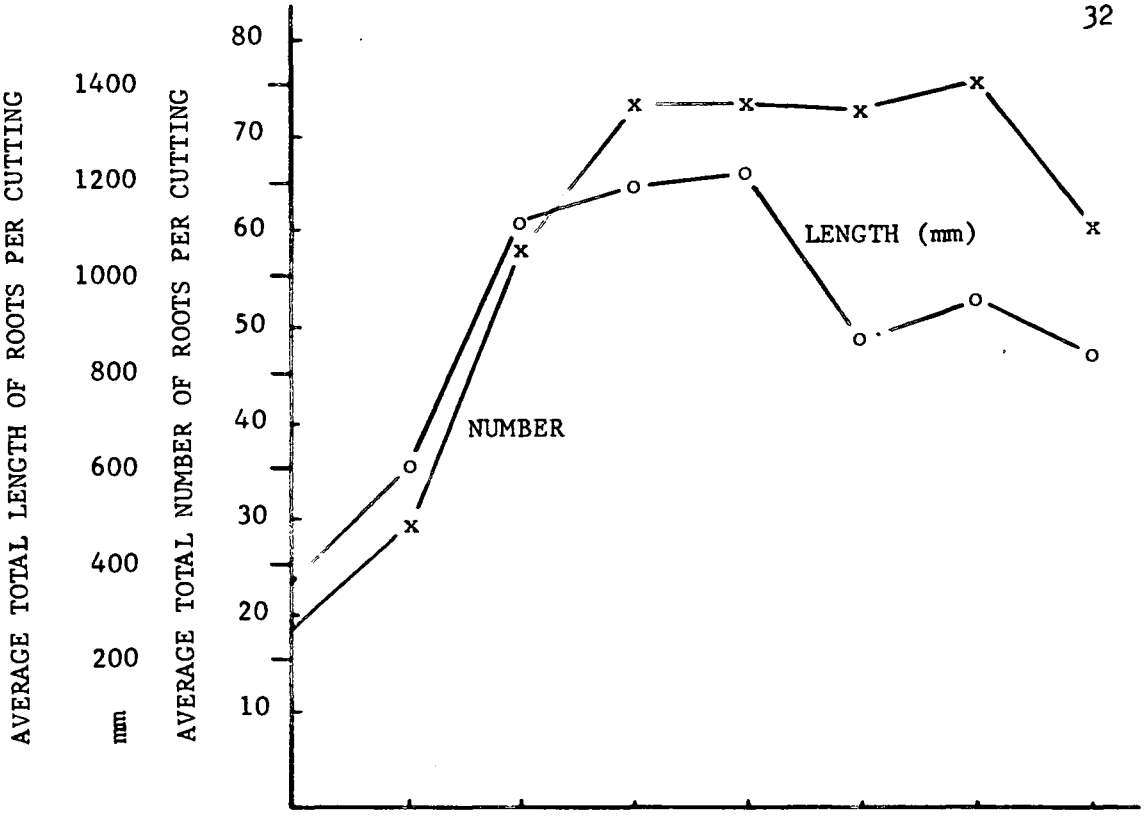


Figure 2. The effect of boron level on length and number of roots.

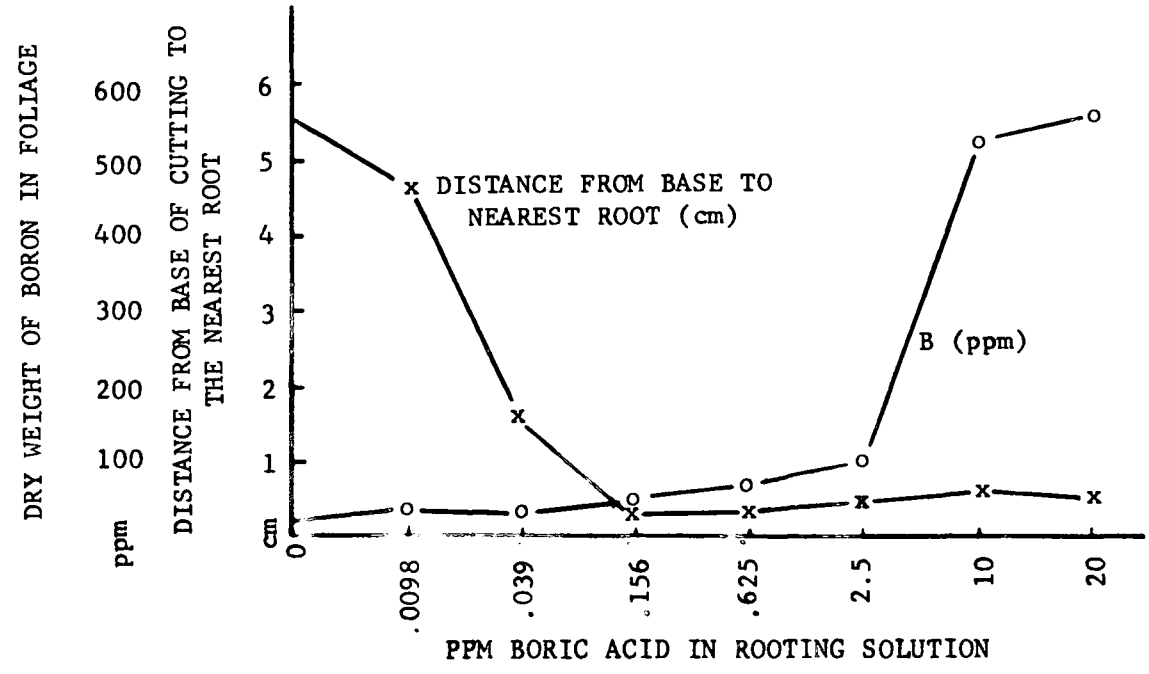


Figure 3. The effect of boron level on boron content of leaves and the distance from the base of the cutting to the nearest root.

Figure 3 and Plate 1 show that cuttings rooted in boron developed roots near the base of the cutting, while those rooted in water formed roots nearer the surface of the rooting solution. Inasmuch as oxygen is known to be necessary for root growth, this observation prompted later work on the possible role of boron in oxidative processes.

2. Root distribution.

A more critical examination of the distribution of roots along the length of the hypocotyl was made in conjunction with the anatomical study described in the Methods section. Figures 4, 5, and 6 show the average number of root initials produced on successive half-inch sections along the length of the hypocotyl. The number of root initials is the sum of the number of roots plus the number of "bumps" which were visible along the length of the hypocotyl. Anatomical examination revealed that these "bumps" were root initials which had not emerged from the hypocotyl tissue. The $LSD_{.05}$ for the average number of roots was 3.5, for average length of roots 25.3 millimeters, and for the average number of root initials, 5.9. Each point on the curves is the average of four cuttings.

These data show that cuttings rooted in boron formed more roots at the base of the cutting. Longer roots, however, were produced on the upper inch of submerged hypocotyl. On the other hand, cuttings rooted in water rooted very sparingly near the base of the

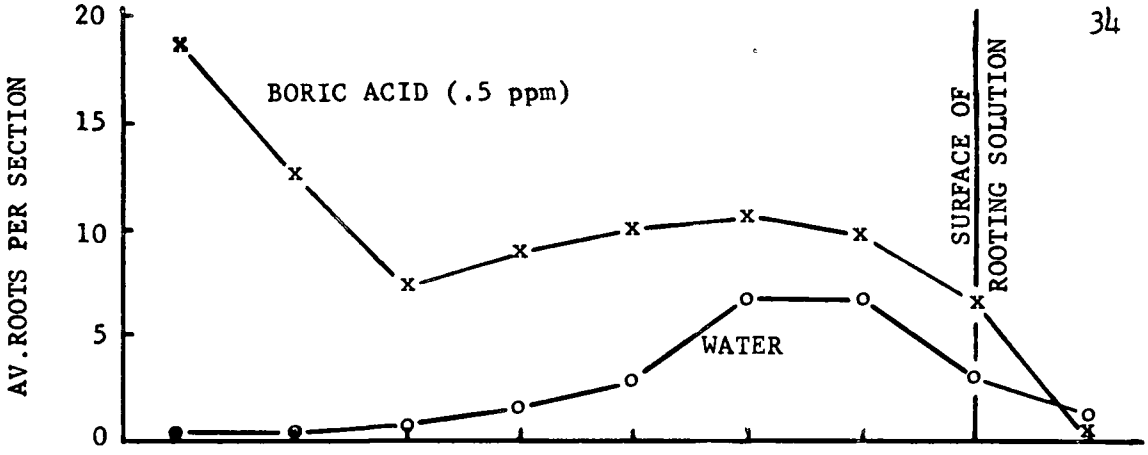


Figure 4. Numbers of roots produced on ½-inch sections of hypocotyl.

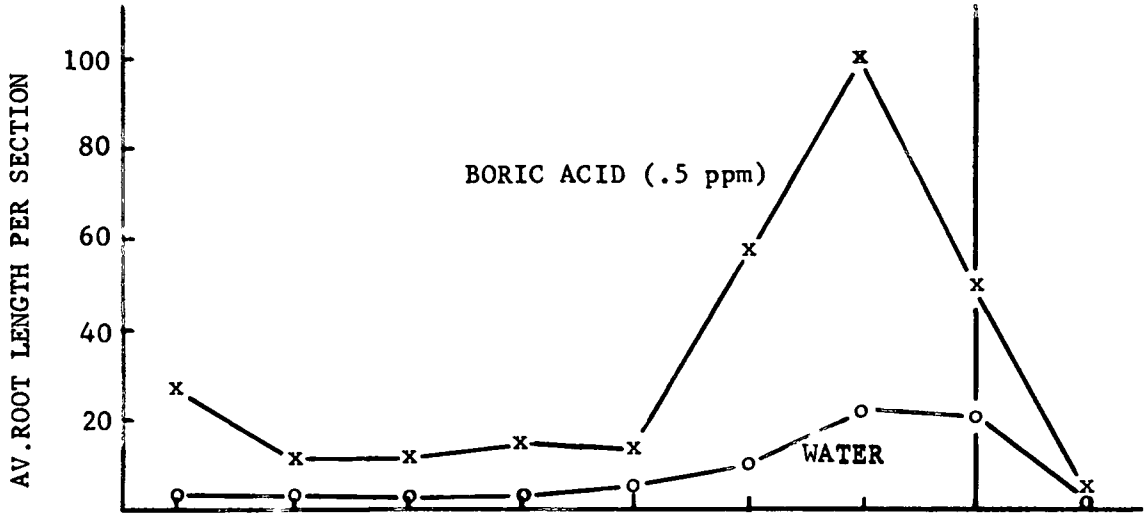


Figure 5. Length of roots produced on ½-inch sections of hypocotyl.

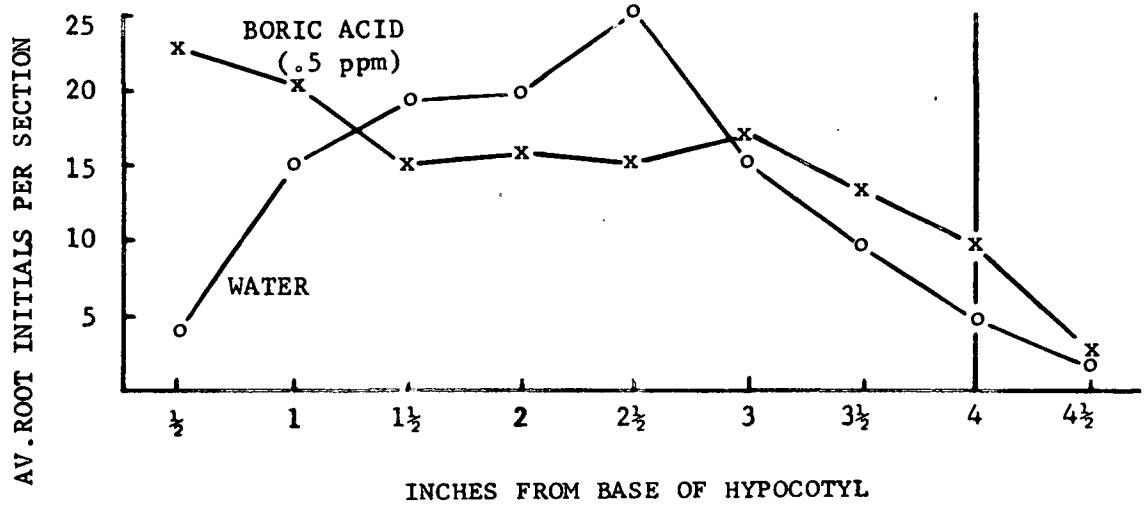


Figure 6. Root initials produced on ½-inch sections of hypocotyl.

cutting and produced both the greatest number and length of roots near the surface of the water. The total number of root initials in the two treatments was remarkably similar, although proportionally more initials were produced at the base of the boron-rooted cuttings but near the center of those rooted in water. Considerably more roots were evident on cuttings rooted in boron. This supports the views of Hemberg (15, vol. 4, p. 358-369) and Gorter (14, vol. 11, p. 1-9) that boron promotes root growth but not root initiation.

Visual counts of root initials agreed well with counts made microscopically and no basic anatomical differences were evident between cuttings rooted in the two treatments. This fact, plus the observation that boron-deficient plants showed numerous striking anatomical abnormalities, (1, vol. 103, p. 475-491) (31, vol. 118, p. 53-71) supports the contention that the plants in these studies were not boron-deficient.

Microscopic examination of sections of hypocotyl revealed that the roots arose endogenously from the pericycle in four longitudinal rows along the hypocotyl, and opposite the four groups of endarch protoxylem cells.

3. The chronological sequence of rooting.

It was observed on clematis (44, vol. 183, p. 559-560) and English holly (45, vol. 75, p. 704-710) cuttings that boron accelerated the rooting process. Critical visual observations of rooting of

bean cuttings were made to study this accelerated rooting and any other effects that boron might have on the sequence of events that are part of the rooting process.

Cuttings for this study were taken from 10-day-old, soil-grown bean seedlings. Rooting was carried out in either 10^{-4}M boric acid (6.25 ppm) or water. At 24-hour intervals the cuttings in each treatment were observed. One cutting from each treatment was removed from the rooting solution, killed in alcohol, and examined carefully under a binocular scope. Notes made over the eight day rooting period are summarized in Table 1.

Microscopic examination of hypocotyl sections, sampled daily in connection with the anatomical study, agreed well with the chronological rooting sequence described in Table 1. Although no anatomical differences were noted between the cuttings rooted in water or boron, there were faster rooting, more and longer roots, and a more general distribution of roots on the boron-rooted cuttings.

Table 1. Visual daily observations of rooting on cuttings in water or 10^{-4}M boric acid.

Observation Time (days)	Notes on Rooting	
	Water	Boric Acid
1	No change	No change
2	Basal swelling of hypocotyl.	Basal swelling of hypocotyl.
3	Four longitudinal cracks along length of submerged hypocotyl. Spongy callus-like cell proliferation in the cracks.	Four cracks plus dense root initials forming in the cracks. Spongy callus-like cell proliferation in the cracks.
4	Root initials evident in cracks.	Roots about 0.5 mm long have pushed through the epidermal tissues, especially at the base of the cuttings.
5	A few roots 1 mm long have emerged near and immediately above the surface of the water.	About 80% of the obvious root initials have pushed through the epidermis and average 2 mm in length. Rather uniform root development over the entire length of the hypocotyl.
6	About 50% of the root initials have emerged on the upper hypocotyl. No roots have emerged on the basal 4-5 cm although initials are present.	About 95% root emergence.
7	A few of the roots on the upper hypocotyl have elongated to 10-15 mm.	Numerous roots 5-6 mm long. Roots on the upper part of the hypocotyl are longer. Roots on the lower part of the hypocotyl are more numerous.
8	A few long (10-20 mm) roots near the surface of the water.	Numerous roots 1-7 mm long. The inverse relationship of number and length of roots as noted above was quite evident.

4. The effect of shading the rooting zone.

Fernquist and Leopold (10, vol. 34, p. iv) reported that light applied to the rooting zone strongly inhibited the rooting of bean cuttings. Because bases of cuttings in these experiments were not shaded, a study was run to determine the effect of basal shading on the boron stimulus. The cuttings from 14-day-old, soil-grown bean plants were rooted for eight days in 0.5 ppm boric acid or water with half of the hypocotyls in each treatment shaded with aluminum foil around the jar. Each figure in Table 2 is an average of 18 cuttings.

Table 2. Effect of shading the rooting zone on boron response of bean cuttings (18 cuttings per treatment).

Rooting Treatment	Av. Number of Roots	Av. Length of Roots (mm)	Av. Distance from Base of Cutting to Nearest Root (cm)	Boron in Foliage (ppm)
Water-light	7.6	8.3	4.6	23.2
Water-shade	12.5	28.7	6.1	23.2
Boron-light	36.4	125.7	0.1	36.8
Boron-shade	33.8	174.8	2.3	25.6
$LSD_{.05}$	10.9	100.6	1.4	

Table 2 shows that cuttings either with lighted or shaded rooting zones responded to boron. Although shading enhanced rooting and altered the root distribution of both water- and boron-rooted cuttings, it did not minimize the boron effects. It appears,

therefore, that the stimulatory effects of shade and boron are not related. For this reason no effort was made in subsequent experiments to shade the rooting zone, but care was taken to insure that the hypocotyls of cuttings in all treatments were exposed to approximately the same amount of light.

5. The rooting response to different forms of boron.

In these studies and studies by others (15, vol. 4, p. 358-369) (14, vol. 11, p. 1-9), boric acid has been used exclusively to supply boron. Two different forms of boron were tested in this study to determine whether the rooting response is a response to boron or more specifically to boric acid.

Cuttings from 18-day-old, soil-grown bean plants were rooted for 10 days either in water, 0.5 ppm boric acid, or in one of two commercial boron fertilizers: Solubor (20.5% B) or Borospray ($Na_2B_{10}O_{16} \cdot 10\ H_2O$, 18% B). The amounts of Solubor and Borospray added to the rooting solutions were calculated to supply the same amount of boron as 0.5 ppm boric acid (17.5% B). There were five cuttings per treatment.

Table 3. Effects of boric acid, Solubor, and Borospray on the rooting of bean cuttings (5 cuttings per treatment).

Rooting Treatment	Average number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)
Water	12.1	68.4
Boric Acid	63.3	229.2
Solubor	52.7	212.2
Borospray	59.2	207.3
$LSD_{.05}$	31.8	114.1

The data indicate that the boron response was not limited to boric acid.

6. The pH of different concentrations of boric acid.

The acidity of boric acid rooting solutions could possibly have an influence on the rooting of cuttings. The pH of different boric acid concentrations was measured with a pH meter to determine the extent of the influence of boric acid concentration on the acidity of rooting solutions. The solutions were made up with the same distilled, ion-exchanged water used for rooting.

Table 4. The pH of different concentrations of boric acid.

Boric Acid Concentration (ppm)	pH
0.0	6.1
0.5	6.1
1.0	6.0
10.0	5.9
1000.0	5.8

Table 4 shows that boric acid had little effect on the acidity of the rooting solutions, especially at the low concentrations most commonly used for rooting. It is unlikely that this slight lowering of pH could account for the profound effects of boron on rooting. This conclusion is supported by the fact that non-acidic sources of boron (see preliminary study 5) had the same effect on rooting as did boric acid.

7. Response of cuttings of other bean cultivars to boric acid.

Hemberg (15, vol. 4, p. 358-369) and Gorter (14, vol. 11, p. 1-9) have used only Black Valentine beans in their rooting studies. An experiment was conducted to determine whether other bean cultivars responded similarly to boron.

Cuttings were taken from 14-day-old, soil-grown bean plants of three cultivars grown under the same conditions. Half of the 10

cuttings of each cultivar were rooted in water and the other half in 0.5 ppm boric acid. The 0.3 ppm copper sulfate, which was added to the rooting solutions to inhibit microorganisms, appeared to have an overall depressing effect upon rooting.

Table 5. The rooting of Topcrop, Wade, and Black Valentine bean cuttings in boric acid and water (5 cuttings per treatment).

Bean Cultivar	Water		Boric Acid	
	Av. No. Roots Per Cutting	Av. Length Roots Per Cuttings (mm)	Av. No. Roots Per Cutting	Av. Length Roots Per Cuttings (mm)
Topcrop	0	0	36	121
Wade	0	0	25	148
Black Valentine	0	0	25	98
$LSD_{.05}$	20	94	20	94

It is apparent that the boron response was not limited to Black Valentine bean cuttings.

8. The effect of boric acid on rooting of cuttings initially high in boron.

The effect of the initial level of boron in the plants on the subsequent rooting response to boron was studied. As would be expected, when bean plants were fertilized with an excess of boron fertilizer prior to cuttage, the cuttings rooted as well in water as in boron rooting solutions. Even cuttings from plants showing severe boron toxicity symptoms demonstrated this enhanced rooting.

Table 6 shows the results of a study in which 10-day-old, soil-grown bean cuttings, which had been watered heavily with 0.025 per cent Borospray five days before cuttage, were rooted for 10 days in either water or 0.5 ppm boric acid.

Table 6. The effect of boron fertilization of bean plants on the subsequent response of cuttings to boron (8 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Treatment	Average Length of Roots Per Treatment (mm)
Water	45.9	361.6
Boric Acid	51.8	378.0
$LSD_{.05}$	30.7	351.3

Cuttings in both treatments rooted well although they had marginal necrotic spots on the cordate leaves because of the excess of boron fertilizer.

9. The boron response of cuttings from plants grown in different media.

To aid in the choice of a suitable growing medium, the rooting of cuttings from plants grown in soil, sand, and sand plus a complete nutrient were compared. Plants were grown in one of the three media for 14 days before cuttings were taken. Ten cuttings from each medium were rooted 10 days in either water or 5 ppm boric acid. Boron analyses of the foliage were run at the start of the rooting period. Results of the study are summarized in Table 7.

Table 7. The rooting of cuttings from plants grown in sand, soil, or sand plus complete nutrient solution (5 cuttings per treatment).

Growing Medium	Water		Boron		Boron in Foliage (ppm)
	Av.No. Roots Per Cutting	Av.Length Roots Per Cutting (mm)	Av.No. Roots Per Cutting	Av.Length Roots Per Cutting (mm)	
Soil	18	171	37	632	46.4
Sand	16	97	38	299	48.4
Sand & Nutrients	24	137	41	601	54.4
LSD $_{.05}$	16	209	16	209	

Although the boron content of the foliage was similar in all cases, the cuttings from plants grown in soil or sand plus nutrients looked healthier and generally rooted better than those grown in sand alone. Soil was used as a growing medium in the majority of subsequent trials.

The plants grown in all three media were more vigorous and darker green than vermiculite-grown plants used in several of the earlier trials. As will be noted in the next two studies vermiculite-grown plants gave inconsistent and confusing results.

10. The influence of complexing elements other than boron on the rooting of bean cuttings.

In view of the well-known ability of boron to form polyhydroxy complexes and also to stimulate rooting, the polyhydroxy complex-

forming elements strontium, germanium, and aluminum, used by Skok (38, vol. 32, p. 308-312), were tested to determine their influence on rooting.

Cuttings from 12-day-old, soil-grown bean plants were rooted nine days in water or 0.5 ppm solutions of boric acid, strontium chloride, germanium dioxide, or aluminum chloride. Table 8 shows the rooting results based on 10 cuttings per treatment.

Table 8. The effect of water, B, Sr, Ge, and Al on the rooting of soil-grown bean cuttings (10 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)
Water	17.3	42.1
Boric Acid	58.2	176.6
Strontium Chloride	14.4	37.8
Germanium Dioxide	11.8	23.9
Aluminum Chloride	15.1	40.2
$LSD_{.05}$	18.5	78.3

In this study, in which soil-grown plants were used, boron was the only element which stimulated rooting, but when cuttings were taken from plants grown in vermiculite the picture was quite different. Table 9 summarizes the rooting effects of the same five treatments: water, boron, strontium, germanium, and aluminum, on 14-day-old, vermiculite-grown bean cuttings. The six cuttings in each treatment were rooted for 13 days.

Table 9. The effect of water, B, Sr, Ge, and Al on the rooting of vermiculite-grown bean cuttings (6 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)
Water	41.7	108.3
Boric Acid	33.8	116.3
Strontium Chloride	17.1	46.2
Germanium Dioxide	8.6	9.9
Aluminum Chloride	45.5	296.3
$LSD_{.05}$	33.2	156.1

These results indicate that boron-rooted cuttings rooted no better than those in water. In addition to this atypical failure to respond to boron, aluminum appeared to stimulate rooting slightly. The response to aluminum and the unusual lack of response to boron by cuttings from vermiculite-grown plants are considered in more detail in the following study.

11. The influence of aluminum on rooting.

A consideration of the aluminum effects on rooting is of special importance because, if aluminum enhances rooting, it implies that the complexing ability, common to both aluminum and boron, may be related to the rooting stimulus. Conversely, if aluminum and other complexing elements do not stimulate rooting, it would appear

that boron's role in rooting is not directly related to its complexing ability. Based on the findings of several studies reported here, the relation of aluminum to rooting can be summarized by the following observations: a) Vermiculite-grown plants were generally yellow in color, dwarfed, and atypical when compared with soil- or sand-grown plants. The presence of a water soluble inhibitor in vermiculite was suggested by the observation that a water extract of vermiculite inhibited growth and caused yellowing of bean plants growing in sand. Galston and Warburg (11, vol. 34, p. 16) have previously reported a water soluble inhibitor in vermiculite. b) Cuttings from plants grown in vermiculite from one source sometimes showed strikingly enhanced rooting in aluminum solutions. Conversely, cuttings from plants grown in soil or in vermiculite from another source consistently showed no rooting response to aluminum. c) As a result of these inconsistencies, it is concluded that the aluminum rooting response was limited to cuttings from plants grown in a certain lot of vermiculite, and were therefore not typical of normal plants. Soil was used exclusively as a growing medium in all later experiments.

Representative studies, which illustrate the responses mentioned in b) above, are presented in the following paragraphs:

In one study cuttings from 13-day-old, vermiculite-grown plants were rooted for 10 days in water, three levels of boric acid (0.1, 1.0, or 5 ppm), or the same three levels of aluminum chloride.

For simplicity, only results from the best boron (0.1 ppm H_3BO_3) and the best aluminum (5 ppm $AlCl_3$) treatments are tabulated in Table 10. Each treatment included four cuttings. The vermiculite used to grow plants for this trial was from the same lot as that used in the second trial in study 10 (Table 9).

Table 10. The effect of boron and aluminum on the rooting of vermiculite-grown bean cuttings (4 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)
Water	31.6	97.4
Boric Acid	52.4	313.1
Aluminum Chloride	48.7	272.3
$LSD_{.05}$	19.9	122.4

The results show that cuttings from plants grown in the original lot of vermiculite were stimulated to root by aluminum. The rooting response to aluminum approached that produced by boron.

The next trial compared the rooting response to aluminum of soil-grown and vermiculite-grown cuttings. The cuttings were taken from plants grown 15 days either in soil or in vermiculite from the same source used in the previous study. The five cuttings in each treatment were rooted in water or in 1 ppm aluminum chloride. Note in Table 11 that soil-grown plants rooted much better than those

grown in vermiculite. The rooting of the vermiculite-grown plants again appeared to be stimulated by aluminum, while soil-grown plants were unaffected.

Table 11. Rooting response of soil and vermiculite-grown cuttings to aluminum (5 cuttings per treatment).

	Water		Aluminum	
Growing Medium	Av.No.Roots Per Cutting	Av. Length of Roots Per Cutting (mm)	Av.No.Roots Per Cutting	Av.Length of Roots Per Cutting (mm)
Soil	42.2	634.3	34.6	662.8
Vermiculite	6.4	6.3	16.5	209.2
$LSD_{.05}$	21.3	189.7	21.3	189.4

The results show that cuttings from vermiculite-grown plants responded to aluminum while those from soil-grown plants did not. The much weaker rooting of the vermiculite-grown plants illustrates their lack of normal vigor.

In other trials, the rooting response of vermiculite-grown plants to aluminum was found to be specific in that aluminum acetate or aluminum chloride were equally effective in enhancing rooting. Chloride containing salts such as sodium chloride and potassium chloride evoked no rooting response.

The studies thus far have shown that cuttings from vermiculite-grown plants responded to aluminum but that cuttings from soil-grown plants did not. A further modification of this situation was found when, in the course of the studies, the original supply of

vermiculite was exhausted and more of the same type was obtained. The plants grown in the new lot of vermiculite were more vigorous and darker green than those grown in the original lot.

Table 12 shows that cuttings from plants grown in this second lot of vermiculite did not respond to aluminum. The plants were grown 14 days in soil, vermiculite, or vermiculite plus a complete nutrient. Cuttings from each medium were rooted for 10 days in water or 10^{-5}M concentrations of boric acid, aluminum chloride, or sodium chloride. There were 10 cuttings in each treatment. As before, the figures in the table for number and length represent the average number and length of roots per cutting.

Table 12. The rooting response of cuttings to 10^{-5}M H_3BO_3, $AlCl_3$, or NaCl, from plants grown in soil, vermiculite, or vermiculite plus nutrients (10 cuttings per treatment).

	Rooting							
	Water		H_3BO_3		$AlCl_3$		NaCl	
Growing Medium	Av. No.	Av. Length (mm)	Av. No.	Av. Length (mm)	Av. No.	Av. Length (mm)	Av. No.	Av. Length (mm)
Soil	34	246	86	854	39	142	25	173
Vermiculite	31	58	79	395	20	123	50	87
Vermiculite Plus Nutrients	53	379	94	658	41	212	57	207
$LSD_{.05}$	26	212	26	212	26	212	26	212

Cuttings from plants grown in this lot of vermiculite, with or without the addition of nutrients, responded normally to boron

and were not stimulated to root by aluminum. Because the stimulatory effects of aluminum on rooting of vermiculite-grown cuttings were not duplicated on cuttings from plants grown in soil, sand, or in a different lot of vermiculite, it appears that the response of cuttings to aluminum, observed in the earlier trials, was not typical of normal plants.

The rooting response of cuttings to boron does not appear to be dependent on its complexing ability because other complexing elements do not stimulate the rooting of cuttings from normal plants.

12. Warburg study.

The study of the respiration of hypocotyl sections, as described in the Methods chapter, was a rather abrupt departure from the foregoing rooting trials. This trial is discussed under the heading of Preliminary Studies because the type of information it provided is general and is difficult to interpret in terms of the four hypotheses proposed to explain the role of boron in rooting. Figure 7 shows that boric acid had essentially no effect on the respiration of bean hypocotyl sections under the conditions of this test. Because of the many differences between hypocotyl sections and intact cuttings, it is difficult to know what significance, if any, these results have with respect to the rooting of cuttings. Gauch and Dugger (13, vol. 28, p. 457-466) found that 5 ppm boric acid increased oxygen uptake by excised roots only when respiratory

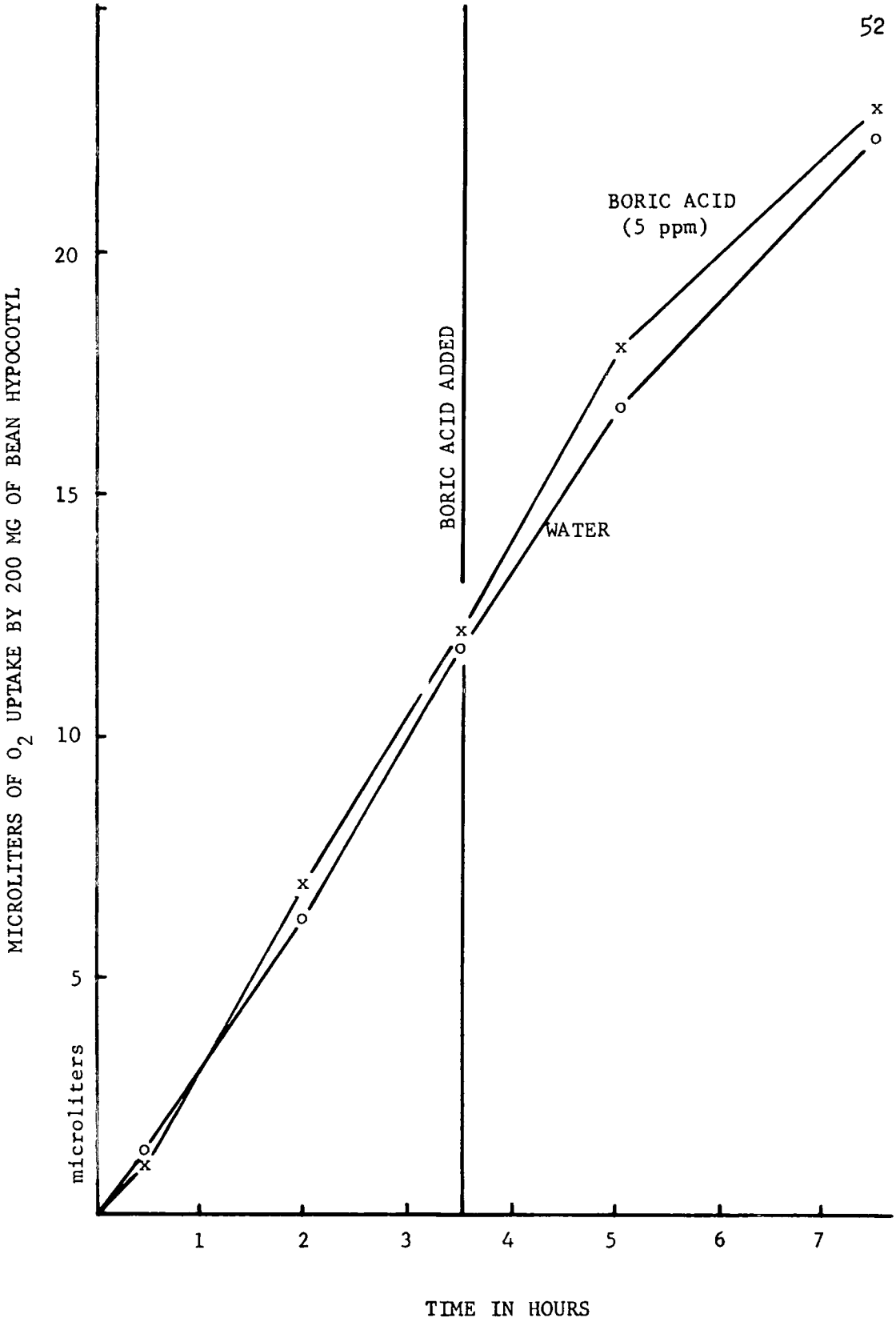


Figure 7. The effect of boron on O_2 uptake by bean hypocotyl sections.

substrate (sucrose) was added. It is possible that the addition of sucrose to the hypocotyl sections in this study would have elicited a similar response, but it would still be difficult to say whether the enhanced respiratory activity resulted from changes in auxin metabolism, sugar translocation, oxidative processes, cell wall metabolism, or some other equally general plant process. For this reason the study was not pursued further.

Evaluation of Hypotheses

The balance of the results presented here evaluate the studies which led to the acceptance or rejection of the four hypotheses presented earlier as possible roles for boron in rooting.

First Hypothesis: Boron Stimulates Rooting of Cuttings Through Its Effects on Auxin Metabolism.

Evidence was presented in the Review of Literature which suggested that boron may be directly or indirectly involved with auxin (IAA) in plants. It is possible, by a simple experiment, to establish whether or not such a boron-auxin interrelation is responsible for the boron effects on rooting. To test the hypothesis, three levels of IAA were chosen which supplied either sub-optimal, near-optimal or supra-optimal levels of IAA for rooting. If boron were involved either in directly stimulating auxin production or in indirectly enhancing auxin translocation, the addition of boron to the three levels of IAA should give evidence of such interactions. For

example, if the hypothesis were true, the addition of boron, at the sub-optimal IAA level, would increase rooting. At the near optimal level, however, rooting would probably be depressed and it would definitely be depressed at the supra-optimal concentration. If boron and auxin stimulations to rooting were not one and the same, then addition of boron to the IAA treatments would cause no such stimulatory and inhibitory pattern of modified response. The lack of such a pattern would not disprove the existence of a boron-auxin relation in plants but it would indicate that this relationship was not responsible for the enhancing effects of boron on rooting. Figures 8 and 9 show the results of the study. Appendix Plate 2, page 109, shows a typical cutting from each treatment. The cuttings in the study were taken from 14-day-old, soil-grown bean plants. Eight cuttings were rooted for nine days in each of eight treatments consisting of water, 0.1 ppm IAA, 0.5 ppm IAA, 2.5 ppm IAA, or in the same four treatments with the addition of 0.5 ppm boric acid. The $LSD_{.05}$ for the number of roots was 26; for length of roots, 368 millimeters.

The curves show that the stimulatory effects of boron and IAA were additive, indicating that they were unrelated in their action on rooting. The results are in perfect agreement with those of Gorter (14, vol. 11, p. 1-9). On this basis, the hypothesis that boron stimulates rooting through effects on auxin metabolism can be rejected.

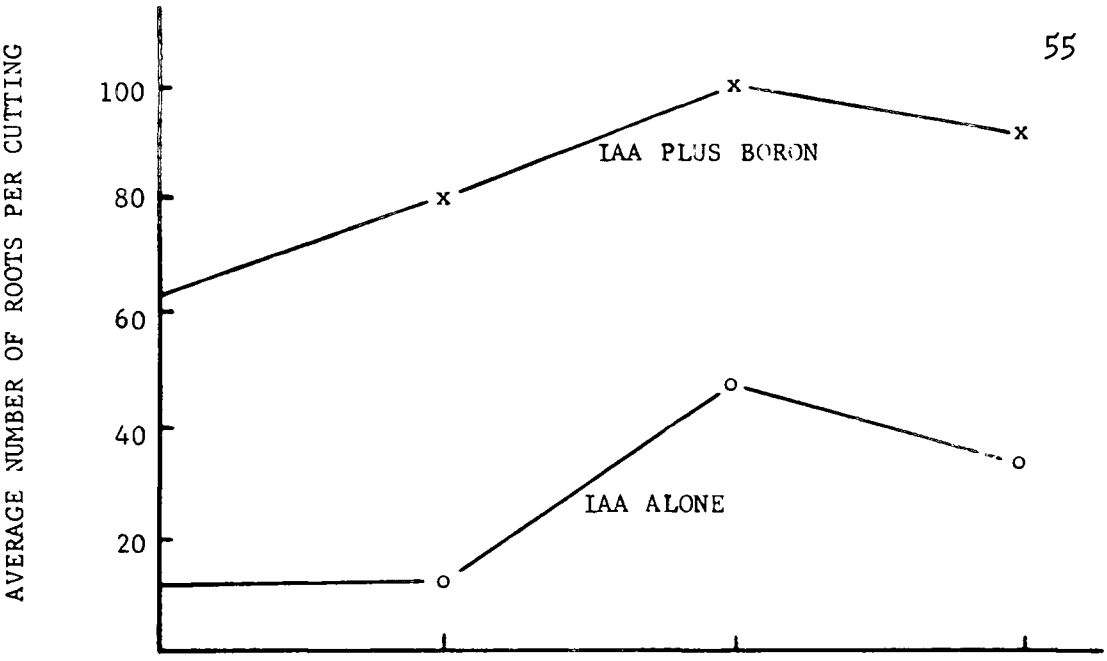


Figure 8. The effect of IAA and boron, separately and combined, on the number of roots produced on bean cuttings.

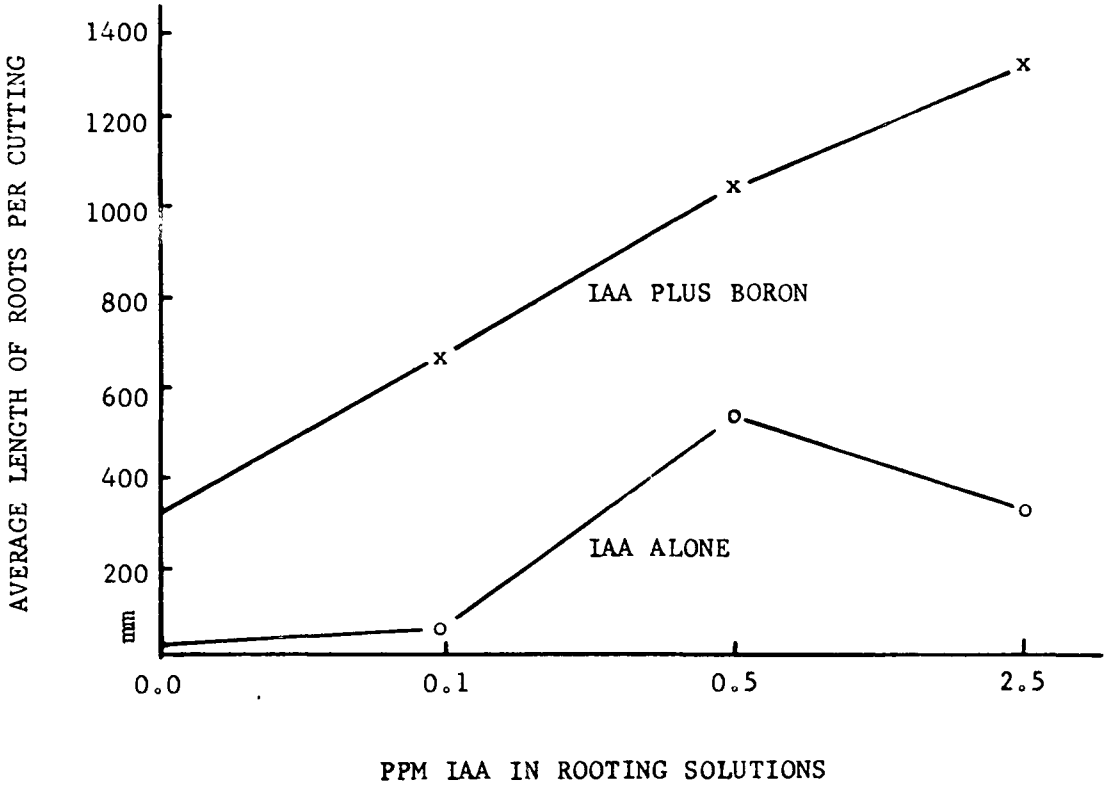


Figure 9. The effect of IAA and boron, separately and combined, on the length of roots produced on bean cuttings.

Second Hypothesis: Boron Stimulates Rooting of Cuttings by Enhancing Sugar Translocation.

Acceptance of this hypothesis is dependent upon two basic assumptions: 1) sugar is a limiting factor in root formation on bean hypocotyls, and 2) boron facilitates sugar translocation from the leaves to the hypocotyl and hence enhances rooting. At first glance, both of these assumptions appear tenable, but, as noted in the Review of Literature, many workers think that boron plays no role in sugar translocation. The first assumption that sugar limits rooting does not seem too logical because there should be ample sugar produced by the actively photosynthesizing young bean plants. The objections are, however, inconclusive. Three methods were used to test the hypothesis experimentally. The methods included rooting studies, application of labelled sucrose to leaves of cuttings, and feeding $C^{14}O_2$ to cuttings.

Rooting studies with sugar.

The approach in the rooting studies was to supply sugar (sucrose) exogenously to the cuttings and to observe the effect on rooting. If sugar supply were the factor which limits rooting, its addition to the rooting solution would enhance root development, especially on hypocotyls of cuttings rooted in water. The hypocotyls of boron-rooted cuttings would supposedly be getting sugars from the leaves due to the boron-facilitated translocation. Sugar supplied

to the leaves of cuttings, rather than to the rooting solution, however, would not enhance rooting in the water treatment if boron were necessary for its translocation. This picture oversimplifies in that the ubiquitous occurrence of microorganism contaminants in the sugar rooting solutions is disregarded, and also the taking-up through the leaf epidermis of sugar painted on the leaves is assumed.

The following rooting trial exemplifies such a study. Half of 48 cuttings from 18-day-old, soil-grown bean cuttings were rooted for seven days in either water, two per cent sucrose, or in water plus daily foliar application of two per cent sucrose. The other half of the cuttings were rooted in the same treatments with the addition of 0.5 ppm boric acid to the rooting solutions. There were eight cuttings in each of the six treatments. Table 13 shows that sucrose, either painted on the leaves or added to the rooting solution, failed to stimulate rooting.

In other studies sucrose had no effect on rooting when supplied to the cutting by immersing one of the cordate leaves in a vial of one per cent sucrose.

Table 13. The effect of sucrose on the rooting of cuttings in water or 0.5 ppm boric acid (8 cuttings per treatment).

Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)
Water	4.0	2.5
Water Plus 2% Sucrose	0.0	0.0
Water Plus 2% Sucrose on Leaves	1.4	0.7
Boron	24.1	50.8
Boron Plus 2% Sucrose	25.9	46.2
Boron Plus 2% Sucrose on Leaves	0.0	0.0
$LSD_{.05}$	5.9	24.8

The implications to be drawn from these results, although inconclusive, are that either boron does not enhance sugar translocation, as suggested by Gauch and Dugger, or that sugar was not a factor limiting rooting under these conditions.

The next experimental approach to this hypothesis, that of supplying the leaves of cuttings with C^{14}-sucrose, helps clarify which of the possible suggestions in the preceeding paragraph is correct.

U-C^{14}-sucrose study.

The U-C^{14}-study was designed to evaluate the uptake and distribution of sucrose or its hydrolysis products in water-, boron-, and aluminum-rooted cuttings. As described in the Methods section, the leaves of a single cutting rooted in each of the solutions were supplied with U-C^{14}-sucrose after seven days of rooting. Nine additional cuttings in each treatment, which were not treated with U-C^{14}-sucrose, were allowed to root three more days (a total of 10 days) before the usual rooting notes were taken. Boron analyses were run on the cordate leaves plus petioles and on trifoliolate leaves plus petioles. The data for rooting and boron analyses are summarized in Table 14.

Table 14. Rooting of cuttings and boron content of cuttings rooted in either water, 10^{-5}M boric acid, or 10^{-5}M aluminum chloride (8 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)	Boron in Cordate Leaves (ppm)	Boron in Trifoliolate Leaf (ppm)
Water	13.2	98.2	32.0	14.3
Boron	32.2	392.7	210.0	178.0
Aluminum	15.6	102.9	30.3	15.3
$LSD_{.05}$	10.7	807.2		

It is apparent that boron enhanced rooting while water and aluminum did not.

In the cuttings treated with U-C^{14}-sucrose the speed of movement of radioactivity from the leaves to the rooted hypocotyl was studied by sampling roots from cuttings in each of the three treatments at timed invervals. Figures 10, 11, and 12 show radioautographs of the roots sampled from cuttings rooted in water, boron, or aluminum, respectively. Time of sampling and distance in centimeters on the cutting from the sampled root to the cordate leaf blade are written below each root radioautograph on the X-ray film in each case. The lack of an image on the film at the earlier sampling times indicates that radioactivity was not yet present. The radioautographs show that the radioactivity supplied to the leaves as U-C^{14}-sucrose moved to the roots more rapidly in water-rooted cuttings than in those rooted in boron or aluminum. For example, activity was present in the sampled root in measurable quantity after six hours in the cutting rooted in water, but not until 14½ hours in those rooted in boron or aluminum. This indicated that the rate of translocation of sucrose, or its hydrolysis products, was slowed down by both aluminum and boron rather than accelerated.

Radioautographs of intact cuttings 72 hours after treatment provide a comparative picture of the quantity and distribution of radioactivity in different parts of the cuttings. Figures 13, 14, and 15 show prints from the radioautographs of water-, boron-, and aluminum-rooted cuttings, respectively. The dark areas in the figures indicate the presence of radioactive material.

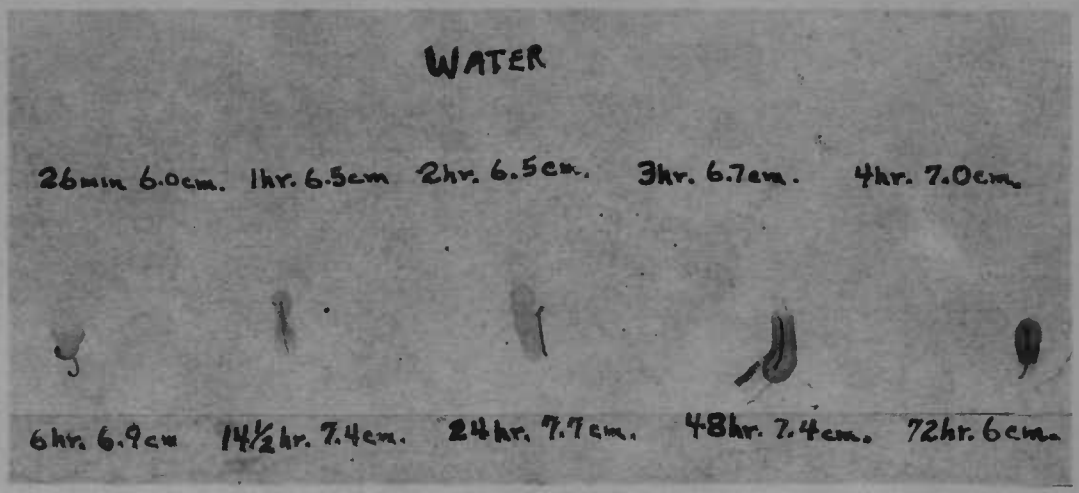


Figure 10. Radioautograph of root samples from water-rooted cutting.

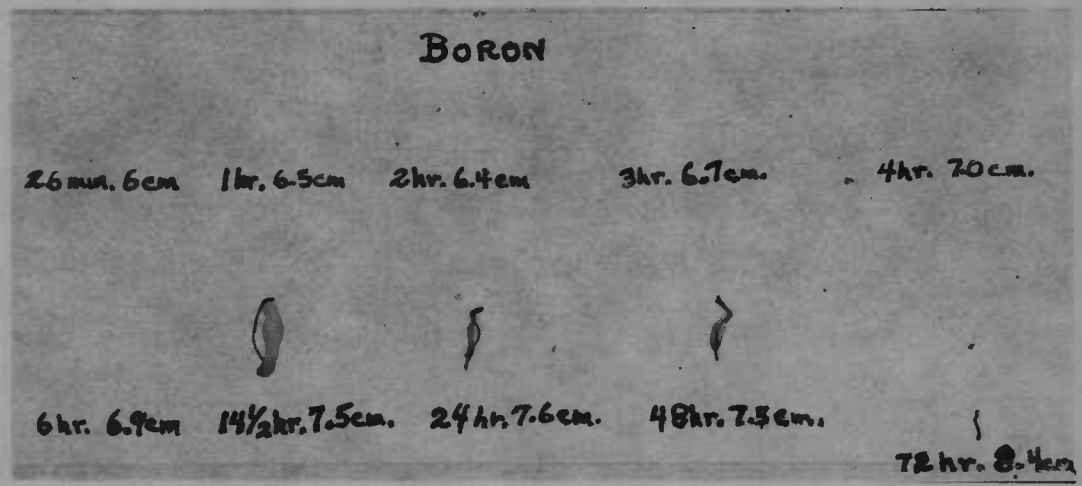


Figure 11. Radioautograph of root samples from boron-rooted cutting.

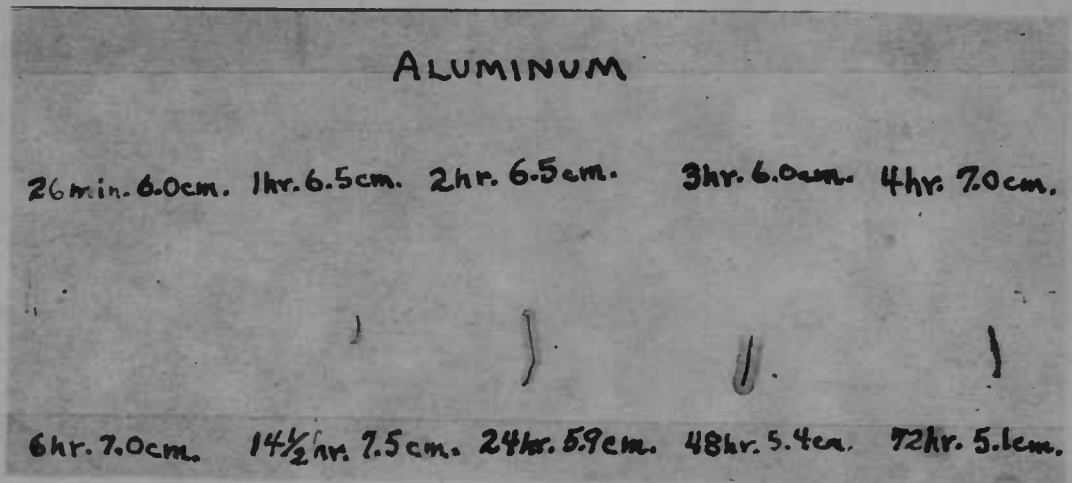


Figure 12. Radioautograph of root samples from aluminum-rooted cutting.

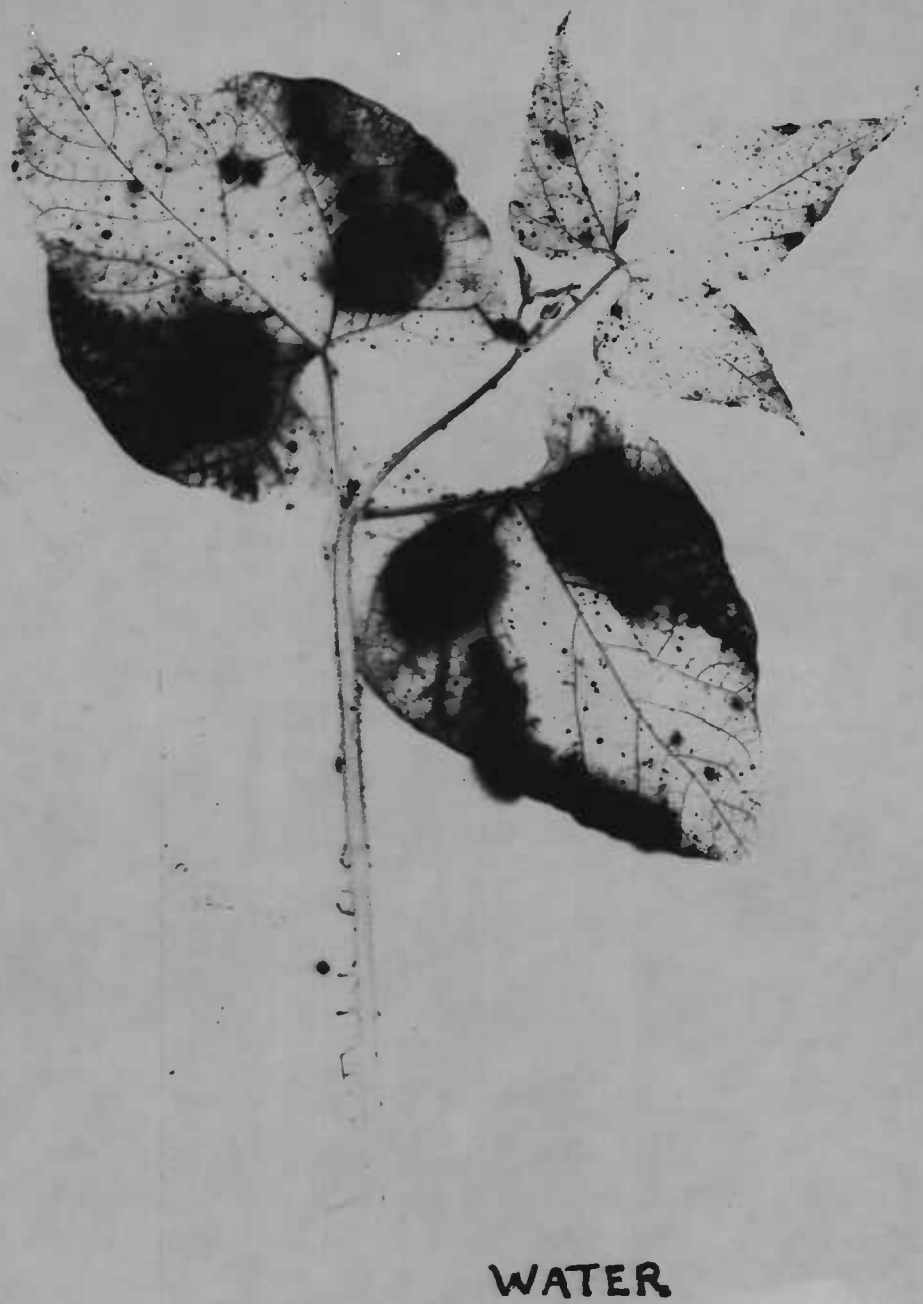


Figure 13. Radioautograph of water-rooted bean cutting 72 hours after application of U-C^{14} sucrose to the leaves.

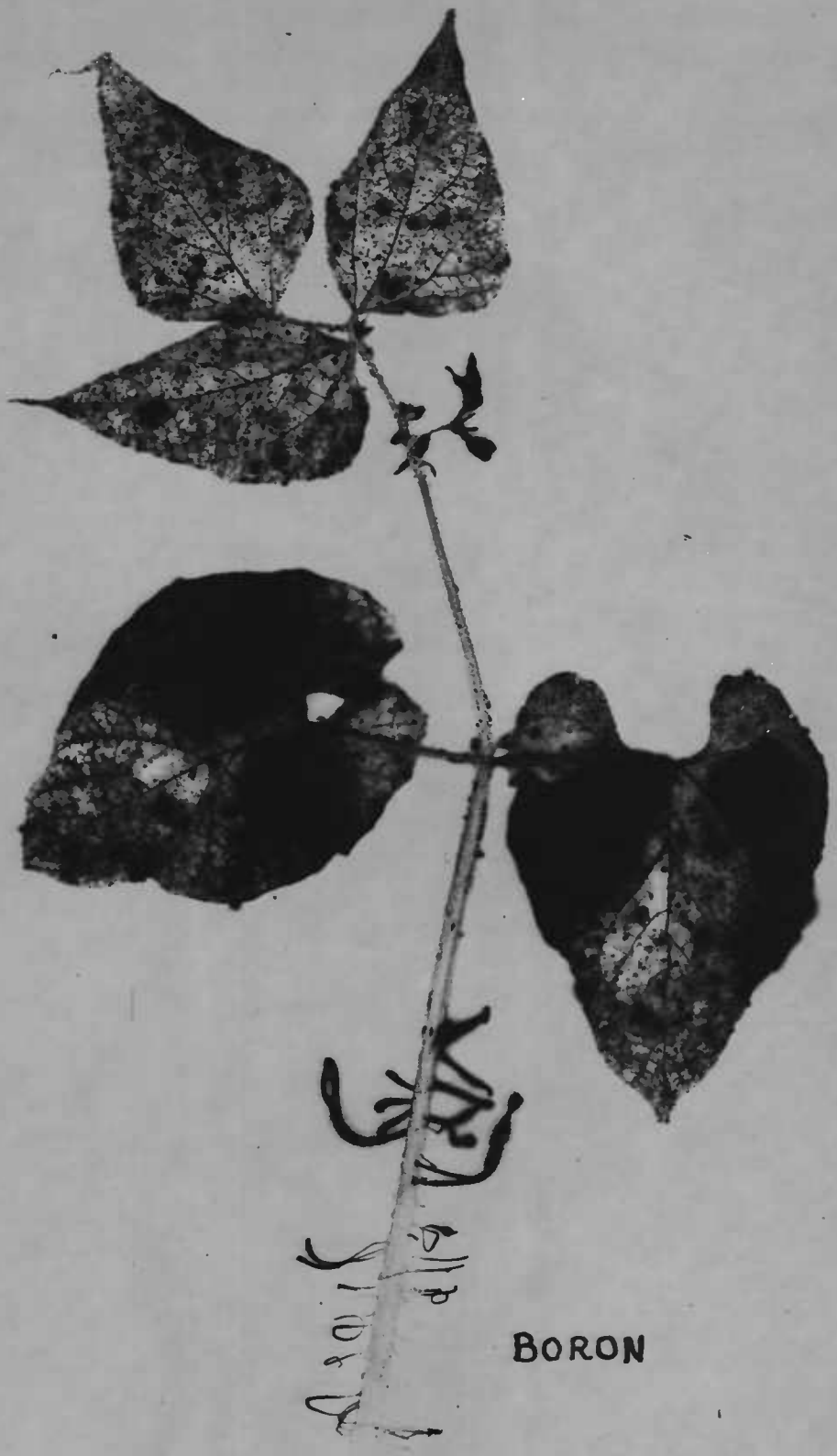


Figure 14. Radioautograph of boron-rooted bean cutting 72 hours after application of U-C^{14} sucrose to the leaves.

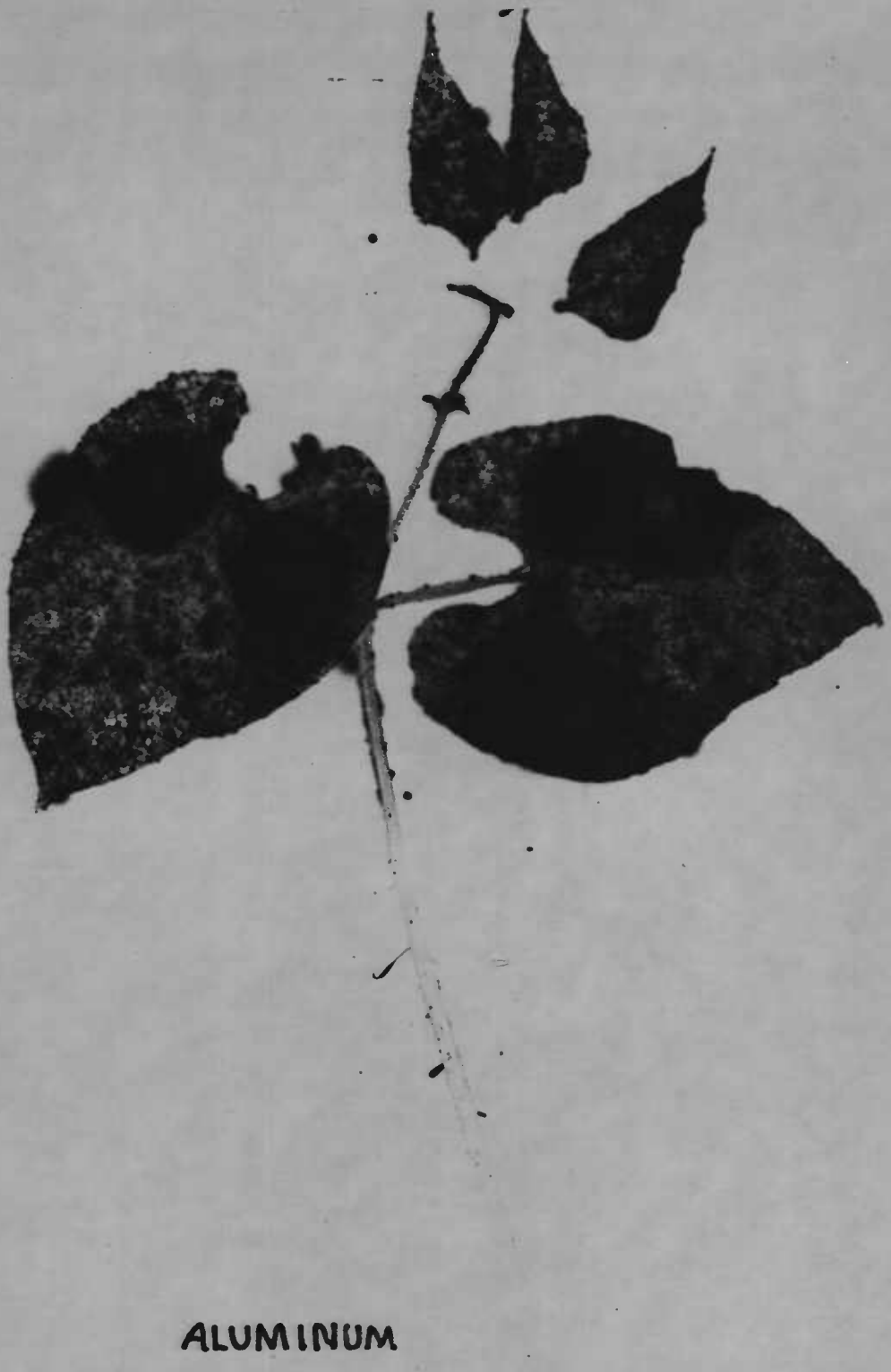


Figure 15. Radioautograph of aluminum-rooted bean cutting 72 hours after application of U-C^{14} sucrose to the leaves.

Radioautography is a poor way to estimate the amount of radioactive material present because the relation between the quantity of radioactive material present and the density of the exposed film is logarithmic. For example, a 10X increase in the amount of radioactivity would cause only a 2X increase in the density of the exposed film. In spite of this limitation, the radioautographs of the cuttings in Figures 14, 15, and 16 show that boron caused a profound and unmistakable increase in the amount of radioactive material taken up by the leaves. These results support the view of Gauch and Dugger that boron facilitates the uptake and movement of sugar or its hydrolysis products in plants when C^{14}-sucrose is applied to the leaves. It is interesting to note, however, that aluminum, which neither enhances rooting nor is an essential element, stimulated the uptake and distribution of C^{14}-sucrose in the foliage even more so than boron. It would appear, then, that both boron and aluminum enhanced sucrose uptake, or perhaps, both uptake and translocation of sucrose or its hydrolysis products were enhanced, when sucrose was applied to the leaves. Because aluminum was more effective than boron in enhancing sugar uptake, and yet did not stimulate rooting, it appears that enhanced sugar uptake and translocation cannot account for stimulated rooting with boron. A weakness in this study is the impossibility of telling whether boron and aluminum enhanced only foliar uptake of externally applied sugar, which would be of no consequence in nature, or whether they were involved in the more basic

phenomenon of sugar translocation as well. The study was also limited to the comparison of quantitative differences in uptake of radioactive sucrose, which obviously was not responsible for the boron stimulus.

The $C^{14}O_2$ feeding experiment was designed to circumvent both of these weaknesses. To summarize the situation before proceeding to this new topic, however, it can be stated that: a) Sugar added to the rooting solution or applied to leaves did not enhance rooting. This lack of response does not conclusively prove that boron does not enhance rooting by facilitating sugar translocation, because it is not known whether sugar painted on the leaves was actually taken up by the cuttings or whether the presence of microorganisms in all rooting solutions containing sugar nullified its possible beneficial effects. b) Uptake of sugar by leaves was found to be enhanced in both boron-and aluminum-rooted cuttings. It would appear, therefore, that boron did affect sugar movement, but apparently this is not the key to its effect on rooting. It is not possible to state at this point whether boron enhanced foliar uptake of applied sucrose only or translocation, as Gauch and Dugger have concluded.

$C^{14}O_2$ feeding experiment.

As mentioned, the $C^{14}O_2$ feeding experiment was designed to answer the questions: Does boron affect only sugar uptake by leaves and not sugar translocation? What quantitative and qualitative

effects does boron have on the movement of photosynthate or other respiratory intermediates from the leaves to the hypocotyls of cuttings? Answers to these questions will allow an evaluation of Gauch and Dugger's theory of boron enhanced sugar translocation, and also point to the possible role of boron in rooting.

The experimental procedure, described in detail in the Methods section, can be divided into several phases: a) The rooting trial to observe the influence of water, boron, and aluminum on rooting, b) radioautography of whole cuttings six hours after $C^{14}O_2$ feeding to see if photosynthetic CO_2 fixation was the same in all treatments, c) radioautography of whole cuttings 16 hours after $C^{14}O_2$ feeding to see if the pattern of distribution of photosynthate was comparable in all treatments, d) Sohxlet extraction and scintillation counting of extracts from fractionated cuttings in each treatment after 16 hours to obtain a qualitative measure of the distribution of radioactive carbon in the various parts of the cutting, and e) chromatography of the Soxhelet extracts of leaves and hypocotyls to obtain a qualitative picture of the 80 per cent ethanol soluble compounds, and their distribution, in the cuttings. These five phases of the study will be discussed in the order listed.

a. Rooting trial.

To study the rooting of cuttings used in the $C^{14}O_2$ study, seven soil-grown cuttings were rooted in each of the three treatments: water, 10^{-5}M boric acid or 10^{-5}M aluminum chloride, as

described in the Methods chapter. Three cuttings from each treatment were selected for exposure to $C^{14}O_2$ and the remaining four cuttings were rooted for a total of nine days to compare the rooting in the three treatments. Table 15 summarizes the rooting results and the boron analyses of the cordate leaves at the end of the rooting period.

Table 15. Rooting of cuttings and boron content of cuttings rooted in either water, 10^{-5}M boric acid, or 10^{-5}M aluminum chloride (4 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)	Boron Content of Cordate Leaves (ppm)
Water	0.0	0.0	38
Boron	51.2	197.6	184
Aluminum	0.0	0.0	41
$LSD_{.05}$	30.8	118.4	

The stimulatory effects of boron were obvious in that the boron-rooted cuttings were well-rooted, but no roots were present on cuttings rooted in water or aluminum.

b. Radioautographs after six hours of $C^{14}O_2$ fixation.

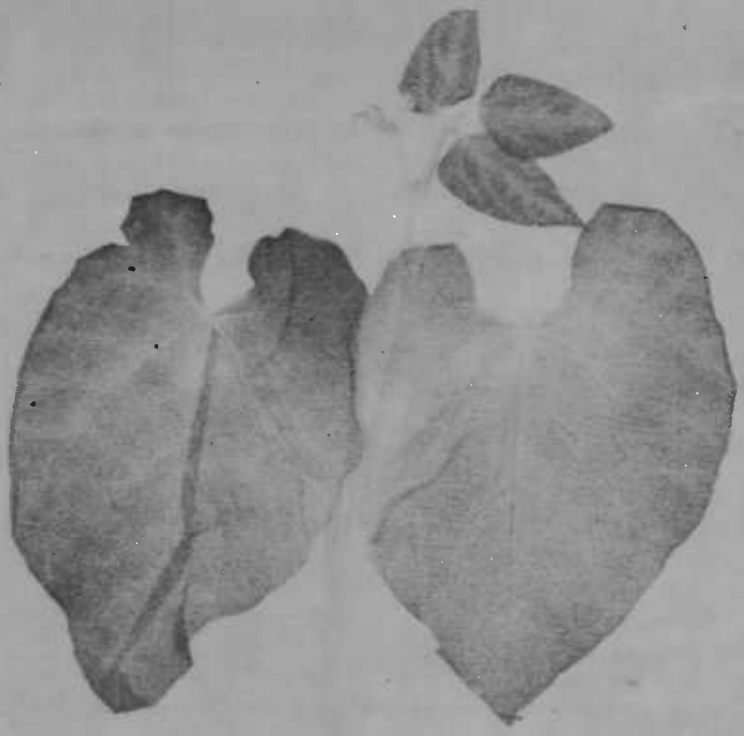
After three hours of exposure to $C^{14}O_2$, plus an additional three hours, one cutting from each treatment was killed and radioautographed to compare the amounts and distribution of radioactive

photosynthate in cuttings from the three rooting treatments. Figures 16, 17, and 18 which follow show radioautographs of the cuttings rooted in water, boron, and aluminum, respectively, six hours after the beginning of exposure to $C^{14}O_2$. There appears to be little difference at this stage in the amount or the distribution of radioactive photosynthate. Most of the radioactive photosynthate is in the leaves with a limited amount in the hypocotyls. The film was exposed to the cuttings for five minutes.

c. Radioautographs after 16 hours of $C^{14}O_2$ fixation.

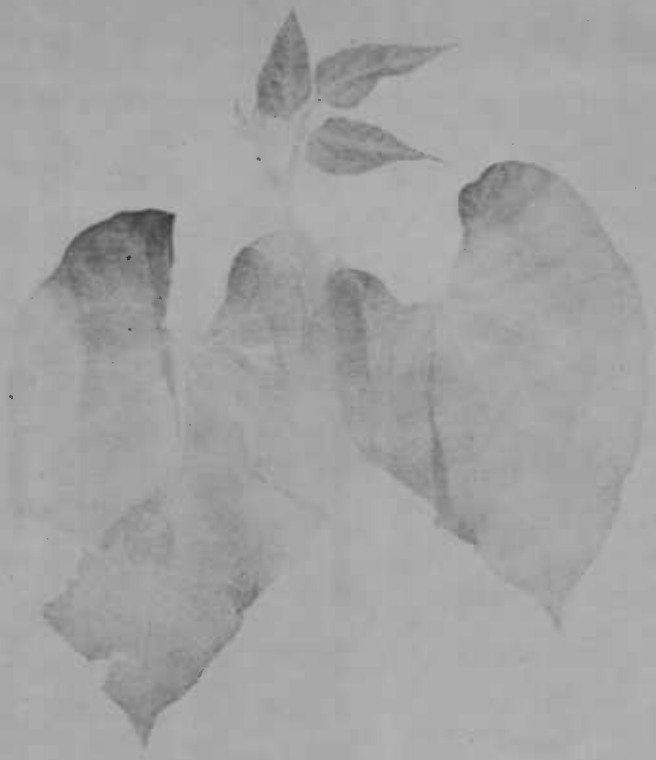
Sixteen hours after the start of the $C^{14}O_2$ liberation another cutting from each treatment was killed and radioautographed to compare the translocation of photosynthate in cuttings from the three rooting treatments. Figures 19, 20, and 21 show radioautographs of cuttings rooted in water, boron, and aluminum, respectively. The cuttings were exposed to the film 10 minutes.

A comparison of these radioautographs with those made after six hours shows that some of the photosynthate from the cordate leaves had moved into the hypocotyls and into the younger trifoliolate leaves during the 10 hours. The hypocotyl of the boron-rooted cutting appeared to contain slightly more radioactivity than those rooted in water or aluminum. The quantitative analysis by scintillation counting, to be discussed shortly, bore out this observation. There was no doubt, however, that the magnitude of the treatment



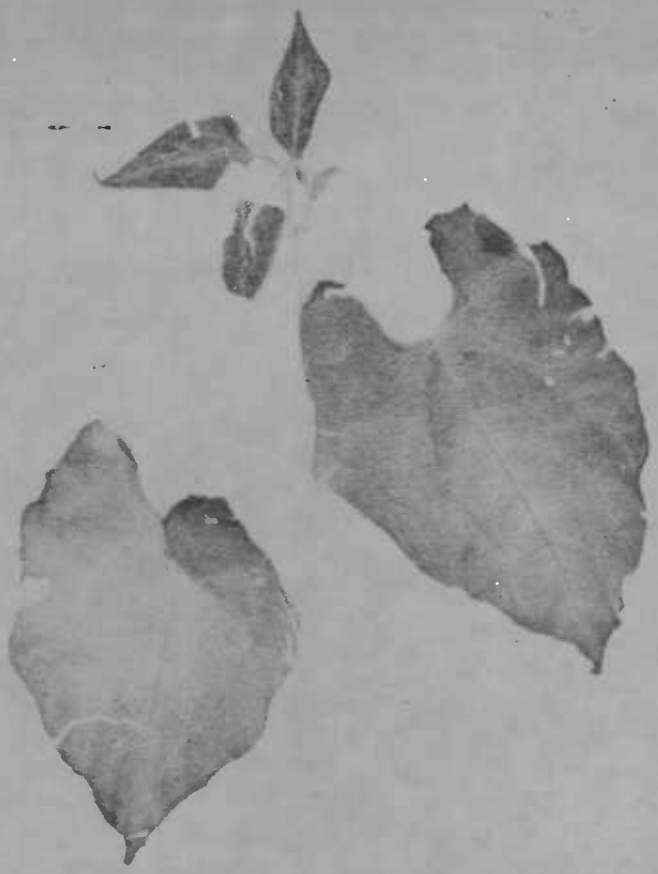
WATER

Figure 16. Radioautograph of water-rooted bean cutting six hours after exposure to $C^{14}O_2$.



BORON

Figure 17. Radioautograph of boron-rooted bean cutting six hours after exposure to $C^{14}O_2$.



ALUMINUM

Figure 18. Radioautograph of aluminum-rooted bean cutting six hours after exposure to $C^{14}O_2$.

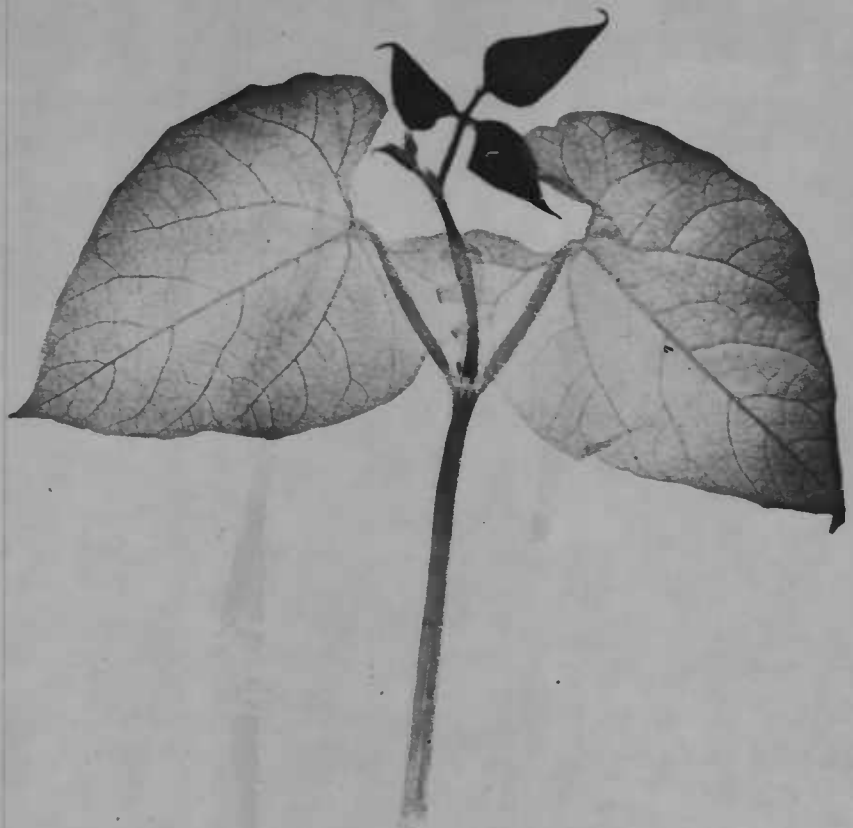


Figure 19. Radioautograph of water-rooted bean cutting 16 hours after exposure to $C^{14}O_2$.

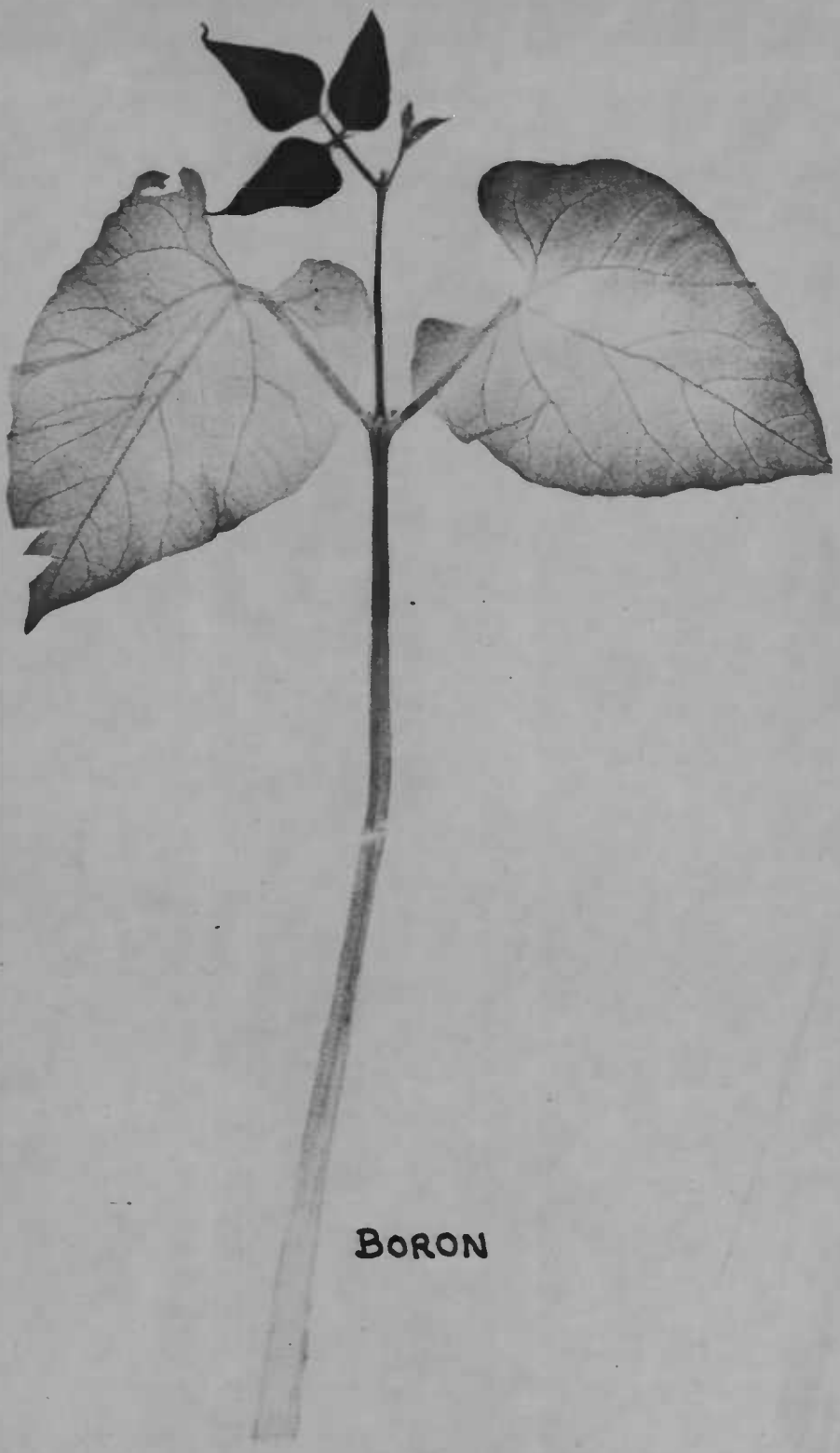


Figure 20. Radioautograph of boron-rooted bean cutting 16 hours after exposure to $C^{14}O_2$.

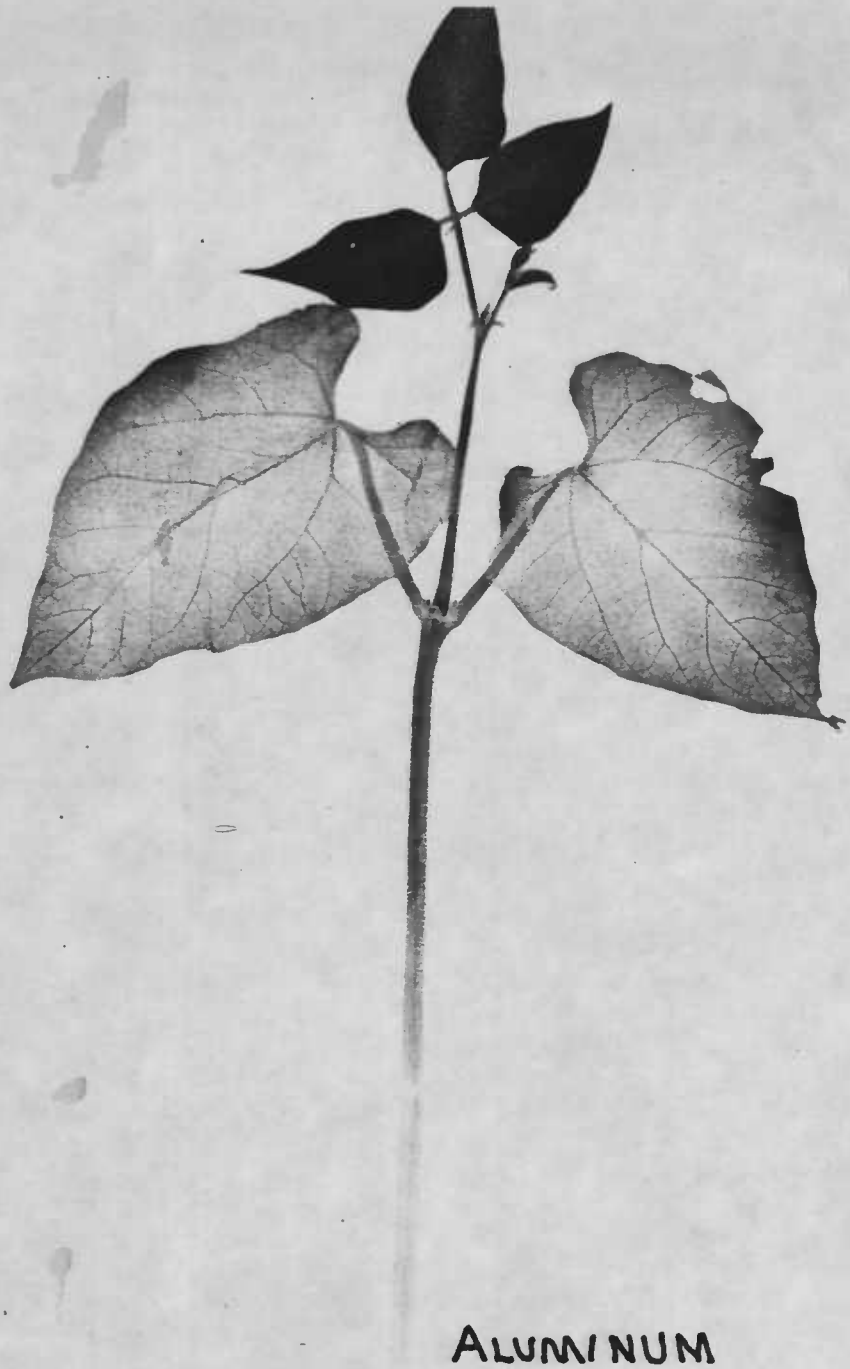


Figure 21. Radioautograph of aluminum-rooted bean cutting 16 hours after exposure to $C^{14}O_2$.

effects on distribution of radioactivity in this study was much less than in the previous U-C^{14}-sucrose study (Figures 13, 14, and 15), or in the comparable study by Gauch and Dugger (13, vol. 28, p. 457-466). It would appear, therefore, that the striking enhancement of sugar "translocation" described by Gauch and Dugger, and suggested by the preceeding U-C^{14}-sucrose study, was not enhanced translocation at all, but rather enhanced foliar uptake of sucrose. This phenomenon would be of little significance under normal conditions and would not explain the boron rooting stimulus.

This surprising evidence suggesting that boron facilitates uptake of sugar by leaves verifies the work of Nelson and Gorham (28, vol. 35, p. 339-347), who found that boron enhanced the entry of sucrose or glucose into cells of non-wounded leaves. Although high pH is known to favor the formation of sugar-borate complexes, these workers found that pH over the range of 4.2 to 8.4 had no effect on uptake. They concluded that their evidence discounted the theory that formation of a sugar-borate complex is necessary for entry of sugar into cells.

d. Sohxlet extraction and scintillation counting of extracts.

The objective of this study was to compare the relative amounts of activity in various fractions of the cutting.

While the second set of radioautographs were made, 16 hours after the start of exposure of cuttings to $C^{14}O_2$, another cutting

from each treatment was cut into various fractions such as cordate leaves, hypocotyls, epicotyls, trifoliolate leaves, and petioles. These sections were weighed, blended in 90 per cent ethanol, and extracted in 80 per cent ethanol by Sohxlet extraction. After combining and concentrating extracts from the same fraction of the same cutting to near-dryness, the dry residue from the cordate leaves and hypocotyls was redissolved in successive 25 milliliter volumes of absolute ethanol, petroleum ether, and water. Equal aliquots from each of these solvents were counted in a liquid scintillation spectrometer. Tables 16 and 17 show the net counting rate per 0.2 milliliter aliquot of the extracts in five milliliters of 95 per cent ethanol and 10 milliliters of phosphor solution. The net counting rate was the observed counting rate less the background counting rate. Inasmuch as the objective was to compare the relative amounts of activity in each fraction, the absolute total radioactivity was not calculated. No radioactivity was found in the petroleum ether soluble fractions so they are not included in the tables. All fractions were counted to a minimum total count of 10,000, giving a standard deviation of no more than one per cent.

Table 16. Net counting rate 16 hours after the start of exposure to $C^{14}O_2$ of the absolute ethanol and water soluble fractions from the cordate leaves of cuttings rooted in water, 10^{-5}M boric acid, or 10^{-5}M aluminum chloride, (counts based on 1 g. fresh weight of leaves).

	Net Counting Rate		
Cordate Leaves	Water-Rooted Cutting (cpm)	Boron-Rooted Cutting (cpm)	Aluminum-Rooted Cutting (cpm)
Ethanol Soluble Fraction	38,482	39,116	42,260
Water Soluble Fraction	6,350	3,046	7,516
Totals	44,832	42,162	49,776

Table 17. Net counting rate 16 hours after the start of exposure to $C^{14}O_2$ of the absolute ethanol and water soluble fractions from the hypocotyls of cuttings rooted in 10^{-5}M boric acid, or 10^{-5}M aluminum chloride, (counts based on 1 g. fresh weight of hypocotyl).

	Net Counting Rate		
Hypocotyls	Water-Rooted Cutting (cpm)	Boron-Rooted Cutting (cpm)	Aluminum-Rooted Cutting (cpm)
Ethanol Soluble Fraction	19,825	27,158	26,564
Water Soluble Fraction	11,243	14,182	9,405
Totals	31,068	41,340	35,969

When the absolute ethanol and water extracts recorded in Tables 16 and 17 were chromatographed it was found that several of the same compounds were present in both fractions. For this reason the total count rates in Tables 16 and 17 indicated better the treatment effects than do the net counting rates for the two solvent fractions. A comparison of the total count rates indicated one basic difference, which is revealed by the ratio $\frac{\text{counting rate in leaves}}{\text{counting rate in hypocotyls}}$ for the three treatments. For the water-rooted cutting this ratio amounted to 1.44, for the boron-rooted cutting 1.02, and for the aluminum-rooted cutting 1.38. The ratios indicate that a relatively greater proportion of the total radioactivity was present in the hypocotyls of the boron-rooted cutting than in those rooted in water or aluminum. This difference was observed in the radioautographs of the cuttings after 16 hours (Figures 19, 20, and 21), but the magnitude of the difference in neither case approached that reported by Gauch and Dugger (13, vol. 28, p. 457-466) or observed in the U-C^{14}-sucrose study (Figures 13, 14, and 15). It is unlikely that such a relatively small increase in the amount of radioactivity in the hypocotyl of the boron-rooted cutting could account for the striking effects of boron on rooting unless the increase in radioactivity reflected the movement of a compound specifically capable of enhancing rooting. The chromatographic study to follow explored this possibility.

e. Chromatography of Sohxlet extracts from leaves and hypocotyls.

The possibility of qualitative differences among the compounds in cuttings from different rooting treatments was investigated by running two-dimensional, 14 x 17 inch chromatograms of the ethanol and water soluble extracts from leaves and hypocotyls. For example, the water-, boron-, and aluminum-rooted cuttings were each represented by four chromatograms, one of the water fraction and one of the ethanol fraction for both leaves and hypocotyls. The solvent system for the first dimension was 72 per cent phenol and 28 per cent distilled water by weight. The second solvent phase was a mixture of equal parts of a solution of n-butanol (1,246 milliliters) plus water (84 milliliters) and a solution of proprionic acid (620 milliliters) plus water (790 milliliters).

After the final run and following drying, the chromatograms were put in contact with X-ray film for 50 hours for radioautography. No qualitative differences were found between compounds present in the hypocotyls in the three treatments. Figure 22 shows a section of a typical chromatogram of the hypocotyl extract, in this case from the boron-rooted cutting. The part of the chromatogram which is not shown, because of the limitations of page size, did not show any spots. The same compounds were present in both the alcohol- and water-soluble extracts of the hypocotyl so the radioautographs of these two fractions are superimposed on each other in Figure 22 to give a complete picture of the labelled compounds. Almost all of

the radioactivity was present in sucrose, with glucose, fructose, and an organic acid spot evident. The sugars---sucrose, glucose, and fructose---were identified by their general position on the chromatogram, their R_f values in several one dimensional systems, and their typical staining properties with an aniline (930 milligrams), phthalic acid (1.6 grams) plus water-saturated n-butanol reagent when heated (3, p. 181).

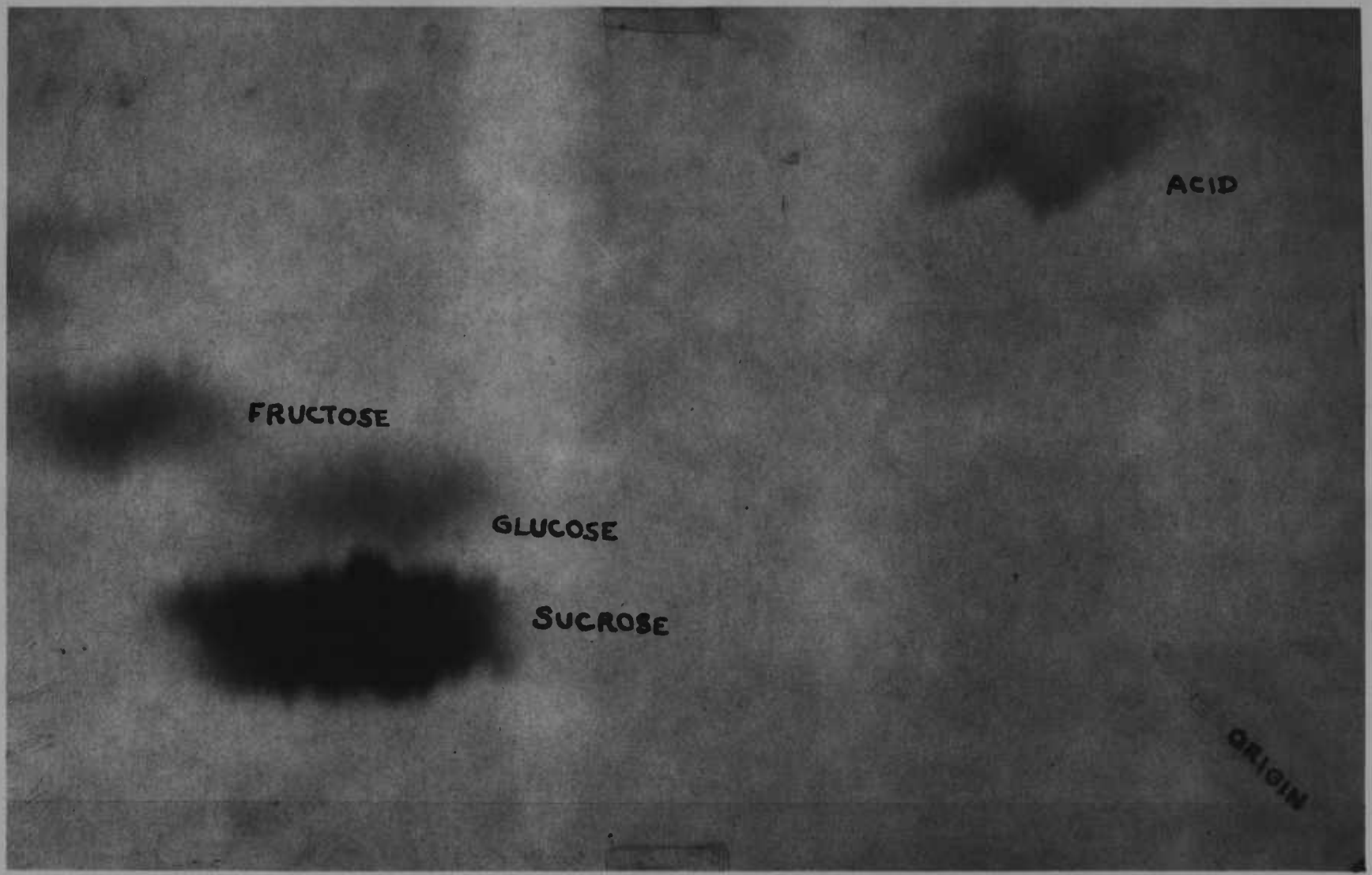


Figure 22. Chromatogram of the 80% ethanol extract from the boron-rooted bean hypocotyl. (Radioautograph)

The chromatograms of the absolute-ethanol-soluble fraction of the leaf extracts also revealed no qualitative differences between the treatments. Figure 23 shows a section of a typical chromatogram of the fraction, in this case from the aluminum-rooted cutting. Sucrose, glucose, and fructose were the only compounds present in detectable quantities.

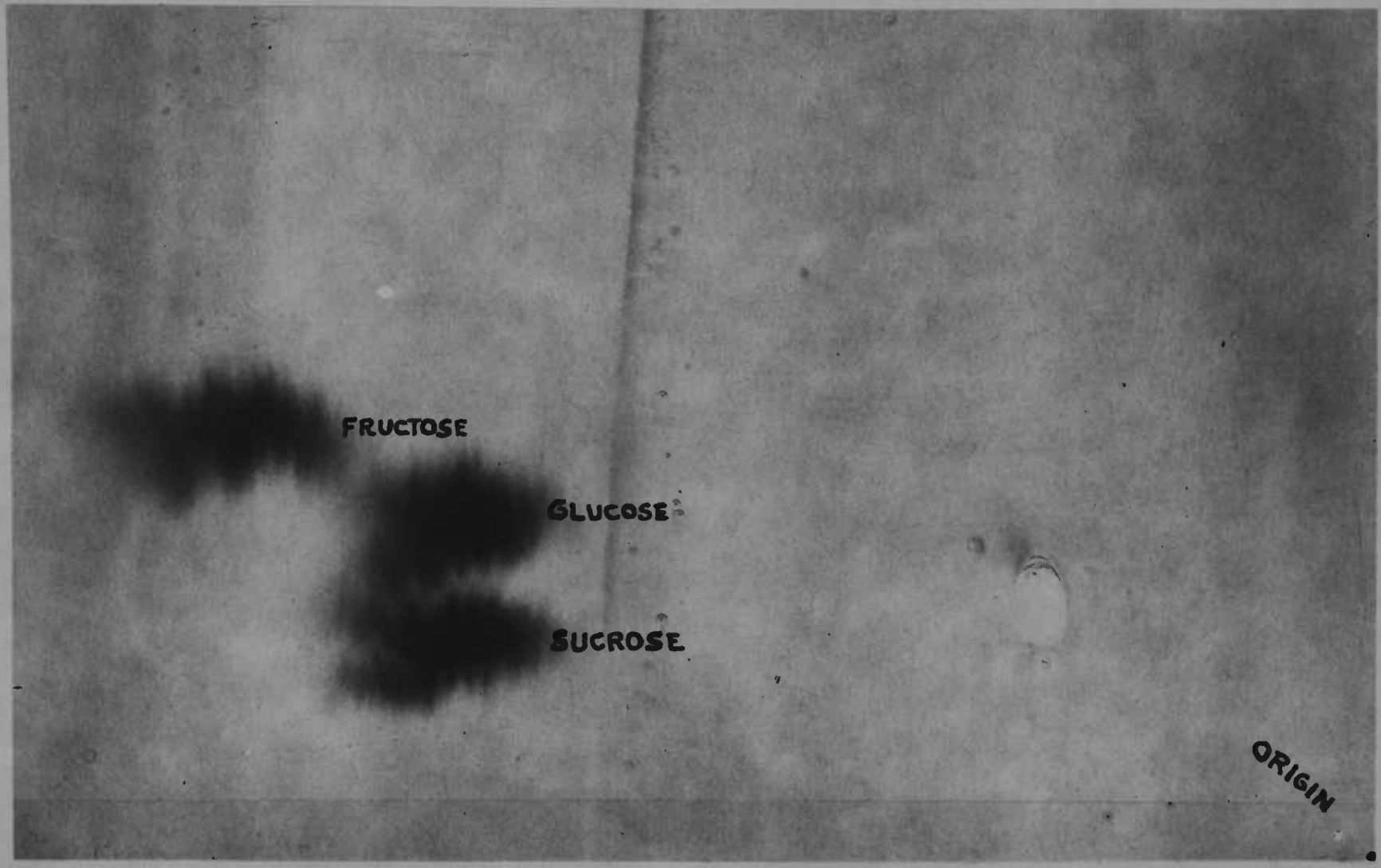


Figure 23. Chromatogram of the absolute ethanol soluble fraction of the 80% ethanol extract from leaves of the aluminum-rooted bean cutting. (Radioautograph).

Chromatograms of the water-soluble fraction from the leaves showed a striking difference between the boron-rooted cutting and those rooted in water or aluminum. Sisler, Dugger, and Gauch (36, vol. 31, p. 16) reported that there were no large differences in organic acids between boron-sufficient and deficient plants. The chromatograms, pictured in Figures 24, 25, and 26, were prepared by spotting the chromatographic paper with two milliliters of extract from the water- and aluminum-rooted cutting and three milliliters from the boron-rooted cutting. The origin was at a point corresponding to the upper right hand corner of the page.

Although more extract was used for the chromatogram of the boron-rooted cutting, the relatively large amount of organic acid in the water and aluminum treatments, (Figures 24 and 26) was not present in the boron extract (Figure 25). Sugars, especially glucose and fructose, were also less abundant. The significance of the presence or absence of the acid in the leaves of the cuttings is discussed in detail in the section concerning the postulated role of boron in oxidative processes.

On the basis of the results of the studies in this section, the hypothesis that boron stimulates rooting of cuttings by enhancing sugar translocation is rejected because: 1) a comparison of $C^{14}O_2$ and U-C^{14}-sucrose studies showed that the effect of boron on the distribution of labelled sucrose in the plant was primarily an effect on foliar uptake and not on translocation, 2) from the U-C^{14}-sucrose

trial aluminum was found to be more effective than boron in enhancing the distribution of labelling from sucrose (Figures 14 and 15), but had no effect on rooting (Table 14), and 3) it is unlikely that the small quantitative increase in the movement of labelled photosynthate from the leaves to the hypocotyl of the boron-rooted cutting (Figures 19, 20, and 21 and Tables 16 and 17) could account for the pronounced effects of boron on rooting.

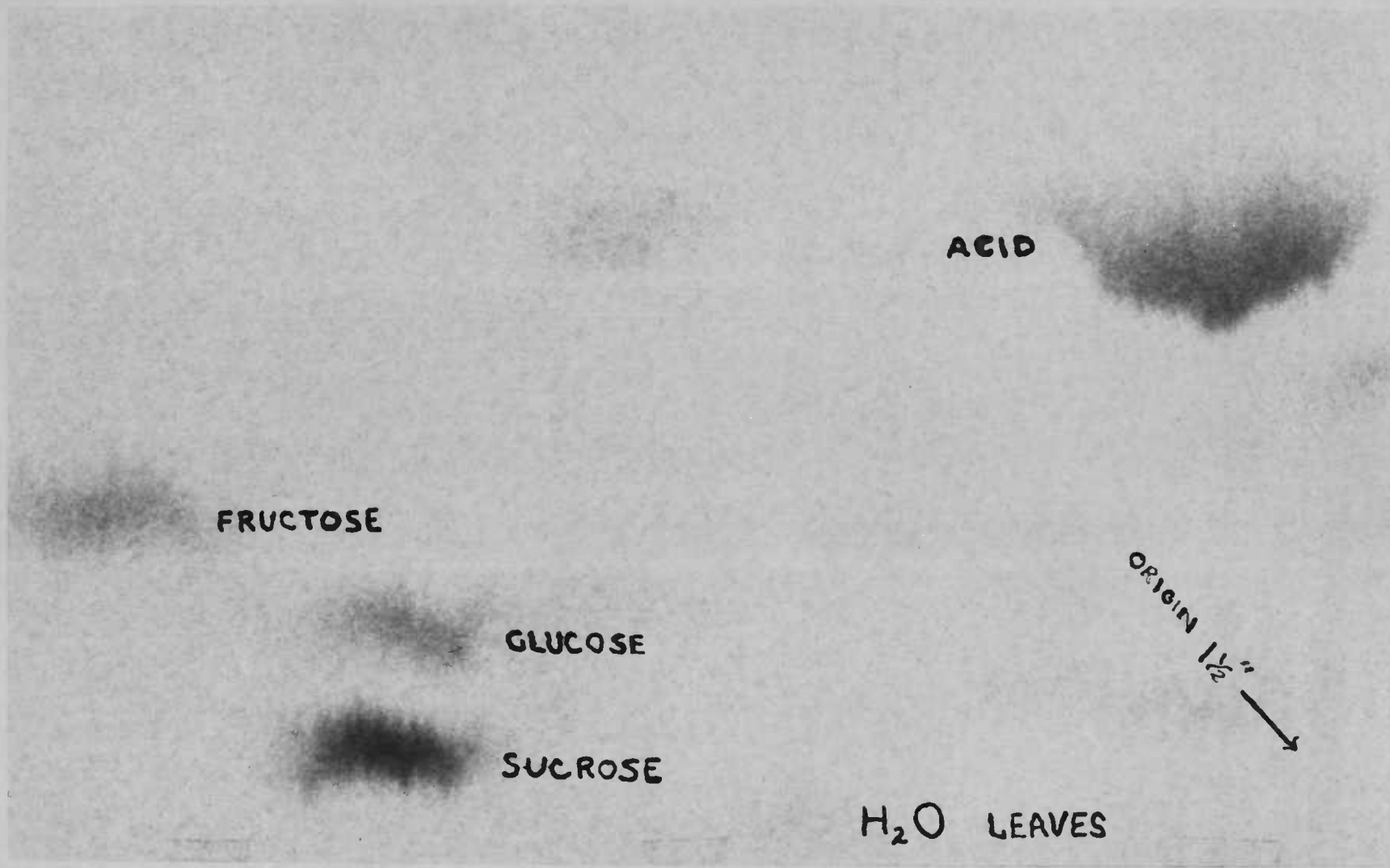


Figure 24. Chromatogram of the water soluble fraction of the 80% ethanol extract from leaves of the water-rooted bean cutting. (Radioautograph).

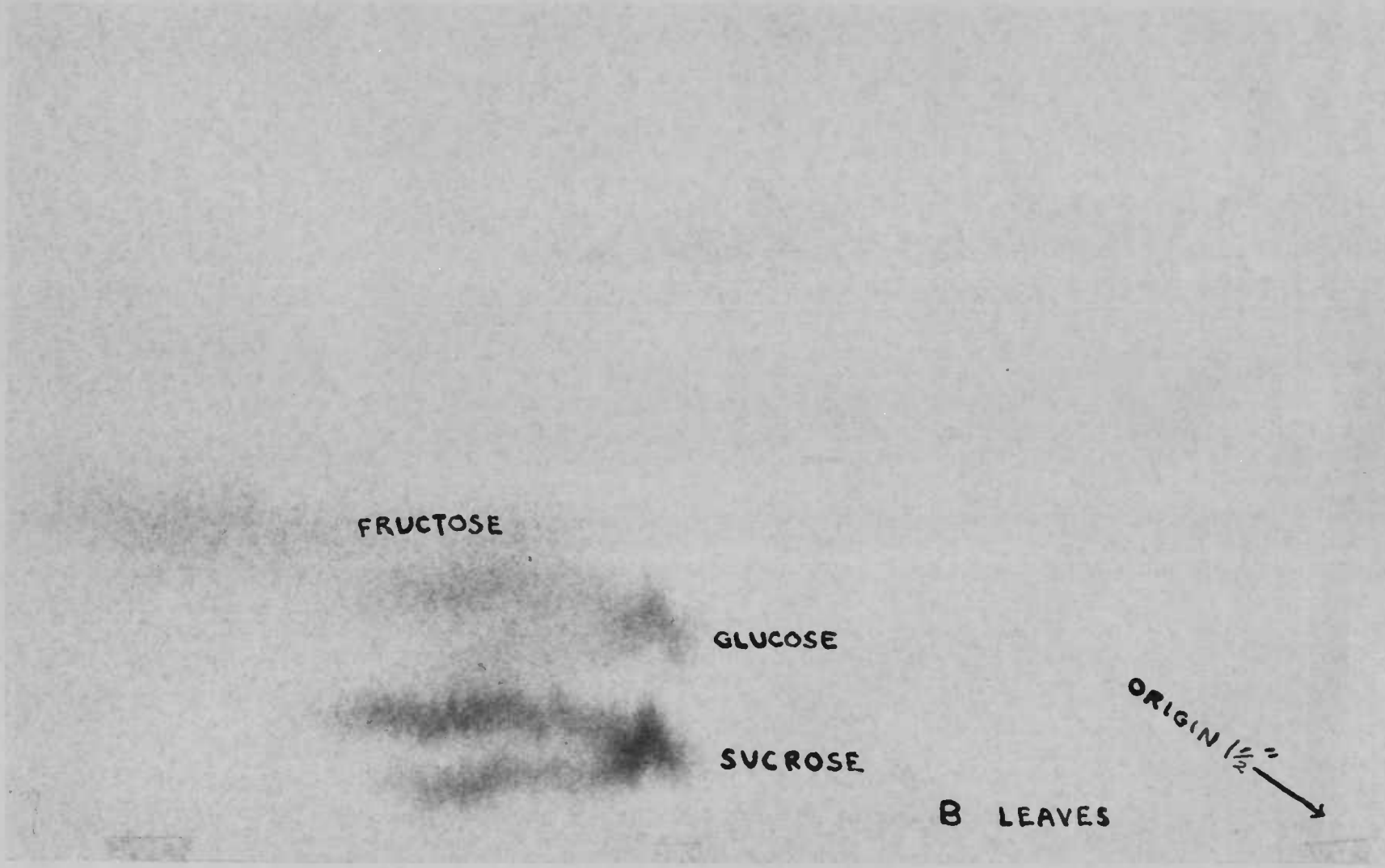


Figure 25. Chromatogram of the water soluble fraction of the 80% ethanol extract from leaves of the boron-rooted bean cutting. (Radioautograph).

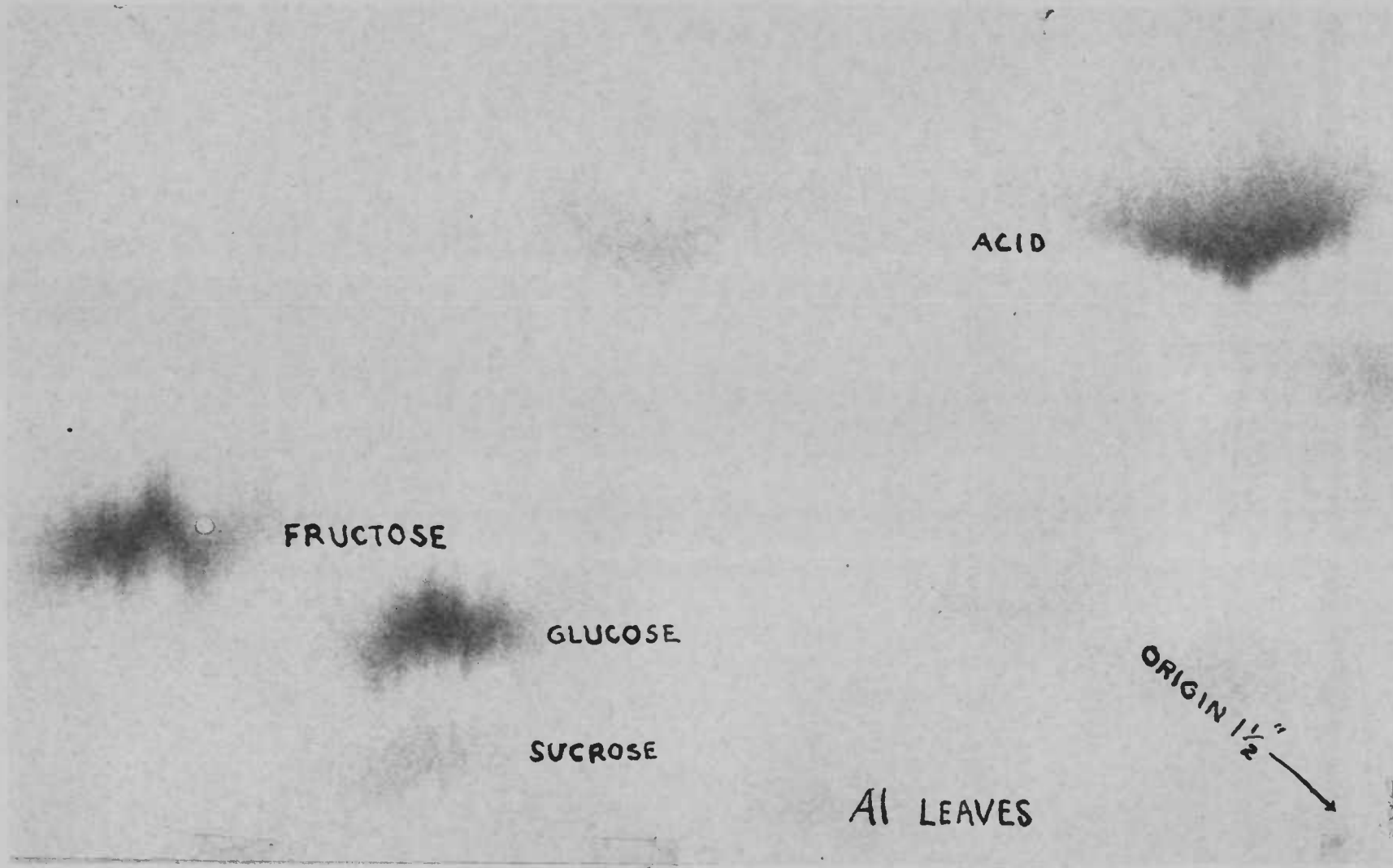


Figure 26. Chromatogram of the water soluble fraction of the 80% ethanol extract from leaves of the aluminum-rooted bean cutting. (Radioautograph).

Third Hypothesis: Boron Stimulates the Rooting of Cuttings by Influencing Cell Wall Metabolism.

As discussed earlier, Torssell (42, vol. 9, p. 652-664) found that such organic boron compounds as the arylboric acids, in concentrations between 10^{-6}M and 10^{-4}M, strongly promoted growth of roots. Boric acid, on the other hand, did not stimulate root growth in the Triticum root test. Torssell's work indicated that the dihydroxyboron groups of the arylboric acids promoted root growth by enhancing cell elongation through an inhibition of cellulose "crystallization" in the cell wall. Phenylboric acid, an arylboric acid, was more effective than boric acid in preventing "crystallization", as indicated by studies of amylose retrogradation.

Consequently, phenylboric acid, the most active of the arylboric acids, was used as a rooting agent to test Torssell's hypothesis that boron stimulates rooting through an effect on cell wall metabolism. If the boron rooting stimulus were a result of the mechanism proposed by Torssell, then phenylboric acid would be more effective than boric acid in promoting rooting.

Table 18 shows the results of the rooting trial testing this hypothesis. Ten-day-old, soil-grown bean cuttings were rooted in either water, 0.1 ppm, 1.0, or 10.0 ppm phenylboric acid, or 0.5 ppm boric acid. The 10 cuttings in each treatment were rooted for nine days. The concentrations of phenylboric acid used in this trial corresponded closely to the optimum root growth range of 10^{-6} to

10^{-4}M proposed by Torssell. (10^{-6}M is 0.122 ppm, 10^{-5}M is 1.218 ppm, and 10^{-4}M is 12.182 ppm). Cuttings rooted in the two higher levels of phenylboric acid showed signs of toxicity.

Table 18. The effect of boric acid and three levels of phenylboric acid on the rooting of bean cuttings, (10 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)
Water	6.8	20.9
Phenylboric Acid (0.1 ppm)	12.8	43.8
(1.0 ppm)	10.1	38.1
(10.0 ppm)	0.0	0.0
Boric Acid (0.5 ppm)	28.8	118.3
$LSD_{.05}$	5.0	29.7

The data show that phenylboric acid was not superior to boric acid as a rooting treatment, in fact the reverse was true. These results indicate that Torssell's postulated role of organic boron compounds in cell wall metabolism does not account for the stimulatory effects of boron on rooting.

Fourth Hypothesis: Boron Stimulates the Rooting of Cuttings by Influencing Oxidative Processes.

Shkol'nik and Steklova (34) observed that flax plants which received hydrogen peroxide in the substrate grew almost as well without boron as with it. Aeration alone was less efficient than hydrogen peroxide in producing the response. These observations suggested that boron is involved in oxidative processes which in some manner supply oxygen to the roots. Indirect support was lent to this argument by the observation, noted earlier, that boron-rooted cuttings rooted along the entire length of the hypocotyl while water-rooted cuttings rooted only near the surface of the solution.

The hypothesis that the boron effects on rooting are due to an oxidative mechanism, suggested by Shkol'nik and Steklova, was tested by adding hydrogen peroxide to rooting solutions to supply oxygen. Its effects on rooting were compared with those of boron. Cuttings from 15-day-old, soil-grown bean plants were rooted in either water, 0.5 ppm boric acid, 0.5 per cent of 3 per cent hydrogen peroxide, or 0.5 ppm boric acid plus 0.5 per cent of 3 per cent hydrogen peroxide. (0.5 per cent of 3 per cent hydrogen peroxide equals 0.015 per cent or 150 ppm of actual hydrogen peroxide.)

Table 19 and Appendix Plate 3 on page 110 show that hydrogen peroxide strikingly increased the number and length of roots per

cutting. Cuttings rooted in hydrogen peroxide also had roots along the entire length of the hypocotyl in the rooting pattern typical of boron-rooted cuttings.

Table 19. Rooting of cuttings in boric acid, hydrogen peroxide, and a combination of the two, (5 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)	Average Distance From Base of Hypocotyl to Nearest Root (cm)
Water	15.8	89.8	5.4
Boric Acid	72.2	813.6	0.0
Hydrogen Peroxide	60.6	265.6	0.0
Boric Acid & Hydrogen Peroxide	83.6	584.0	0.0
$LSD_{.05}$	34.9	162.8	0.9

The ability of hydrogen peroxide to increase the number and the length of roots, and to cause a rooting pattern typical of boron-rooted cuttings, substantiated the hypothesis that boron is involved in some oxidative mechanism.

As an additional test of the hypothesis, rooting trials were conducted to compare the effects of aeration and boron on rooting. These trials indicated that limited aeration of rooting solutions caused little rooting stimulus when compared with boron. By increasing the rate of aeration, however, rooting was greatly

stimulated even in the absence of boron. As noted by Shkol'nik and Steklova, aeration was generally not as effective as hydrogen peroxide in enhancing rooting.

Table 20 summarizes the results of a rooting trial in which cuttings from 14-day-old, soil-grown bean plants were rooted for eight days in water or 0.5 ppm boric acid, or in the same two treatments plus vigorous aeration. There were 12 cuttings in each treatment. Cuttings in aerated solutions rooted along the entire length of the hypocotyl in the manner typical of boron-rooted cuttings. The length of roots was measured rather than estimated in this trial.

Table 20. The effects of boric acid and aeration on the rooting of cuttings, (12 cuttings per treatment).

Rooting Treatment	Average Number of Roots Per Cutting	Average Length of Roots Per Cutting (mm)	Average Distance From Base of Hypocotyl to Nearest Root (cm)
Water	16.0	55.8	6.03
Boric Acid	55.3	182.8	0.80
Water & Aeration	59.2	1190.7	0.00
Boric Acid & Aeration	58.4	1398.0	0.00
$LSD_{.05}$	10.3	731.1	2.55

The ability of both hydrogen peroxide and aeration to increase the number and length of roots and to cause a rooting pattern similar to that produced by boron strongly supports the hypothesis

that boron is involved in oxidative processes which enhance rooting. This hypothesis would be further strengthened if a mechanism were known by which boron could supply oxygen to the immersed hypocotyls. The possibility of an *in vitro* oxygen-supplying reaction involving boron in the rooting solution does not seem likely in view of the fact that cuttings from plants fertilized heavily with boron rooted well without additional boron in the rooting solution. (See preliminary study 8). A tentative *in vivo* oxidative mechanism is suggested by the observation that leaves of boron-rooted cuttings did not contain an organic acid which was present in relatively large amounts in leaves of both water- and aluminum-rooted cuttings. The absence of the acid in leaves of the boron-rooted cutting could indicate several things. First, the acid was not formed in the leaves of the boron-rooted cutting. Second, it was formed but for some reason disappeared rapidly because of an accelerated acid metabolism perhaps arising from the activation by boron of certain enzymes. Third, it could simply mean that boron facilitated the movement of the acid out of the leaves into the hypocotyl. If the last were true, an oxidative mechanism could be postulated involving the mobilization of oxygen-rich plant acids from the leaves to the hypocotyl. These acids, of course, would not actually supply oxygen to the hypocotyls *per se* but, compared to sugars, would require less exogenous oxygen from the rooting solution for the respiratory processes which provide energy and substrates for root development.

Work was done to identify the acid in order to help evaluate these possibilities.

Wiltshire (47, vol. 44, p. 239) found that the organic acids present in bean leaves included fumaric, succinic, malonic, glycolic, malic and citric plus isocitric. Citric plus isocitric acid are often reported together because their structures and properties are so similar that they are hard to separate. He reported citric plus isocitric and malic acid to be the major acids in bean leaves. The respiratory quotient of these three acids is 1.33 as compared to 1.00 for a simple hexose sugar. The position of the acid spot from the water- and aluminum-rooted cuttings (Figures 24 and 26) corresponded closely to citric acid on Bassham and Calvin's (2, p. 23) chromatographic map. Chromatography revealed that the spot for isocitric acid, not shown on Bassham and Calvin's map, fell at the same point as citric acid in the phenol: ammonia: water and butanol: proprionic acid: water systems used for the two-dimensional chromatograms (Figures 22, 23, 24, 25, and 26). A definite indication that the acid from the leaves was a mixture of citric and isocitric acids was obtained by co-chromatography of the leaf extracts against standards of citric and isocitric acids as well as other common plant acids including lactic, malic, oxalic, tartaric, succinic, α-keto glutaric, fumaric, cis-aconitic, pyruvic, benzoic, aspartic, glutamic, glyceric, and malonic. Good agreement was obtained between the acids in the leaf extracts and the mixed citric-isocitric

standard. None of the other acids matched the leaf acid spot. The spot produced by a citric acid standard alone matched well with the trailing half of the larger leaf acid spot, while the spot produced by an isocitric acid standard coincided with the most advanced half of the leaf acid spot. The spot produced by a mixture of the citric and isocitric standards agreed perfectly with the leaf acid spot in all of the solvent systems used. The solvent systems used were ether: acetic acid: water (13 to 3 to 1); n-butanol: proprionic acid: water, as used in the two dimensional chromatography; and tert-butyl alcohol: benzyl alcohol: isopropyl alcohol: water: 90 per cent formic acid (1:3:1:1:2%), as used by Stark, Goodban and Owens (41, vol. 23, p. 413-415). The latter system was the easiest to use because the small amount of formic acid in the solvent system was easily driven off the paper with steam, whereas the large amounts of acetic or proprionic acid in the other systems required long periods of steaming to drive the solvent acids off the paper to the point where the bromcresol green indicator could be used to detect the acid spots.

Citric and isocitric acidswere also detected in the leaves by the gradient elution method of Hulme and Woolerton (16, vol. 9, p. 150-158) described in the Methods section. Titration of an eluate containing the leaf acids and a chromatographic comparison of the acids in cordate and trifoliolate leaves revealed that citric and isocitric acids were the major acids common to both cordate and trifoliolate leaves. As reported by Wiltshire (47, vol. 44, p. 239),

malic and succinic acids were present, but, as noted earlier, standards of these acids did not match with the major leaf acid spot in question.

The identification of the major leaf acid spot on the chromatograms (Figures 24 and 26) as a mixture of the universally occurring citric and isocitric acids essentially ruled out the possibility that the absence of the acids in the leaves of the boron-rooted cutting was indicative that they were not formed there. This means that, if the observed absence of citric and isocitric acids in the leaves of the boron-rooted cutting stands the test of repetition, further study should show that these acids are either utilized rapidly in the leaves or moved out of the leaves into the hypocotyl. At present the latter possibility seems most likely because rooting experiments indicate that the boron rooting response is an oxygen-like response, and the hypothesis that boron facilitates the movement of acid to the hypocotyl could explain how boron lowers the oxygen requirement for root development.

It was found that 10 ppm citric acid added to the rooting solution or sprayed on the leaves of cuttings had little effect on rooting, but, as in the case of the sugar-rooting studies, the significance of such results are questionable because it is not known whether the microorganism contaminants used the citric acid before it was taken up by the cuttings or whether the acid, if available, was taken up in appreciable quantity by the cuttings.

Results of these studies support the hypothesis that boron enhances rooting through an influence on oxidative processes. It is hypothesized that this effect may be due to a boron enhanced mobilization of oxygen-rich citric and isocitric acids into the hypocotyls or by some other effect on the acid metabolism of the cuttings.

SUMMARY AND CONCLUSIONS

The objective of this dissertation was to elucidate the role of boron in the rooting of bean hypocotyls. It was hoped that a study of this phenomenon might contribute to an understanding of rooting, increase the useful applications of boron as a rooting agent, and contribute knowledge to the role of boron in intact plants.

Preliminary experiments were conducted to obtain background information characterizing the boron response of bean cuttings and to evaluate four hypothetical roles for boron in rooting. These four roles, developed from the literature dealing with the roles of boron in intact plants, envisioned boron being involved in either: 1) auxin metabolism, 2) sugar translocation, 3) cell wall metabolism, or 4) oxidative processes. Theoretically, each might explain the enhanced rooting of bean cuttings supplied with boron.

Preliminary studies indicated that approximately 10^{-5}M boric acid gave best rooting. Roots were distributed along the entire length of the hypocotyl on boron-rooted cuttings, but those in water rooted only on the upper hypocotyl near the surface of the rooting solution. It was thought that this might result from the lower oxygen tensions in this region. The cuttings rooted sooner with boron and had more and longer roots than water-rooted cuttings, but the cuttings from both treatments had about the same total number of root initials. This supports the view that boron stimulates root growth and not root initiation.

Shading the rooting zone enhanced rooting but did not change the response of cuttings to boron. The boron rooting response was not limited to boric acid because other forms of boron were equally effective; nor was it limited to Black Valentine bean because other cultivars responded also. It is doubtful that the lowering of pH of rooting solutions by boric acid could account for the boron rooting response because the concentrations used had little effect on the pH of the solutions. Cuttings from plants heavily fertilized with boron rooted as well in water as in boric acid rooting solutions. Soil-grown or sand plus nutrient-grown plants rooted better and responded more strikingly to boron than those grown in sand alone or in vermiculite. Vermiculite-grown plants were dwarfed and yellow and did not give reproducable rooting results. The complexing elements---aluminum, strontium, and germanium, did not enhance the rooting of cuttings from soil-grown plants. This suggests that the boron rooting stimulus is not dependent on the complexing ability of boron. Aluminum stimulated the rooting of cuttings from plants grown in a certain lot of vermiculite, but the stimulation could not be repeated on plants grown in sand or soil or even in a different lot of vermiculite. Boron did not affect the respiration of bean hypocotyl sections which were not supplied with an exogenous supply of respiratory substrate. Even if boron stimulated respiration upon the addition of a substrate, it would be difficult to interpret the results in terms of the rooting stimulus.

A rooting study showed no interaction or specific similarity between the rooting responses of bean cuttings to IAA and boron. As a consequence, the hypothesis that boron stimulates rooting through effects on auxin metabolism was rejected.

The test of the hypothesis that boron enhanced rooting by facilitating the translocation of sugar provided information on a variety of topics. It was found that sugar added to rooting solutions or painted on leaves did not enhance rooting, but these results were inconclusive because of microorganism contaminants in the rooting solutions and lack of information on the uptake of sugar by the cuttings. The application of U-C^{14}-sucrose to the leaves of water-, boron-, and aluminum-rooted cuttings showed that boron has a profound stimulatory effect on the distribution of labelling in the cutting. Aluminum, which did not stimulate rooting, had a similar and even more profound effect on distribution of labelling, however, so it was concluded that the stimulation of rooting by boron did not result from its quantitative effects on sugar translocation. Although boron stimulated the distribution of labelling in the cuttings in this study, one cannot ignore the possibility of enhanced foliar uptake of sugar, and, as Gauch and Dugger did, attribute this to enhanced sugar translocation alone. The feeding of $C^{14}O_2$ to water-, boron-, and aluminum-rooted cuttings, in fact, showed little quantitative difference in sugar translocation between the treatments, indicating that the prime effect of boron in the C^{14}-sucrose study

was on foliar uptake and not on translocation. Chromatography of 80 per cent ethanol extracts from the cuttings revealed a qualitative difference between the treatments in the water-soluble fraction of this extract of the leaves. This fraction from the water- and aluminum-rooted cuttings contained relatively large amounts of citric and isocitric acids, while that from the boron-rooted cutting contained only a trace. The possible significance of this difference is discussed in relation to the hypothesized role of boron in oxidative processes.

Phenylboric acid, purported to be more effective than boric acid in preventing the "crystallization" and stiffening of cell walls, was found to be less effective than boric acid as a rooting agent. This led to rejection of the hypothesis that boron enhances rooting by affecting cell wall metabolism according to the scheme of Torssell.

Hydrogen peroxide, a strong oxidizing agent, increased the number and length of roots on bean cuttings and caused rooting along the entire length of the hypocotyl just as did boric acid. Aeration produced the same effects and masked the influence of boron on rooting.

The similarity in the rooting response of cuttings to hydrogen peroxide, to aeration, and to boron supports the hypothesis that boron enhances rooting through effects on oxidative processes. The striking difference in the acids in cuttings as revealed by chromatography of leaf extracts provides a possible explanation for the

oxygen-like response of cuttings to boron. It is suggested that boron may lower the exogenous oxygen requirement for rooting by facilitating the movement of partially oxidized substrate (citric and isocitric acid) to the submerged hypocotyls. Such acids would require less oxygen than would sugars for the respiratory processes which provide the energy and substrate for synthesis of new cell constituents in developing roots. In this manner boron may not supply the hypocotyls with oxygen per se but rather with a substrate which can be utilized in an oxygen-deficient environment.

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APPENDIX

Plate 1. Rooting of bean hypocotyls at different levels of boric acid.
Bottom row, left to right: 0, 0.0098, 0.039, and 0.156 ppm boric acid.
Top row, left to right: 0.625, 2.5, 10, and 20 ppm boric acid.

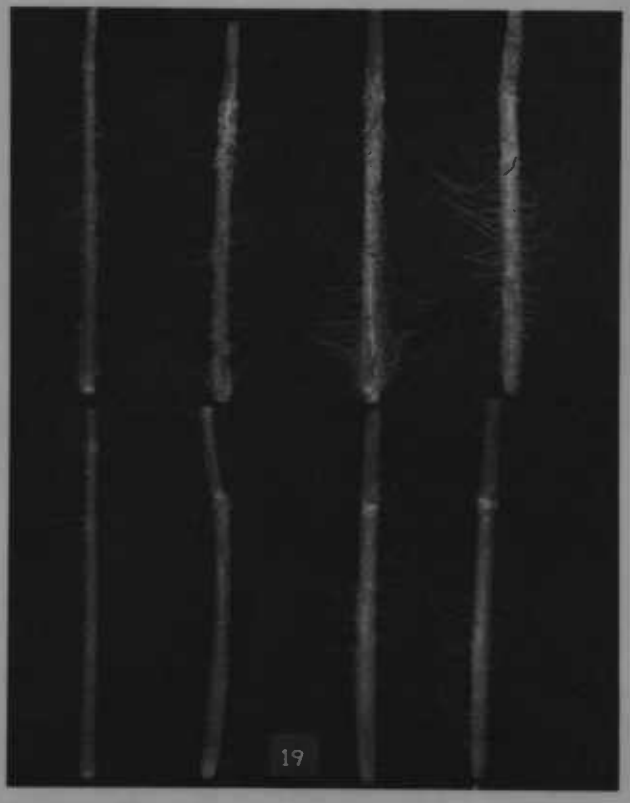

Plate 2. Rooting of bean hypocotyls at three levels of IAA and three levels of IAA plus 0.5 ppm boric acid.
Bottom row, left to right: 0.0, 0.1, 0.5, and 2.5 ppm IAA.
Top row, left to right: 0.0, 0.1, 0.5, and 2.5 ppm IAA plus 0.5 ppm boric acid in all cases.

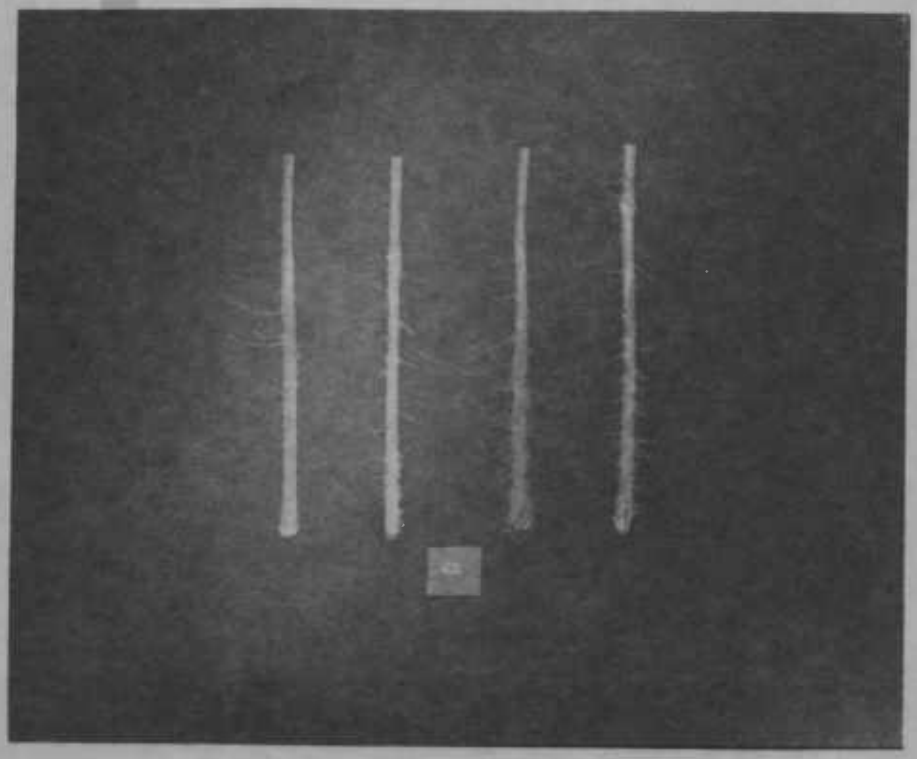

Plate 3. Rooting of bean hypocotyls as influenced by boric acid and dilute hydrogen peroxide. Cuttings left to right rooted in water, 0.5% H_2O_2 (30%), 0.5 ppm boric acid, or 0.5 ppm boric acid plus 0.5% H_2O_2 (30%).