5-1959

A Comparison of Internal Bicarbonate of Some Chlorosis-Resistant and Chlorosis-Susceptible Plants

Ralph Barlow Clark
Utah State University

A COMPARISON OF INTERNAL BICARBONATE OF SOME CHLOROSIS-RESISTANT AND CHLOROSIS-SUSCEPTIBLE PLANTS

by

Ralph Barlow Clark

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Soil Chemistry

UTAH STATE UNIVERSITY
Logan, Utah

1959

ACKNOWLEDGMENT

The author wishes to express gratitude to Doctors R. L. Smith and Herman H. Weibe for their helpful suggestions leading to the subject selection and completion of this research problem. I wish to thank Dr. Raymond W. Miller who served on the committee for his helpful suggestions. I also wish to thank my wife Lynore for her untiring efforts in proofreading and typing. Appreciation is also due the Atomic Energy Commission for providing the funds to make this research possible.

Ralph B. Clark

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

Iron chlorosis has been a srious problem for many years. This physiological disease has occurred so frequently on calcareous soils that it has been called lime-induced chlorosis by many of the workers. Because the western United States has so many soils of this type, the chlorosis problem has been of primary concern. Untold economic losses have been the result of this physiological disease.

Interveinal yellowing of the leaves along with loss of plant vigor and retarded growth characterize the disease. In severe cases die-back of the terminal growth and eventual death of the plant result. No single factor has been established as being the cause of this condition. Consequently, no permanent cures have been recommended.

Of the many theories that have been advanced as to the cause of this chlorotic condition, much emphasis has been placed upon the effect of the bicarbonate ion (HCO_3).[a] In past years many experiments have been conducted concerning plant growth in relation to HCO_3 and the results have been varied. Although the results of most of the early workers have been variable, generally a toxic effect of HCO_3 was reported. This was particularly true with high concentrations of HCO_3 in the nutrient solution. Kearney and Cameron (1902) compared the effects of bicarbonate salts with that of poisons and observed that very dilute solutions of HCO_3 stimulated growth whereas greater concentrations inhibited growth. Harley and Lindner (1945) found that

---

[a]Henceforth this will be written simply as HCO_3.

irrigating with water high in HCO_3 caused a chlorotic condition in certain varieties of pear and apple.

This chlorotic condition has brought about closer investigation of the effects of HCO_3 in its effects on plant growth. It has been noted by Wadleigh and Brown (1952), Miller (1954), and Goss (1957) that high concentrations of HCO_3 inhibited respiratory processes and cation uptake of many plants. It has also been reported by Ulrich (1941) that HCO_3 caused an increase in sap pH, a greater change in individual organic acids, and a decrease in the respiratory quotient of some plants in comparison to other salts.

Baxter and Belcher (1955) indicated that the internal environment of chlorotic citrus roots was disturbed by an accumulation of HCO_3. This seemed to effect the hydrogen ion concentration and the buffer capacity of the plant sap. Goss (1957) postulated that the HCO_3 inhibits the translocation of minerals more than it does their absorption. Honda (1955) also indicated that the internal pH increased as a result of HCO_3 influences. This resulted in decreased iron solubility and an upset of the cytochrome oxidase system which initiated chlorosis in chlorotic-resistant varieties. Carlson (1957) observed that the translocation of iron tended to decrease with an increased pH and HCO_3 of the plant sap.

These observations suggest that the build up of HCO_3 concentration internally enhances chlorotic conditions in plants. The objective of this study is to compare the internal HCO_3 concentration of plants, both those susceptible and those resistant to chlorosis.

REVIEW OF LITERATURE

General Factors Affecting Lime-induced Chlorosis

The chlorosis problem is believed to be one of iron deficiency; however, other nutritional disorders have been related to this problem. McGeorge (1948) observed that most chlorosis in Arizona occurred on soils which contained more than three percent calcium carbonate. Other workers found no relation between the percent lime in soils where the plants were green and adjacent areas where they were chlorotic (Haas, 1942, and Thorne et al., 1950). Thorne et al. (1950) also found no correlation between the severity of chlorosis and the percent lime in the soil.

Chlorosis found in pineapple in Hawaii has been attributed to a decreased ion assimilation in the plant because of high manganese in the soil (Johnson, 1917). Similar results of the relationship between iron and manganese in solution culture experiments were reported by Sommers and Shive (1942). They found that iron deficiency resulted with excess manganese and manganese deficiency resulted with excess iron. An unbalance of iron and such minor elements as copper and zinc have been reported to cause chlorosis of lemon and orange trees (Chapman et al., 1939).

It has been observed by Lindner and Harley (1944) and Iljin (1952) that chlorotic plants tend to have higher potassium concentrations than green ones. They suggest that the potassium replaces iron on enzymes responsible for chlorophyll formation. Thorne et al. (1950) and Iljin (1952) suggested that a higher potassium:calcium ratio existed in

chlorotic plants. Wadleigh and Brown (1952) and Lunt et al. (1958) showed that decreased amounts of calcium existed in the plant with an increased concentration of HCO_3. Wadleigh and Brown (1952) suggested that the higher HCO_3 treatments lowered the activity of iron.

In investigating chlorosis of wheat plants, Loehwing (1930) found the degree of chlorosis increased with an increased intensity of sunlight. He observed that wheat plants were chlorotic when grown under conditions of full sunlight, whereas similar plants grown on the same soil under shaded conditions did not produce the same degree of chlorosis. Wann (1941) found similar results with grapes.

Chlorotic and non-chlorotic plants have been found growing side by side in the same plot or orchard. Varieties as well as branches of an individual plant may vary in their susceptibility to chlorosis. Wann (1941) grafted a chlorotic variety of grape on a non-chlorotic grape rootstock and immunity to iron chlorosis resulted. Brown (1955) grafted P.I.-54619-5-1 (PI) soybean, a chlorosis-susceptible variety, on the rootstock of the Hawkeye (HA) soybean, a chlorosis-resistant variety, and found similar results.

Burgess and Pohlman (1928) showed that by keeping soil moisture near field capacity chlorosis was intensified. They demonstrated that by permitting the soil to approach the wilting point before irrigation and then applying a relatively heavy irrigation, this chlorotic condition was reduced. Thorne and Wann (1950) suggested that approximately fifty percent of the chlorosis in Utah could be prevented by controlled soil moisture. Thorne et al. (1950) observed that fine soil texture, high soil moisture, poor aeration, and cool temperatures intensified the chlorosis problem. Excessive soil moisture in calcareous soils was

paralleled by an increase in pH and with an increase in ferrous iron precipitate (Thorne and Wallace, 1944).

Thorne et al. (1950) found that the pH-aeration interaction in calcareous soils had a decided influence upon the prevalence of available iron. They observed that the leaves of deciduous trees had less acid soluble and ferrous iron and postulated that iron is taken up by the plant and translocated in the ferrous form. Lindsay and Thorne (1954) noticed that translocation of iron from roots to the aerial portions of the plant was affected by the oxygen supply to the roots. They found that plants aerated with twenty percent oxygen had less iron than those aerated with one percent. DeKock (1955) reported similar results. He found that chlorosis could be alleviated with a reduction of oxygen to the roots.

Certain plants have marked differences in their tolerance to salts. Gauch and Wadleigh (1941) found Rhoades grass tolerant to relatively high concentration of $NaHCO_3$ (12 me./l), whereas Dallis grass became extremely chlorotic and died at the same concentration of HCO_3. Wall and Cross (1943) found this same type of tolerance with carnations in comparison to chrysanthemums. Heller et al. (1940) noted that $NaHCO_3$ added to the nutrient solution used for irrigating tomatoes in sand culture experiments was more harmful than equimolar concentration of NaCl. Harley and Lindner (1945) and Wadleigh and Brown (1952) also observed differences in tolerances of plant species to HCO_3.

Function of Iron in Plants

Marcour (1952) found that HCO_3 depressed the uptake of iron and decreased the formation of chlorophyll. Lindsay (1953) found that when iron salts were applied to plants, made chlorotic due to HCO_3 in the

nutrient medium, they recovered and became healthy. Warnock (1952) found that the activity of iron was not the same in different plants and not all plants were equally efficient in chlorophyll formation. It was suggested that protochlorophyll was reduced in the presence of light to yield chlorophyll, and that iron functioned as an electron carrier. Bonner (1950), however, suggested that the function of iron might be related to the production of a chloroplastic protein rather than the formation of chlorophyll.

Brown and Hendricks (1952) showed that different plants have different enzyme systems. Brown (1953) indicated that plants appearing to have iron-mediated metabolic systems were susceptible to lime-induced chlorosis and relatively resistant to copper deficiency. Copper-mediated metabolic systems seemed to predominate in the plants not susceptible to iron chlorosis. Fritz and Beevers (1955) and Honda (1955) also substantiated that chlorosis-susceptible plant species were predominantly iron-requiring terminal oxidase enzyme systems and that chlorosis-resistant species were predominantly copper-requiring metabolic systems. Further work by Honda (1955) indicated that a copper-requiring enzyme, ascorbic oxidase, functioned at a higher pH than an iron-requiring enzyme, cytochrome oxidase. It would then suggest that a decreased solubility of iron would upset the iron-requiring enzyme, resulting in a chlorotic condition of the plant.

Unavailability and Immobilization of Iron

Many investigators have postulated that the lack of available iron in the soil has been the cause of lime-induced chlorosis. Mann (1930) found that lime added to an acid soil reduced the solubility of iron but did not affect its absorption by the plant. Chapman (1931) and Thorne and Wallace (1944) found no relationship between the occurrence

of chlorosis and water-soluble iron in a calcareous soil. Thorne and Wallace (1944) did show, however, that soils with a chlorosis problem had less reducible iron than soils where chlorosis seldom occurred. Olsen and Carlson (1949) found that extractable iron from soils producing chlorotic sorghum was less than from soils producing normal plants.

Ingalls and Shive (1931) found a direct relationship between the hydrogen-ion concentration of tissue fluids and the soluble iron content of leaves. They found that during the day the pH of the tissue fluids of stems and leaves increased. With the increased pH the total iron increased but the soluble iron decreased. Rogers and Shive (1932) reported similar results.

Milad (1924) and Marsh and Shive (1925) reported that iron in chlorotic plants was immobilized within the plant and tended to accumulate in the stems of soybeans. Warnock (1952) found similar results with beans. Chapman (1931) found that wood from chlorotic trees contained more iron than the normal and that this iron accumulated in the phloem and the xylem.

When an iron chelate was added to chlorotic orange trees, the leaves became green within a few weeks (Stewart and Leonard, 1952). In nutrient culture studies with corn and beans, Carlson (1957) found that by adding a chelate compound to the solution the translocation of foliar applied iron was enhanced in nearly all cases.

pH Effects on Chlorosis

The pH of the nutrient media has a decided influence upon the metabolism of plants. The occurrence of chlorosis at low hydrogen-ion concentrations of the growth media was observed by Franco and Loomis

(1947) and McGeorge (1951). Epstein and Stout (1951) found no relationship between the wide range of hydrogen-ion concentration and the uptake of iron by tomatoes. Arnon and Johnson (1942) and DeKock (1955) found that the pH of the nutrient solution had no significant effect on the pH of the expressed sap of roots and leaves.

Baxter and Belcher (1955) showed that the root sap of orange trees that were grown on alkaline soil had a lower hydrogen-ion concentration than the sap from tree roots grown on an acid soil. They also reported that soils containing lime had a further decrease in hydrogen-ion concentration of the plant sap. Small (1946) found similar results. He also found that the pH of roots was affected more than the stems. Rogers and Shive (1932) found the highest pH values of specific tissues occurred in the phloem, with the cortex slightly lower, and the lowest pH in the xylem. Steep pH gradients occurred between the xylem and the phloem.

Biddulph (1951), using radioactive iron and phosphorus, reported that when the pH of the solution was neutral and the supply of phosphorus in the nutrient solution moderate the phosphorus precipitated iron within the veins of bean plants. Rediske and Biddulph (1953) further substantiated the theory that pH did influence the absorption and translocation of iron. They found that the rate of distribution of injected iron within beans depended on the pH of the external nutrient media. They also observed that the amount of phosphorus available to the plant and the concentration of the iron in solution was critical. With low phosphorus at pH 4, the iron entered the plant and was rapidly distributed throughout. At the same phosphorus concentration and at pH 7 the iron entered the veins but not the mesophyll. However, at pH

7 with high phosphorus ferric phosphate precipitated on the surface of the roots. They concluded that the accumulation of the insoluble material in the vein was similar to the precipitate of insoluble material on the root surface. Marcour (1952) reported that the uptake of iron seemed to be slowed down by the presence of HCO_3 at the surface of the root and that the iron already present in the cell was immobilized. DeKock (1955) observed that the concentration of phosphorus in mustard plants decreased and that the severity of chlorosis was intensified with HCO_3. Other investigators (Iljin, 1943; McGeorge, 1946; and Lindner and Harley, 1944) have failed to find any relationship between phosphorus and incidence of chlorosis.

Carlson (1957) found that even though the pH and the HCO_3 of corn sap was higher than that of beans, corn had highly significant increases in translocation of iron over that of the bean. He observed a difference in metabolic function of chlorosis-susceptible and chlorosis-resistant plants. A review of previous work done on the pH of plant sap indicates much variability between plant species with a tendency for chlorosis-resistant varieties to have a higher sap pH than susceptible species (Small, 1946; Hurd-Karrer, 1939; and Thornton, 1933).

Bicarbonate as a Factor in Chlorosis

Porter and Thorne (1955) tried to separate the pH and the HCO_3 effects on plant chlorosis. With the pH of the solution culture held constant, increases in HCO_3 resulted in significant increases in chlorosis, and with HCO_3 held constant and the pH decreased, chlorosis decreased slightly. It was concluded that the HCO_3, and not the pH, was the primary causitive factor in lime-induced chlorosis. Carlson (1957) provided information to show that while the pH and the HCO_3

effects can hypothetically be separated in the nutrient solution they are actually inseparable when considering the pH and the HCO_3 concentrations of the expressed sap.

In solution culture studies it was found that HCO_3 reduced the movement of radioactive iron into the leaves and stems and increased its accumulation in the roots (Goss, 1957). He found that HCO_3 was associated with a decreased uptake of radioactive cations to the shoots. It was observed that the uptake of radioactive cations in $NaHCO_3$ treatments in comparison to the nutrient solution was greater in barley than in beans. He assumed that the HCO_3 interacted with plant metabolism, probably through its effect on translocation.

Brown (1953) suggested that the pronounced effect of HCO_3 might be on the iron requiring enzyme systems, and that copper requiring enzyme systems would not be affected as much. HCO_3 may affect the system proposed by Lundegardh (Broyer, 1951). This system is concerned with the terminal oxidase of the roots, especially the cytochrome-cytochrome oxidase system. Broyer (1951) suggested that HCO_3 could inhibit either the terminal oxidase suggested by Lundegardh or the organic acid cycle (Krebs cycle) from which the energy necessary for the migration of anions is derived. The external factors which influence HCO_3 concentrations could in this way be connected to iron. Miller and Thorne (1956) observed that whereas the inhibition of oxygen uptake by excised roots due to carbon monoxide was reversed by light, the inhibition due to HCO_3 was not light sensitive. They advanced the suggestion that the specific effect of HCO_3 is more complicated than a simple inhibition of the cytochrome oxidase enzyme system.

It was noted that plant species differ in their sap pH, depending on their metabolic system, and the change in pH of the sap due to

external conditions was correlated with the buffer capacities of the specific plant sap (Small, 1946). Baxter and Belcher (1955) found that roots from chlorotic orange trees had the highest internal pH, and the buffering capacity of the sap increased in the same direction as the pH. The buffer capacity of the chlorotic trees was very high in the range pH 6.2 to 6.4. High buffer activity at this pH range coincided with the buffer action of HCO_3 which indicated an accumulation of this ion in the roots of chlorotic trees. Small (1946) pointed out that the external influences on the pH of the expressed sap depend largely on the buffer capacity of the sap and that plants vary considerably in this respect. Stewart and Preston (1941) studied the effect of pH and components of the HCO_3 buffer system on the metabolism of potato discs and their ability to absorb ions. It was noted that HCO_3 more than any other component of the buffer system suppressed the uptake of ions. The increased concentrations of $KHCO_3$ progressively depressed protein synthesis and oxidase activity. They observed that this was the first time a potassium salt decreased respiration and metabolism of potato discs and concluded that this effect was due to HCO_3. Baxter and Belcher (1955) indicated that the internal environment of chlorotic citrus roots was disturbed by accumulation of HCO_3 with its resultant effect on the hydrogen-ion concentration and buffer capacity of the sap.

Jacobs (1922) found that an increased CO_2 pressure on bean roots caused an increased uptake of monovalent ions over divalent cations. Wadleigh and Brown (1952) found similar results in their work. Ulrich (1941) found that the increased absorption of cations resulted in an increase in organic acids. Thorne et al. (1950) and Iljin (1951) observed that chlorotic leaves had a higher concentration of organic

acids. An increased concentration of citric acid was directly correlated with potassium concentration. Carlson (1957) suggested that an excess uptake of cations due to an increased formation of organic acids would result in an increased sap pH. He also suggested that higher sap pH would decrease the activity of iron by its being precipitated out.

Recently, Lunt et al. (1958) and Miller et al. (1959) found increased solubility of phosphorus in the nutrient solution with increased HCO_3. They found that with this increased soluble phosphorus in the nutrient solution there was less phosphorus taken up by the plant and suggested that the phosphorus was precipitated by the iron in the nutrient solution. It was also suggested that the additional phosphorus did not enter the plant, or if it did, it was precipitated in the roots before it could be translocated to the stems or leaves.

Uptake of Carbon by the Root

Jacobs (1920) found that plants in a CO_2 saturated condition yielded a pink hue in the pigment of the root cells. This indicated an acidic reaction and he suggested that CO_2 was taken up by the plant root. Poel (1953), Jacobson (1955), Goss (1957), and Jackson (1957) found that CO_2 was taken up by roots and fixed in the plant. Iljin (1952) found that in chlorotic leaves of fruit trees there was an extra ordinarily high concentration of organic acids. Other investigators (Rhoads, 1959, and Jacobson, 1955) found an accumulation of organic acids associated with the Krebs cycle. Jackson (1957) found similar results in all his treatments of HCO_3 above pH 7.

Jacobson (1955) found that excised roots treated with CO_2 in solutions of water, KBr, $CaBr_2$, and KH_2PO_4 had the greatest accumulation of organic acids associated with KH_2PO_4. The magnitude of fixation was

determined largely by the concurrent ion absorption treatment, and he attributed the increased production of organic acids with KH_2PO_4 to an excess cation absorption. He found the greatest concentrations of organic acids to be malic and pyrrolidone carboxylic (PCA), respectively.

GENERAL EXPERIMENTAL METHODS

Growing, Treating, and Harvesting the Plants

It was the purpose of this study to use chlorosis-susceptible and chloris-resistant plant species. The plants chosen were red kidney bean and PI soybean for the chlorosis-susceptible species and kingcrost Ky. 7 hybrid corn and HA soybean for the chlorosis-resistant species. The red kidney bean, PI soybean, and HA soybean were germinated in the following manner. Paper towels were wrapped around the seeds with the bottom portion of the towels immersed in a beaker of water. They were then placed in a germinator for two days, after which the seedlings were transplanted to well-drained pans of fine crushed gravel. The plants grew until they were three or four inches high and the root systems were large and sturdy enough for transplanting. The plants were then maintained in gallon jars with nutrient solution in the greenhouse.

The corn was germinated in the following manner. A large desicator was cleaned and a small wire handle was put on the porcelain plate. Water was put in the bottom of the desicator with a thin film of water covering the porcelain plate, which contained small holes. The seeds were placed on the plate and a piece of glass tubing, connected to an air stream, was put into the desicator to provide sufficient aeration. The top was covered with a parafilm cover to prevent evaporation. The seeds germinated and were allowed to grow until time of transplanting. This method proved very satisfactory.

The jars were painted black to prevent algal growth. There were

three plants per jar, and these three plants combined constituted one treatment. The plants were supported by split corks padded with cotton or sponge rubber and held in waxed paper lids so that the roots could extend into the nutrient solution below. All jars were uniformly aerated with forced air through glass tubing with a fine capillary at the end.

The nutrient solution used was a modification of Hoagland's #2 solution, suggested by Hoagland and Arnon (1950). The modification was in using the chelated form of iron (Chel 330-Fe) rather than $FeSO_4$. The Chel 330-Fe was added at 5 ppm per liter of nutrient solution. This solution was full strength and good growth resulted in all of the plants. Each day supplementary amounts of iron were added to corn to maintain a non-chlorotic condition. The supplement was not needed for the other species.

The nutrient solution was made up in bulk using tap water and stored in a covered 50-gallon polyethylene tank. The pH was adjusted to 5.0 - 5.5 with HCl. The solutions were changed at 5 - 7 day intervals until treatments were applied to the plants. Extra jars of plants were grown to assure a sufficient number of healthy, uniform plants.

When the variables were applied, two days prior to harvest, a new solution using distilled water was used. The treatments consisted of four replications each of four levels of HCO_3 at three different pH levels. Each set of variables consisted of sixteen pots, completely randomized in a single line, with four levels of HCO_3 at a constant pH. The experiment for one species was then repeated over three pH levels. The treatment variables consisted of 0, 10, 30, and 50 me./l $NaHCO_3$ and

pH levels of 7.3, 7.8, and 8.2

After the treatments had been applied, the pH of the solutions was checked every 12 hours until harvest and adjusted, using HCl and NaOH. The pH determinations were checked using a Beckman Model H-2 pH meter or a Photovolt model 125 battery operated pH meter. In spite of frequent adjustments, the pH drifted, especially with 0 me./l $NaHCO_3$.

The pH of the various HCO_3 solutions was held constant by aerating with forced air mixed with required proportions of CO_2. From the Henderson-Hasselbalch equation the relationship of HCO_3 concentration to pH and the CO_2 partial pressure in the system is expressed as:

$$pH = pK' + \log\frac{HCO_3}{CO_2} \qquad (1)$$

HCO_3 and CO_2 are expressed in moles/l and pK is the first dissociation constant for carbonic acid at 38° C (6.317). From the above equation the percent CO_2 in the air stream was calculated to be 1, 3, and 5 percent for 10, 30, and 50 me./l $NaHCO_3$, respectively. The aeration mixture was prepared by transferring 10, 30, and 50 pounds of CO_2 into pressure tanks. Each tank was then filled to 1000 pounds pressure with air.

At the time of harvest the plants were 18 - 21 days of age. Harvesting was done in the afternoon. The method of harvesting consisted of taking the roots from the jar, shaking them quickly, and then putting them under cold mineral oil (see harvesting methods under experiments on methodology). The tops were cut off and the sap of the roots expressed in a cylinder by pressing them in a Carver press at 10,000 to 15,000 pounds per square inch pressure. A small amount of cold mineral oil was placed in the cylinder collecting the sample from the press so that the sap did not make contact with air. The collected

sap was then transferred to a 15 ml. centrifuge tube, covered with a small amount of oil, and immediately cooled.

The cooling system consisted of a refrigerated unit with an extended coil that dipped into a container with ethanol. Another method consisted of putting snow or ice in the ethanol. This made a slushy composition which lasted for better than five hours and proved to be very satisfactory. The ethanol was kept between -5 and -10° C. In this way the samples could be kept cold while harvesting and prior to being transferred to a refrigerator. Samples of the nutrient solution were collected and cooled in this same manner.

Analysis of Root Sap

The samples were stored in a refrigerator from 2 - 6 weeks before analyses were made. The samples were then analyzed for total CO_2 in a Van Slyke Blood Gas apparatus (Figures 1 and 2) using a modified procedure after the work of Van Slyke and Neill (1924), Van Slyke (1927), Van Slyke and Sendroy, Jr. (1927), Hawk et al. (1947), and Kock and Hanke (1948). A detailed description of the procedures was given by Carlson (1957). Enough modifications were made from the procedure of Carlson (1957) that a detailed procedure is given in this section.

Reagents

CO_2 and gas free N NaOH.--A small quantity of CO_2 and air free NaOH can be prepared in the extraction chamber of the Van Slyke Blood Gas apparatus. This is done by admitting approximately 30 ml. N NaOH into the extraction chamber and shaking to extract the gas. Release the gas from the chamber and repeat until there is essentially no gas remaining. This usually takes from three to five shakings. The NaOH is then siphoned directly into the flask prepared for storing CO_2 free

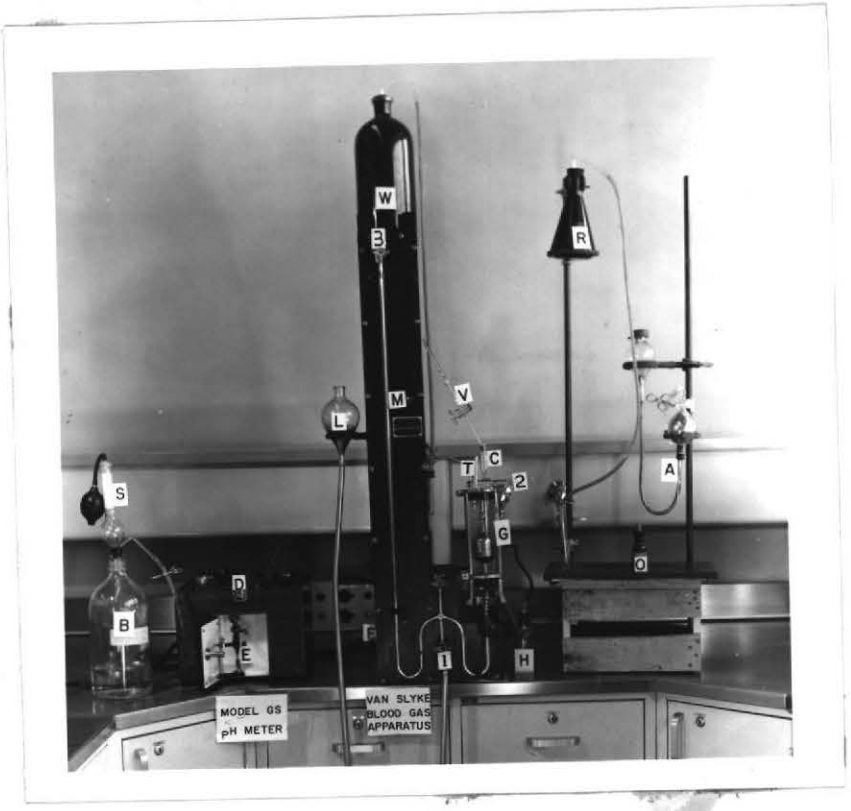

Figure 1. A Van Slyke blood gas apparatus and model GS pH meter

The legend of the numbers and letters on the above figure are as follows: A--NaOH (CO_2 free) apparatus; B--buffer solution; C--calibrated cup; D--dials for pH meter; E--electrodes of pH meter; F--control switches of Van Slyke blood gas apparatus; G--extraction chamber; H--vacuum (suction) for wastes; L--leveling bulb; M--manometric tube; O--octyl alcohol bottle; R--lactic acid reagent; S--soda lime tube; T--thermometer; V--Van Slyke-Neill pipette; W--wash solution; 1--stopcock 1; 2--stopcock 2; 3--stopcock 3.

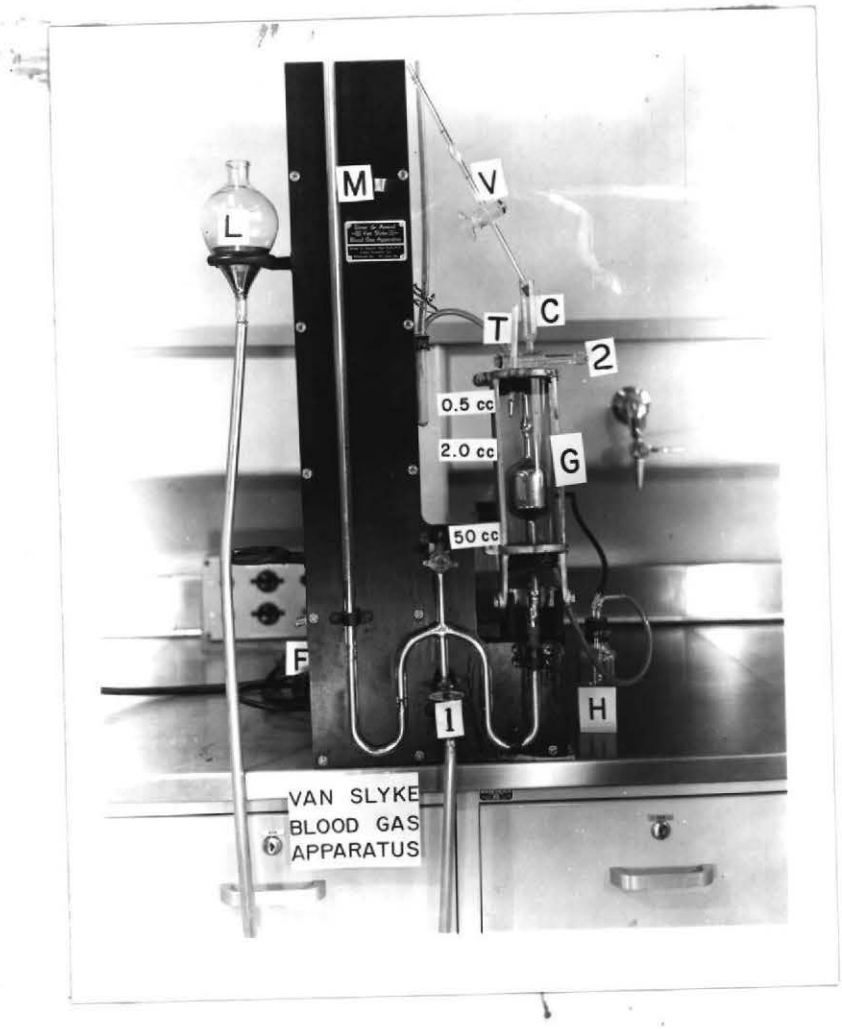

Figure 2. A closeup picture of the extraction chamber of the Van Slyke blood gas apparatus

The legend of the numbers and letters on the above figure are as follows: C--calibrated cup; F--control switches for Van Slyke blood gas apparatus; G--extraction chamber; H--vacuum (suction) for wastes; L--leveling bulb; M--manometric tube; T--thermometer; V--Van Slyke-Neill pipette; 1--stopcock 1; 2--stopcock 2; 0.5 cc. - 0.5 cc. meniscus mark; 2.0 cc. - 2.0 cc. meniscus mark; 50 cc. - 50 cc. volume mark in chamber.

NaOH (A of Figure 1).[a] This is done by lowering the mercury in the leveling bulb, moving the glass tip equipped with a rubber tip into the calibrated cup. The rubber tip fits securely against the glass so that the NaOH will pass into the storing flask without exposure to air.

Lactic acid reagent.--Take 10 ml. of N lactic acid and dilute to volume in a 250 ml. volumetric flask with CO_2 free distilled water. This solution is then stored in flask (R).

The containers for storing the lactic acid reagent should be dark to eliminate algal and bacterial growth. If this reagent stands in a clear container there will be growth within one or two days.

Wash solution.--Take 10 ml. of N lactic acid and dilute to volume in a liter volumetric flask and store in bottle (W). This solution is subject to the same algal growth as the lactic acid reagent solution; therefore, care must be taken to eliminate the growth.

Octyl alcohol.--Stock solution of N octyl alcohol is stored in dropper bottle (O).

Ethylene glycol.--Stock solution of ethylene glycol is stored in a dropper bottle.

Procedures prior to analysis of sample

Make sure all stopcocks are not stuck and are greased properly. It is necessary to have an excellent seal to prevent leakage when vacuum is applied to the system. Stopcocks and other glassware can be cleaned of vaseline by washing them thoroughly with soap and water and then rinsing in ether. To grease the stopcocks apply a thin layer of good rubber base grease, place stopcock in position, and give it

---

[a]All subsequent letters and numbers referred to in the procedure for analysis are found in Figures 1 and 2. The letters and numbers are the same in both figures.

several turns. Best results were given by use of Peters-Van Slyke stopcock grease from Fisher Scientific Company.

The apparatus should be checked against leaks. To do this introduce 3.5 cc. of slightly alkaline distilled water into the extraction chamber. Lower the liquid and shake for two minutes. Then raise the liquid to the 2 cc. mark and read the corresponding pressure of the mercury in the manometer. Repeat the shaking process and read the pressure again. If the temperature was the same, the two pressure readings should be the same. A leak usually results in a steady increase of readings upon repeated shakings.

Any air or moisture should be expelled from the manometer tube. Small amounts of moisture find their way into the manometric tube from the analysis chamber. If the moisture is neglected there will be some error due to the vapor pressure of the moisture above the mercury. To absorb this moisture two or three drops of ethylene glycol are admitted through stopcock 3 at the top of the manometer tube and allowed to flow about 10 cm. down the tube. This should be allowed to flow only this far as it will interfere with subsequent readings of the mercury meniscus. Ethylene glycol should be renewed every few days. Ethylene glycol is used in preference to other organic fluids because it does not char the lubricant used in the stopcock.

Preceeding each analysis the apparatus should be cleaned by introducing 15 to 20 ml. of washing solution into the extraction chamber. Lower the solution in the extraction chamber and shake for 15 to 20 seconds. Then expell the liquid. Any washing solution adhering to the glass will introduce no error in subsequent analysis.

Procedures for analyzing extracted sap samples

The apparatus is now ready to analyze the expressed sap samples

for total CO_2. With the extraction chamber filled with mercury, all stopcocks closed, and the leveling bulb in the lower position, introduce a drop of octyl alcohol and 7.5 cc. of lactic acid reagent into the calibrated cup. Open stopcock 1; then open stopcock 2 and allow the liquid to move into the extraction chamber being careful not to allow the entry of any air. Close stopcock 2 when the liquid has entered the extraction chamber. Lower the mercury and the liquid in the extraction chamber by holding the leveling bulb low. When there is but a little mercury still remaining in the extraction chamber bulb, close stopcock 1. For best results of agitation of the liquid a little mercury should be left to shake in the extraction chamber. Place the leveling bulb in the upper position and shake for three minutes. Now manipulate stopcock 1 until the liquid has reached stopcock 2 and the mercury in the manometer has reached the top. Caution should be taken when letting the liquid back up to prevent the extraction chamber and manometer from being broken because of liquid and mercury slamming against them.

By manipulating stopcock 2 the liquid is raised into the calibrated cup until 1.5 cc. remains in the extraction chamber. Close stopcock 2 and remove all but 3 cc. of the solution in the calibrated cup. For drawing off the excess solution connect rubber tubing to a vacuum and draw off the liquid into a bottle.

Using a Van Slyke-Neill pipette 1 cc. aliquot of the sample to be analyzed is sucked up. The pipette, with a fitted rubber tip, is then inserted into the calibrated cup still containing 3 cc. of the lactic acid reagent. The leveling bulb is lowered and the stopcock of the pipette is opened. Cautiously open stopcock 2 and allow the 1 cc. of

sample into the extraction chamber. Close stopcock 2 and the stopcock on the pipette, and then remove the pipette from the calibrated cup. Care should be taken not to agitate the liquid. Admit one more cc. of the lactic acid reagent still in the calibrated cup into the extraction chamber by manipulating stopcock 2. Draw off the remaining lactic acid reagent in the calibrated cup by suction, add a drop or two of mercury in the mouth of the calibrated cup, and then lower the leveling bulb to bring the liquid and mercury into the extraction chamber for shaking. With the leveling bulb in the upper position, shake for four minutes. During this shaking CO_2 is liberated from the acidified sample. At the conclusion of the shaking period open stopcock 1 and allow the liquid to rise at a fairly rapid rate until the extraction chamber is about three-quarters full. Then gradually close the stopcock to reduce the flow of the mercury until the flow is so slow that when the liquid meniscus reaches the 2 cc. mark there is no difficulty in stopping it exactly on the mark. While raising the liquid turn on the light behind chamber to facilitate seeing the mark easier. Turn on the light for reading the manometer, tap the manometer tube lightly with the finger, and read the height of the mercury as P_1. The temperature of the gas phase is also recorded at this time. If the liquid meniscus passes the 2 cc. mark, readjustment must be made by relowering the mercury meniscus and shaking the mixture for one minute. If this is not done, more reabsorption of CO_2 will take place than is provided for in the calculations.

To absorb the CO_2 the NaOH free from CO_2 is added under reduced pressure. To obtain a reduced pressure, the meniscus of the mercury is lowered about two cm. by opening stopcock 1 and putting the leveling bulb in the lower position. NaOH is admitted to the calibrated cup by

putting the rubber tip connected to the NaOH storing apparatus in the bottom of the calibrated cup and releasing NaOH. The only NaOH exposed to the atmosphere is that which is at the surface. Admit 1 cc. of NaOH into the extraction chamber by manipulating stopcock 2. The excess NaOH is sucked out of the calibrated cup and a drop or two of mercury is added. Lower the liquid and mercury by opening stopcock 1 and lowering the leveling bulb as previously mentioned. Shake for two minutes and then raise the liquid and mercury in the extraction chamber as described until the meniscus is at the 2 cc. mark. Tap the manometer tube as before and read the height of the mercury in the manometer as P_2. The temperature is again recorded. From these readings P_{CO_2} is obtained.

$$P_{CO_2} = P_1 - P_2 \qquad (2)$$

With the leveling bulb in the upper position open stopcock 1 and allow the liquid to move up slowly to the top of the extraction chamber neck and the mercury to move up the manometer. Again care must be taken not to slam the liquids. Stopcock 2 is now opened and the liquid is allowed to go into the calibrated cup. Suck the solution off, lower the leveling bulb, clean the apparatus, and repeat the given procedure for other analysis. After skillful practice, each sample can be run in approximately 15 minutes.

Factor to correct for volume change with added NaOH.--Other constants which enter into the final determination should be mentioned. When adding 1 ml. of NaOH to absorb the evolved CO_2, a factor is needed to correct for the increased height in the aqueous phase above the mercury (Table 1). This correction was determined in a blank analysis. Three and one-half ml. of slightly alkaline water was put in the

Table 1. Correction factors (C_{CO_2}) used when using the 0.5 or 2.0 cc. volume as meniscus mark for measuring CO_2 in a Van Slyke blood gas apparatus

Total volume of solution used	Correction factor[a]	
	a = 0.5 cc.	a = 2.0 cc.
cc.	cm. Hg.	cm. Hg.
7.0	2.5	0.26
3.5	0.64	0.11

[a]Mark at which the liquid meniscus rests when reading P on the manometer.

Table 2. Conversion factors (F_t) used to convert pressure of gas to millimoles per liter (mM/l)[a]

Temperature	F_t	Temperature	F_t
°C.		°C.	
15	0.1229	25	0.1165
16	0.1222	26	0.1160
17	0.1215	27	0.1154
18	0.1208	28	0.1149
19	0.1202	29	0.1143
20	0.1196	30	0.1138
21	0.1190	31	0.1133
22	0.1183	32	0.1128
23	0.1177	33	0.1123
24	0.1171	34	0.1118

[a]Van Slyke and Sendroy (1927).

extraction chamber, shaken, and P_a read on the mercury column. One more ml. of water was added. This would compensate for the increased volume when NaOH is added. The solution was lowered, shaken and P_b read. The difference between readings ($P_a - P_b$) is the correction factor (C_{CO_2}) used in the calculation of mM/l of CO_2 evolved in the sample. The four correction factors listed in Table 1 arise from the possibility of using two total volumes of solution (3.5 or 7.0 cc.) and two calibrated marks on the extraction chamber (0.5 or 2.0 cc.). In the studies reported here the total volume was 3.5 cc. (S) and the mark used was 2.0 cc. (a). Thus the correction factor for CO_2 evolved due to adding the 1 ml. NaOH would be 0.11 cm. Hg. Equation (2) would be corrected as follows:

$$P_{CO_2} = (P_1 - P_2) - C_{CO_2} \qquad (3)$$

Factors to convert P_{CO_2} to mM/l_{CO_2}.--Certain factors are needed to convert P_{CO_2} to mM/l, mg/l, or volume percent (Van Slyke and Sendroy, 1927). In the present work all conversions are to mM/l. Table 2 records these factors (F_t) for different temperature levels. To convert from P_{CO_2} to mM/l_{CO_2} the following would be used:

$$\text{Corrected } mM/l_{CO_2} = P_{CO_2}\,(F_t) \qquad (4)$$

Correcting pK' for temperature changes.--The Henderson-Hasselbalch equation is used to solve for $\frac{HCO_3}{CO_2}$ (equation 1, page 16.) Solving equation (1) for $\frac{HCO_3}{CO_2}$ the following is obtained.

$$\frac{HCO_3}{CO_2} = \text{antilog}\,(pH - pK') \qquad (5)$$

In these equations pK' is the first dissociation constant for carbonic acid. At 38° C. this is 6.317 (Shelovsky and MacInnes, 1935). As the temperature changes the value of pK' changes. Theoretically, this change is equal to 6.317 - 0.5 u, where u is the ionic strength.

Shedlovsky and MacInnes (1935) have determined the value of pK' to increase by 0.005 units per each degree change in the temperature between 30° and 40° C. Value for pK' was therefore figured as:

$$pK' = 6.317 + 0.005\ (38 - T) \qquad (6)$$

where T = temperature (degrees C.) measured on each sample.

Taking pH of samples

The pH was measured on a Beckman GS model pH meter. This instrument is a modification of model G featuring two new operating controls, a helipot with a duodial which provides higher measuring accuracy and a sensitivity switch to change from normal to increased sensitivity. The duodial has 1000 scale divisions, 100 divisions for each turn of the helipot. The total voltage across the helipot is 0.2000 millivolts (mv) per division. The meter has an accuracy of 0.003 of a pH unit relative to the buffer.

The equation for converting to pH is:

$$pH_s = pH_b + \frac{(E_b - E_s)}{\frac{2.3026\ RT\ 1000}{F}} \qquad (7)$$

pH_s = pH of sample

pH_b = pH of buffer solution

E_b = emf of buffer (mv)

E_s = emf of sample (mv)

T = absolute or Kelvin degrees temperature

R = universal gas constant, 8.3144 joules per degree per mole

F = Faraday's constant, 96496 coulombs per equivalent

R_b = duodial reading in the buffer

R_s = duodial reading in the sample

By putting in constants and reducing terms,

$$\frac{2.3026\ RT\ 1000}{F} = 0.19842\ T \qquad (8)$$

and

$$(E_b - E_s) = 0.200\ (R_b - R_s) \qquad (9)$$

Equation (2) then reduces to:

$$pH_s = pH_b + \frac{0.200\ (R_b - R_s)}{0.19842\ T} \qquad (10)$$

After the samples had been analyzed on the Van Slyke apparatus, the pH was taken of the sample. One to 5 ml. pipettes were used to take a sample of the expressed sap or nutrient solution from the centrifuge tube. The sample was then placed in a 5 ml. beaker used with the model GS pH meter. The beaker was put into the meter and the electrodes immersed in the sample. The door was closed to eliminate outside interference. The time between placing the sample into the meter and taking the reading was kept as constant as possible for all samples. The time varied between 15 and 20 seconds. The sensitivity of the meter made this a requisite because the sample could pick up CO_2 and cause the machine to drift. The meter was periodically checked with a buffer throughout the measurements. The buffer was mixed and stored in a bottle with a soda lime tube on the air inlet to protect it from CO_2 in the air.

Example of Calculation of HCO_3, CO_2, and pH of a Sample

From the following measurements the pH, mM/l, HCO_3, and mM/l_{CO_2} will be calculated as an example.

Van Slyke measurements

$P_1 = 22.98$

$P_2 = 12.85$

$T = 27^\circ$ C.

$F_t = 0.1154$ (Table 2)

$C_{CO_2} = 0.11$ cm. Hg. (Table 1) for S = 3.5 cc. and a = 2.0 cc.

pH measurement

$pH_b = 7.00$

$R_s = 386$

$R_b = 531$

$T = 26^\circ$ C.

From equation (3)

$$P_{CO_2} = (22.98 - 12.85) - 0.11 = 10.02 \text{ cm. Hg.} = 100.2 \text{ mm. Hg.}$$

From equation (4)

$$mM/l_{CO_2} = (100.2)(0.1154) = 11.56$$

The value for mM/l_{CO_2} is total CO_2 evolved or a summation of CO_2, HCO_3, and CO_3. Assuming all CO_3 is in the form of HCO_3 or CO_2, then

$$mM/l_{CO_2} = (CO_2 + HCO_3) \tag{11}$$

From the pH readings and equation (10) we get

$$pH_s = 7.000 + \frac{0.200\ (531 - 386)}{0.1984\ (299.2)} = 7.489$$

From equation (6) we get

$$pK = 6.317 + 0.005\ (35 - 26) = 6.377$$

Equation (5) then gives us

$$\frac{HCO_3}{CO_2} = \text{antilog}\ (7.489 - 6.377) = \text{antilog}\ 1.112$$

or

$$\frac{HCO_3}{CO_2} = 12.94$$

From equation (1) we get the identity

$$HCO_3 = \frac{(CO_2 + HCO_3)\ HCO_3/CO_2}{1 + HCO_3/CO_2} \tag{12}$$

Using the values obtained above we get

$$HCO_3 = \frac{(11.56)(12.94)}{1 + 12.94} = 10.73 \text{ mM/l}$$

From the value obtained by equation (5) we get

$$CO_2 = \frac{10.73}{12.94} = 0.83 \text{ mM/l}$$

This is actual CO_2 and not the value obtained from the Van Slyke measurements. Another way to obtain this mM/l_{CO_2} (actual) would be from equation (11).

$$mM/l_{CO_2} \text{ (actual)} = 11.56 - 10.73 = 0.83$$

The results would be tabulated as:

pH	$CO_2 + HCO_3$ mM/l	HCO_3 mM/l	CO_2 mM/l
7.49	11.56	10.73	0.83

Each sample of expressed sap, as well as each sample of nutrient solution, was analyzed in the Van Slyke apparatus and the pH measured on the model GS pH meter, and the obtained values were calculated in the manner above.

EXPERIMENTS ON METHODOLOGY

Harvest Methods

Procedure

Kingcrost Ky 7 corn was germinated and transplanted to the nutrient culture as described in the general experimental methods. The plants grew without difficulty and when the treatment variables were applied at 18 days of age uniform healthy plants were available. Fifteen jars, each containing three plants, were used. The nutrient solution contained 50 me./l $NaHCO_3$ aerated with 5 percent CO_2 and adjusted to pH 7.3. The pH was checked every 12 hours while the plants were under treatment. There were five methods of harvest with three replications per method.

The five different harvest methods, designated as 1, 2, 3, 4, and 5, were as follows.

1. The plants were taken from the nutrient solution and the roots washed successively in cold tap water, 0.1 N HCl, and cold distilled water. The washing time was from 2 to 3 seconds in each solution. The roots were then blotted with paper towels and immersed in cold mineral oil. The tops of the plants were cut off under oil and discarded. The roots were placed in a steel cylinder (2 1/4-inch diameter) and pressed in a Carver press at 10,000 to 15,000 pounds per square inch pressure. The expressed sap, collected under mineral oil, was then transferred to a 15 ml. conical centrifuge tube, covered

with a thin layer of cold mineral oil, and cooled immediately in a cold alcohol bath (Carlson, 1957).

Each of the remaining harvest methods follow method 1 after the plant roots were immersed in oil.

2. After the roots were taken from the nutrient solution, they were washed in cold distilled water for 10 seconds and then immersed in cold mineral.
3. The roots were taken from the nutrient solution, quickly shaken by hand, blotted with paper towels, and then immersed in the oil.
4. The roots were taken from the nutrient solution, quickly swung, and then immersed in the oil.
5. The roots were taken from the nutrient solution, washed in cold distilled water for 10 minutes, and immersed in the oil.

Results and discussion

It is believed that the internal root sap, with a high external concentration of HCO_3 and CO_2, should come nearly to an equilibrium with the external solution. If this is true, the internal HCO_3 and CO_2 values for the plants grown in solutions of 50 me./l $NaHCO_3$ would be expected to be high. When the roots underwent a long wash period (method 5) or were washed by method 1, the HCO_3 of the expressed root sap was very low (Table 3). There was an increase in internal HCO_3 when the roots were washed for 10 seconds in cold, distilled water (method 2), and still greater increase when the roots were simply blotted (method 3) or given a quick swing (method 4). The difference between methods 3 and 4 was due largely to the amount of HCO_3 in the external solution and to

the relationship of HCO_3 to CO_2 from the Henderson-Hasselbalch equation. There was little difference between the total CO_2 and HCO_3 of these two methods compared to the methods where the roots were washed. It would then seem that the values that more nearly equal that of the actual internal HCO_3 and CO_2 would be those where the roots were unwashed. It is felt that the very low values of methods 1 and 5 are due to the wash methods in that a high amount of HCO_3 and CO_2 probably diffused out of the root during the extended time.

Table 3. The pH, bicarbonate, and carbon dioxide of root sap of corn plants grown in 50 me./l $NaHCO_3$ and harvested by five different methods

Method number[a]	Root sap			Nutrient solution		
	pH	$HCO_3 + CO_2$	HCO_3	pH	$HCO_3 + CO_2$	HCO_3
		mM/l	mM/l		mM/l	mM/l
1	5.7	2.6	0.5	7.6	41.3	39.0
2	6.7	4.2	2.9	7.7	47.4	45.3
3	6.5	20.1	11.3	7.5	28.7	26.7
4	7.2	24.4	20.9	7.8	45.9	44.1
5	5.3	1.7	0.1	7.7	40.3	38.2

[a]See text, page 31, for description of methods.

Carlson (1957) reported values similar to those found by method 1, and this was a repeat of his harvest method. Considering the extended time of washing and the acid solution used, it would seem that these values are lower than might be expected. The roots harvested by method 4 have the highest concentration of HCO_3. The external solution still adhering to the root surfaces after a quick shake or quick blot (methods 3 and 4) should be small. The most critical loss would seem to occur

between taking the plant out of the solution and putting it under oil. In a later experiment it was found that there was very little difference in the values when harvested by either methods 3 or 4. If an equilibrium between the external solution and the internal root solution exists, the HCO_3 and CO_2 values would be expected to be higher than the values reported for any of the harvest methods. The results of this study did not show that the internal HCO_3 rises as high as that of the external solution, suggesting that the internal HCO_3 does not reach the same level of HCO_3 as that in the external solution

pH of Roots by pH Papers and Indicators

Procedure

The purpose of this experiment was to estimate the change of internal pH in respect to the external HCO_3 concentration in the nutrient solution. Two methods were used. The first method for measuring the internal pH of plant roots consisted of taking corn roots and immersing them for 30 minutes in beakers containing different treatments. These treatments were (1) 0 me./l $NaHCO_3$ aerated with air, (2) 0 me./l $NaHCO_3$ aerated with 5 percent CO_2, (3) 50 me./l $NaHCO_3$ aerated with air, and (4) 50 me./l $NaHCO_3$ aerated with 5 percent CO_2. When the roots were taken from a given nutrient solution, four different treatments were made before the pH was measured. These four treatments on the roots consisted of (1) washed and not blotted, (2) washed and blotted, (3) unwashed and not blotted, and (4) unwashed and blotted. The washing was accomplished in cold distilled water for 2 to 3 seconds and the blotting was done with paper towels.

The second method for measuring the pH of root sap consisted of putting root sections into solutions of different indicator dyes and then noticing the change in color when put in different concentrations

of HCO_3. Root sections of corn, similar to those in the above experiment, were used. A number of root sections were placed in various indicator solutions and maintained at a pH 4.5 to 5.0 for 22 hours in order that the indicator would be absorbed into the roots. Indicators used were neutral red, bromthymol blue, bromcresol purple, methyl red, and phenol red with a total range of pH between 4.2 and 8.4. The same treatment variables used for testing with pH papers were used. Color changes were observed over a 30-minute period in order to allow the pH of the root sap to come to equilibrium with the solution.

Results and discussion

The pH of roots was determined by expressing root sap directly on pH indicator paper (Table 4). This was done by placing a piece of pH paper on a flat surface, taking a portion of the root and squeezing the juice onto the paper with a glass rod, and measuring the pH of the root sap by comparing the colors of the treated paper with the standard known colors. This was done with a group of different pH papers to check the results of each.

Table 4. The pH of corn root sections determined by pH papers when placed in $NaHCO_3$ solutions of different concentrations, different carbon dioxide pressures, and harvested by four different methods

Treatment		Solution	Root treatment			
CO_2	$NaHCO_3$	pH	No wash no blot	No wash blot	Wash no blot	Wash blot
%	me./l					
0	0	5.3	5.2	5.2	5.4	5.1
5	0	4.4	4.8	4.9	4.8	4.8
0	50	8.8	7.7	7.8	6.8	6.7
5	50	7.2	6.7	7.0	6.9	6.7

It can be seen that with no $NaHCO_3$ in the culture media the media had a lower pH than solutions containing 50 me./l $NaHCO_3$. The corresponding pH of the root sap was lower and close to the range of the pH of the external media. It was also noticed that with CO_2 in the aeration stream the external pH was lower than if no CO_2 was in the air. This was noticed particularly with the high HCO_3 with 5 percent CO_2. When the roots in treatment 3 were not washed, the pH was nearly a whole pH unit higher than those that were washed.

The high pH of treatment 3 would indicate that there might have been contamination on the external surfaces of the unwashed roots. If there was contaimination on the unwashed roots of this treatment, then the HCO_3 would have increased the pH above that of the washed roots. The external pH of treatment 4 was lower than that of treatment 3 due to the CO_2 in the aeration stream of treatment 4. The pH of roots in treatment 4 would not be expected to rise as high as the roots in treatment 3 because of the lower external pH.

With no HCO_3 the pH of the root sap maintained about the same pH as the external medium. This was true down to approximately pH 5. Below pH 5 the sap pH was above that of the solution. Some CO_2, it appears, was absorbed by the roots and caused a slight decrease in pH, but not as low as the external media.

The pH of corn roots under conditions of no HCO_3 or CO_2 in the external media ranged between 5.0 and 5.5. This is in agreement with values recorded by Hurd-Karrer (1939). With the addition of HCO_3 to the external media, the internal pH rose proportionately until, at a high HCO_3 concentration, the pH was in the range of 6.4 to 6.8. After reaching this pH any increase in pH was small, possibly due to the plant's ability to buffer the solution.

It should also be mentioned that it was found in both methods for measuring pH the apex of the roots showed a slightly higher pH than did the lateral roots or main portion of the root. This pH difference was from 0.2 to 0.4 units higher. The reason for this could be due to protoplasmic differences of the cells between the apex and the elongation area.

Table 5 gives the results of the pH found when corn roots had been kept in different indicator dyes for 22 hours, then were put into different solution treatments. The pH determined in this manner was approximately the same as when measured by pH papers. The results show that with an increased HCO_3 concentration in the external solution, there was an increase in root sap pH. The pH of the roots at the 50 me./l HCO_3 levels was slightly higher than the respective pH of roots of treatments 3 and 4 as determined by pH papers. This would suggest that the pH of unwashed roots would represent the actual internal pH and the internal HCO_3, as the HCO_3 is dependent on pH. This would also suggest that some HCO_3 was lost by washing, even if washed for a very short time.

The Effect of Time on the Uptake of Bicarbonate

Procedure

In an attempt to answer what values of HCO_3 in the root are most nearly the actual values, the following experiments were set up. Corn was put into treated nutrient solution with 50 me./l HCO_3 with 5 percent CO_2 in the aeration stream and adjusted to pH 7.3. The plants were left in the treatment solution for different lengths of time (1 minute to 24 hours). The method of harvest consisted of washing the roots in cold distilled water for 20 to 30 seconds before immersing them in the cold mineral oil.

Table 5. The pH of corn root sections determined by indicators when placed in $NaHCO_3$ solutions of different concentrations with different carbon dioxide pressures in the aeration stream

Treatment		Indicator[a]				
CO_2	$NaHCO_3$	N.R.	B.T.B.	B.C.P.	M.R.	P.R.
%	me./l					
		pH Range of Indicators				
		6.8 - 8.0	6.0 - 7.6	5.2 - 6.8	4.2 - 6.2	6.8 - 8.4
		pH of Roots				
0	0	<6.8	<6.0	5.3	5.1	<6.8
5	0	<6.8	<6.0	<5.2	4.8	<6.8
0	50	7.4	7.5	>6.8	>6.2	7.7
5	50	7.2	7.3	6.7	>6.2	7.3

[a]N.R. = neutral red, B.T.B. = bromthymol blue, B.C.P. = bromcresol purple, M.R. = methyl red, and P.R. = phenol red.

In another section of the experiment a larger root was used in an attempt to reduce or eliminate the problem of contamination and diffusion. Carrots were first tried but they were not satisfactory because of the large amount of material needed to get enough sap for analysis. Potato tubers were used even though a potato is not a true root. Its structure, however, resembles that of a fleshy root, and it yielded more sap. At first an omni mixer was used to macerate the material, but too much oil and liquid were needed to insure a well macerated mixture. So, the method used on all previous experiments, that of pressing in a Carver press under a minimum amount of oil, was still used.

U.S. No. 1 russet potatoes weighing between 6 and 10 ounces were used. The treatment variables consisted of four jars of nutrient solutions, two each with 0 and 50 me./l $NaHCO_3$. The jars with 0 me./l

HCO_3 were aerated with air and those with 50 me./l were aerated with 5 percent CO_2. Each jar was adjusted to pH 7.3. The potatoes were peeled down two sides and then immersed in the nutrient solution. Four potatoes were put into each jar to give four replications of each treatment. The pH of the solution was adjusted at 12-hour intervals.

After the treatments had been on one day, the first harvest was carried out. The potato was taken from the nutrient solution and three or four cork bore samples 3/4 inch in diameter were taken from the center of the potato. The ends of the bore samples were cut off to exclude parts that had been in contact with the solution. The sample was then put into a 2 1/4-inch diameter cylinder, covered with a minimum amount of oil, put into a press, and pressed at 10,000 to 15,000 pounds per square inch pressure. The sap was collected in a 15 m. conical centrifuge tube and cooled immediately in a cold alcohol bath. The samples were transferred to a refrigerator and analyzed within a week after harvest. After four days the remainder of the potatoes were taken from the nutrient solution and sap samples collected in the same manner.

Results and discussion

The HCO_3 concentration of the root sap was very low because of the washing procedure. Correlations can be made, however, even at the low HCO_3 concentration. When the samples were left in for one minute (Table 6), the concentration was about half that of the samples left in for 24 hours. This would indicate that the root had a certain amount of HCO_3 in its system at the beginning, showed a rapid rise in HCO_3 concentration at first, then gradually leveled off with increased time. The CO_2 measurement at one minute was about half that of 33 minutes and from that point on it decreased.

Table 6. The pH, bicarbonate, and carbon dioxide of washed corn roots when put in 50 me./l $NaHCO_3$ for different lengths of time

Time	pH	$HCO_3 + CO_2$	HCO_3
min.		mM/l	mM/l
		Root Sap	
1	6.5	0.7	0.5
3	6.5	0.8	0.4
10	6.5	1.0	0.6
33	6.4	1.3	0.7
100	6.7	1.3	0.9
333	6.8	1.2	0.9
1440	7.0	1.2	1.0
		Solution	
	7.7	50.8	48.7

Stolwijk and Thimman (1957) showed that the uptake of CO_2 by plant roots was rapid when the roots were first put into a CO_2 solution. They also found that after the first few hours the internal CO_2 concentration leveled off. In this work it was found that the roots took up CO_2 rapidly first and then only made a gradual increase after the first hour.

Jackson (1957) and Jacobson (1955) suggested an explanation for this. They showed that organic acids accumulated in the root at a gradual rate. The decrease in CO_2 might then be expected if the CO_2 was converted to organic acids.

It is interesting to note that as the roots are left in solution for longer lengths of time the pH of the root sap increased. This correlates with an increase of HCO_3 in the roots.

From a plot of mM/l HCO_3 and mM/l CO_2 against log time (Figure 3), it can be noted that the rise of HCO_3 and CO_2 in the plant root was

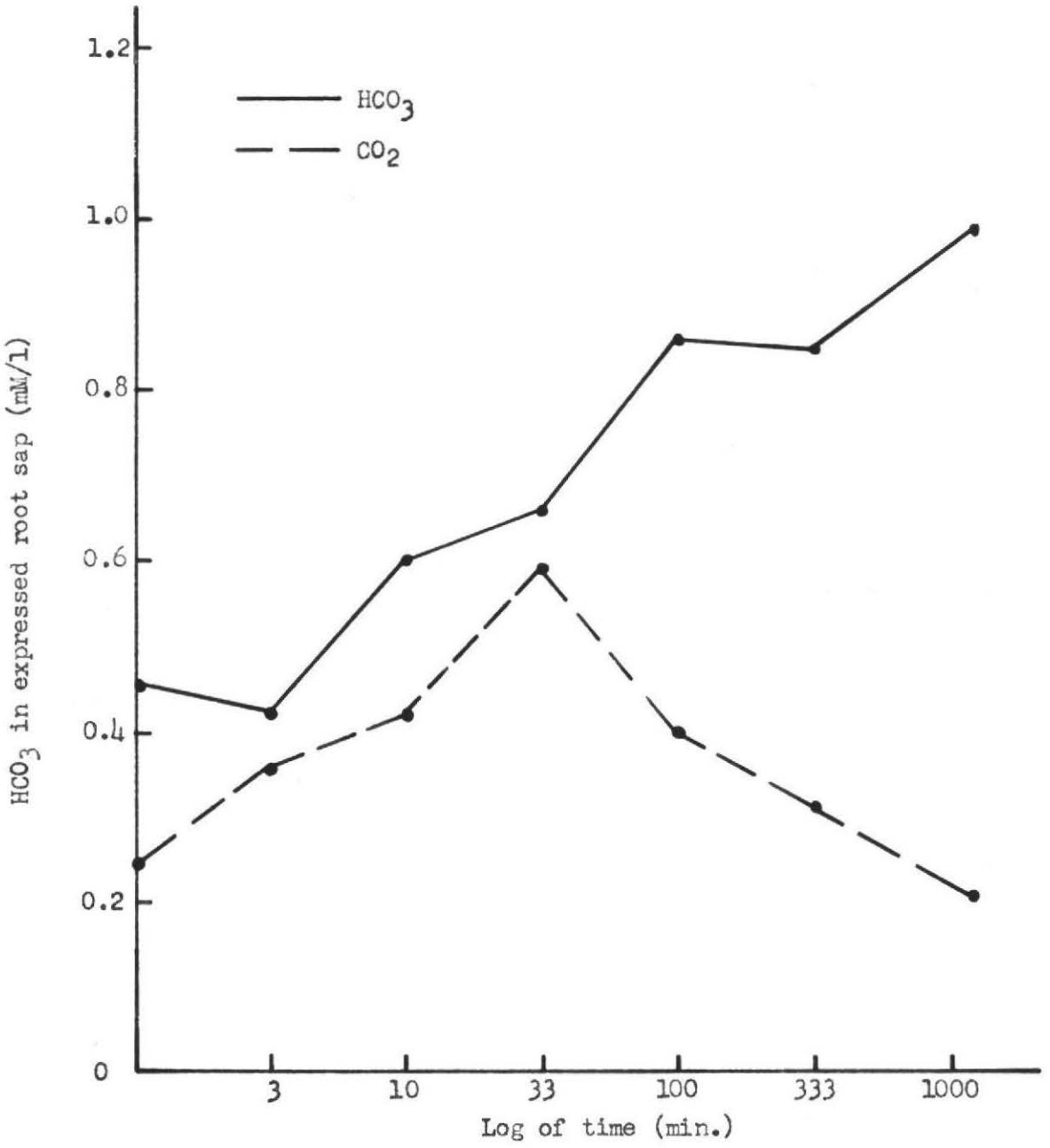

Figure 3. The bicarbonate and the carbon dioxide uptake of corn roots when left in 50 me./l $NaHCO_3$ for different lengths of time

equal until about 33 minutes. After this the HCO_3 increased slightly while the CO_2 decreased about the same rate that the bicarbonate was increasing. The ratio of HCO_3 to CO_2 is nearly equal to 1 up to 33 minutes. By 24 hours the ratio increased to about 5 to 1.

The pH of the expressed sap of potatoes was lower on the samples harvested the fourth day when compared to those harvested the first day (Table 7). Those harvested at the later date showed a continued buildup in CO_2 which would cause a slight decrease in pH. This rise in CO_2 suggests that with time there was either a continued uptake of CO_2 or an accumulation of respiration-produced CO_2 within the tuber due to some block in the normal elimination process brought on by the HCO_3 and/or CO_2 in the nutrient solution. It may also be that potatoes contain fairly high concentrations of CO_2.

Table 7. The pH, bicarbonate, and carbon dioxide of expressed sap of potato tubers after being in bicarbonate treatments for one and four days

Treatment		pH	$HCO_3 + CO_2$	HCO_3
Days	$NaHCO_3$			
	me./l		mM/l	mM/l
			Root Sap	
1	0	5.9	9.7	2.3
1	50	5.9	9.0	2.1
4	0	5.6	10.4	1.6
4	50	5.6	17.6	2.4
			Solution	
	0	7.5	6.9	6.5
	50	7.2	49.3	42.2

Because of the high HCO_3 concentration of the nutrient solution at 50 me./l $NaHCO_3$, it was expected the tuber would take up HCO_3 if an

equilibrium was to be achieved. As the tubers absorbed very little HCO_3 it is assumed that potato tubers do not take up much HCO_3. The low HCO_3 can be accounted for in the calculation because the pH was low. This low concentration of HCO_3 may also occur because this plant does not absorb high HCO_3 due to the numerous layers of cells in the tuber.

Loss of Bicarbonate by Diffusion

Procedure

Another experiment was conducted to show how much HCO_3 and CO_2 diffused out of the plant roots. This was done by putting corn roots in nutrient solution with 50 me./l $NaHCO_3$ aerated with 5 percent CO_2 at pH 7.3 for 24 hours, and then exposing the roots to the atmosphere for different lengths of time. The roots were taken from the nutrient solution, given a quick swing, allowed to hang exposed to the atmosphere for 0, 5, 15, 30, and 60 seconds, and then immersed in cold mineral oil and the root sap expressed.

Results and discussion

There was a decrease in internal HCO_3 of the corn roots with time (Table 8). There was not a significant change in pH or internal CO_2. This suggests a diffusion of HCO_3 outward. Each of the roots were shaken so any external contamination should have been the same for all plants. The loss of HCO_3 was greater just after the plants were taken from the solution than after they had been exposed to the atmosphere for any length of time.

Method and Length of Storage

Procedure

A composite experiment was designed to see if there is a difference between the concentration of HCO_3, CO_2, and the pH of the sample due to harvesting methods, length of storage time, and method of storage.

Table 8. The pH, bicarbonate, and carbon dioxide of corn roots grown in high bicarbonate and exposed to the atmosphere for different lengths of time

Time	pH	$HCO_3 + CO_2$	HCO_3
sec.		mM/l	mM/l
		Root Sap	
0	7.0	29.6	23.5
5	7.0	26.5	21.0
15	7.0	26.3	21.2
30	6.9	24.1	18.2
60	6.9	25.1	19.5
		Solution	
	7.7	47.5	45.3

Corn plants were put in treatment variables of 50 me./l HCO_3 and aerated with 1.5 percent CO_2 at pH 7.8 for 24 hours. At the end of the 24-hour period the plants were harvested.

These harvest methods were:

I. The roots were washed successively in cold tap water, in 0.1 N HCl and in cold distilled water. They were then blotted, immersed in cold oil, and the sap expressed. The washing time was 2 to 3 seconds in each case.

II. The roots were swung quickly and then immersed in the oil at room temperature.

III. This was the same as method II except the oil was cold, having just been taken from the deep freeze.

The different storage times were:

1. The expressed sap was analyzed the same day as harvested.
2. The sap was analyzed 10 days after harvest.

3. The sap was analyzed 7 weeks after harvest.

4. The sap was analyzed 15 weeks after harvest.

The storage methods consisted of:

A. The plant sap was not stored but analyzed the same day.

B. The root sap was stored in a refrigerator.

C. The root sap was stored in a deep freeze.

Results and discussion

In comparing the different storage times (Table 9) there seemed to be a difference between the samples that were analyzed the same day they were harvested and those that were analyzed some time later. The biggest difference seemed to be between the 10-day and 7-week period of time. When the expressed root sap was stored in the refrigerator there was an increase in HCO_3 with increased time. There was, however, a decreased amount of HCO_3 if the sap were stored in a deep freeze for 15 weeks compared to 10 days. These differences might be due to a chemical change during the storage time. The trend was for the CO_2 to rise but not the HCO_3.

Table 9. The effect of storage times, storage methods, and harvest methods on the bicarbonate concentration of expressed root sap of corn grown in 50 me./l $NaHCO_3$

Treatment[a]			Root sap			Nutrient solution		
Storage time	Storage method	Harvest method	pH	$HCO_3 + CO_2$	HCO_3	pH	$HCO_3 + CO_2$	HCO_3
				mM/l	mM/l		mM/l	mM/l
1	A	I	5.5	1.7	0.2	8.0	33.2	32.2
1	A	II	6.6	26.9	16.5	8.1	46.5	45.6
2	C	III	7.7	24.5	23.4	8.1	42.5	41.7
2	B	II	7.4	29.0	26.5	8.2	47.2	46.4
3	B	I	5.8	6.1	1.3	7.7	45.3	43.3
3	B	II	7.2	47.8	42.0	7.7	46.8	44.6
4	B	III	7.2	45.8	39.8	7.7	44.2	42.2
4	C	II	7.6	17.8	16.8	8.2	22.6	22.3

[a]See text, pages 44 and 45, for description of treatments.

The big difference in the HCO_3, as has been pointed out previously, was due to the method of harvest. If the roots were washed thoroughly the values of HCO_3 were low. Methods II and III were essentially the same but method I included an intensive wash.

Carbon Dioxide Solubility in Mineral Oil

An extensive search of the literature revealed little information or acute problems concerning the solubility of CO_2 in mineral oil. Van Slyke and Neill (1924) used mineral oil to cover air and CO_2-free NaOH used in reabsorbing CO_2 in manometric gas analysis. They found it gave sufficient protection against air absorption for at least one working day. Mineral oil has also been used to cover blood samples to prevent gas exchange prior to manometric analysis. No data was found in the works of Seidell (1952), Quinn (1936), and other authors (Scheflon, 1953, and Ridenour et al., 1954) who have compiled physical constants of the solubility of CO_2 in different solvents and materials. Other compounds similar to mineral oil were found to be low in CO_2 solubility (Quinn, 1936). The assumption was made that CO_2 was not appreciably soluble in mineral oil.

However, Kubie (1927) determined the solubility of CO_2, N_2, and O_2 in mineral oil. In all his determinations he used the apparatus described by Van Slyke and Neill (1924). In his calculations he used somewhat different constants than those used by Van Slyke and Neill (1924). He found the value of CO_2 solubility in mineral oil to be 0.841 ± 0.011 at 1 atmosphere pressure and at 24-25° C. (Bunsen coefficient). The value for CO_2 solubility in water in the "Handbook of Chemistry and Physics" is 0.759 at 25° C. and 1 atmosphere pressure. Ridenour et al. (1954) reported the solubility of CO_2 in molten paraffin at 72.2° C. and

752.5 mm. Hg. to be 0.548 (Bunsen coefficient).

In the present study no difference was found between the total CO_2 pressures determined on the Van Slyke for large amounts of nutrient solution with a high HCO_3 concentration covered with a small amount of mineral oil and a small amount of the same nutrient solution covered with a large volume of oil (Table 10).

Table 10. The pH, bicarbonate, and carbon dioxide of nutrient solution when covered with different amounts of mineral oil

Treatment			Nutrient solution		
$NaHCO_3$	Volume		pH	$HCO_3 + CO_2$	HCO_3
	Solution	Oil			
me./l	ml	ml		mM/l	mM/l
0	60	3	7.5	6.9	6.5
50	60	3	7.2	49.3	42.2
0	3	60	7.4	8.9	8.2
50	3	60	7.6	49.7	45.4

It is concluded that there might be some error in the calculation of HCO_3 due to the CO_2 solubility in the mineral oil. The percent error could not be determined because the amount of mineral oil used to cover the sap samples was not measured.

EXPERIMENTS MEASURING INTERNAL BICARBONATE AND pH OF ROOT SAP

Procedures

The methods and procedures of these experiments are discussed under general experimental procedures. The major modifications from these methods were with the PI soybeans. Since these plants were grown in December, lights were assembled overhead to give a light intensity of 1,200 foot candles at a distance of 1 1/2 feet from the light source. All other plants were grown during the summer and early fall when there was ample light.

Only two replications of PI soybeans could be harvested. The growth habits of this variety of soybean tend toward prostrate growth with long internodes. Many of the plants died when their stems were damaged from transplanting, changing nutrient solution, or bending because of increased growth.

It should be mentioned that the yield of root sap of each of the species was different. Corn and bean roots yielded well with corn giving the higher yield of the two species. The soybean varieties yielded less than the corn or beans with the HA variety yielding greater than the PI variety. There was, however, an ample amount of sap for each analysis on the Van Slyke apparatus and for taking the pH.

At the time of harvest there were three plants per jar. In some cases, if individual plants died or were damaged after they had been treated, only two plants were harvested in each replication. Because of the limited number of PI soybean plants, half of the jars had only two plants per replication.

Results and Discussion of the Corn Experiment

As the HCO_3 of the nutrient solution increased, the internal HCO_3 of the root sap gradually increased (Figure 4). The HCO_3 was lower at pH 8.2 than at either pH 7.3 or 7.8 below 30 me./l (Table 11).

When the HCO_3 was held constant and the pH increased, the HCO_3 concentration of expressed root sap decreased (Table 11). However, when the decrease in internal HCO_3 was plotted against the actual external HCO_3 values, it was noticed that the internal HCO_3 increased with pH between 7.3 and 7.8.

Carlson (1957) found a decreased internal HCO_3 when the external HCO_3 was held constant and the pH increased. He suggested that with increased CO_2 pressure due to the increased CO_2 in the aeration stream that the higher pH may have an additional effect upon the internal HCO_3. As pointed out above, this apparent decrease in internal HCO_3 with increasing pH at constant external HCO_3 is not real when plotted against the actual external values.

As the pH of the external solution was increased, the pH of the expressed root sap did not increase significantly (Table 12), although there was a slight increase in internal pH with increased external pH. As the external HCO_3 was increased the expressed root sap did not increase in pH (Figure 5). The increase in internal HCO_3 seemed to be more effected by the increased HCO_3 than by the increased pH in the nutrient solution.

Results and Discussion of the Red Kidney Bean Experiment

The internal HCO_3 of bean roots greatly increased with an increase in external HCO_3 (Figure 6). The results show nearly a tenfold increase in internal HCO_3 from 0 to 50 me./l HCO_3 in the external solution. The increase of internal HCO_3 at pH 7.3 was lower than at either pH 7.8 or

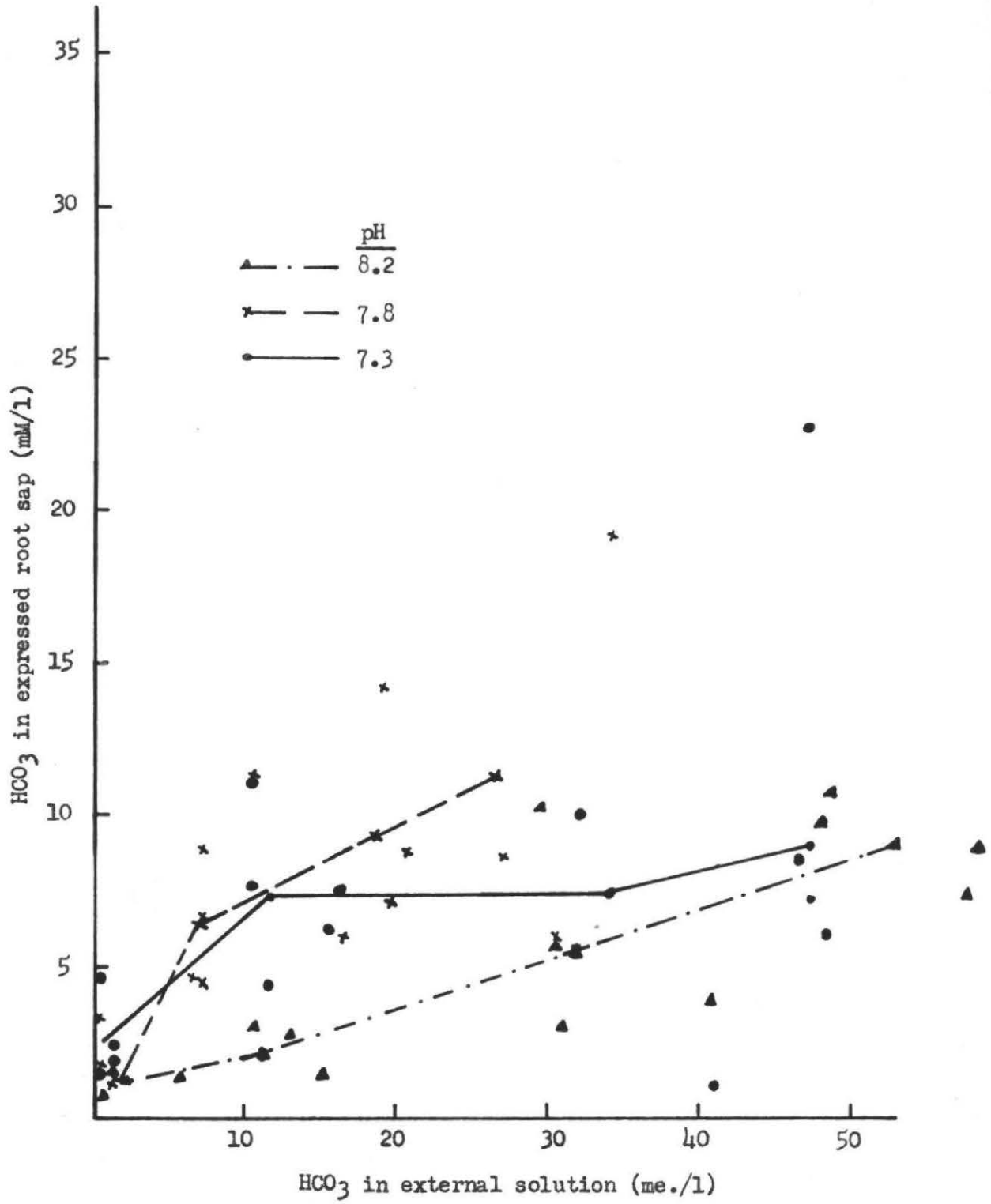

Figure 4. The bicarbonate concentration of expressed root sap of corn compared to the bicarbonate level of the external solution at various pH levels

Table 11. The pH, bicarbonate, and carbon dioxide of corn root sap (mean of four replications)

Proposed treatments		Root sap			Nutrient solution		
$NaHCO_3$	pH	pH	$HCO_3 + CO_2$	HCO_3	pH	$HCO_3 + CO_2$	HCO_3
me./l			mM/l	mM/l		mM/l	mM/l
0	7.3	6.5	5.0	2.7	6.3	1.3	0.9
10		6.7	11.3	7.4	7.5	12.6	11.8
30		6.4	25.8	9.7	7.0	33.8	23.3
50		6.3	24.7	11.2	7.4	50.6	47.4
0	7.8	6.3	4.7	2.2	7.2	2.9	2.6
10		6.6	9.0	5.9	7.2	6.0	5.4
30		6.6	15.5	9.2	7.2	33.0	18.7
50		6.3	23.5	11.2	7.0	35.0	25.6
0	8.2	6.2	3.6	1.3	6.9	2.6	2.1
10		6.3	5.5	2.4	7.2	15.9	12.9
30		6.5	9.3	5.7	7.8	34.2	32.8
50		6.7	14.0	9.2	7.9	55.0	53.0

Table 12. Analysis of variance of bicarbonate and pH of corn roots

Source	D. F.	S. sqs.	M. sqs.	F	Sig.
		HCO_3			
Total	47	1199.02			
pH	2	85.09	42.55	2.51	.10
HCO_3	3	485.05	161.68	9.55	.005
pH x HCO_3	6	19.42	3.24	0.19	N.S.
Error	36	609.45	16.93		
		pH			
Total	47	7.96			
pH	2	0.23	0.12	0.72	N.S.
HCO_3	3	0.41	0.14	0.83	N.S.
pH x HCO_3	6	1.48	0.25	1.52	N.S.
Error	36	5.85	0.16		

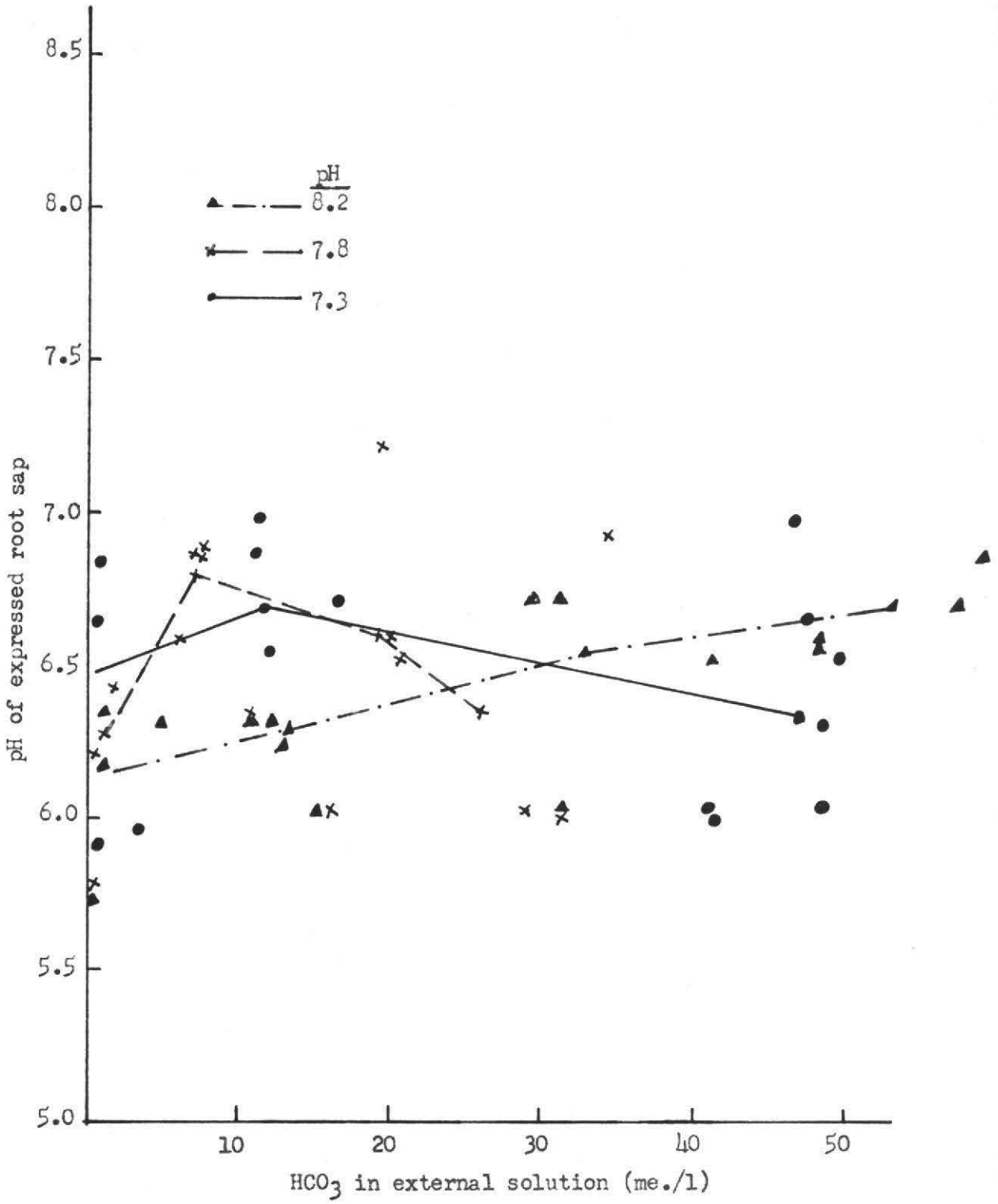

Figure 5. The pH of expressed root sap of corn compared to the bicarbonate level of the external solution at various pH levels

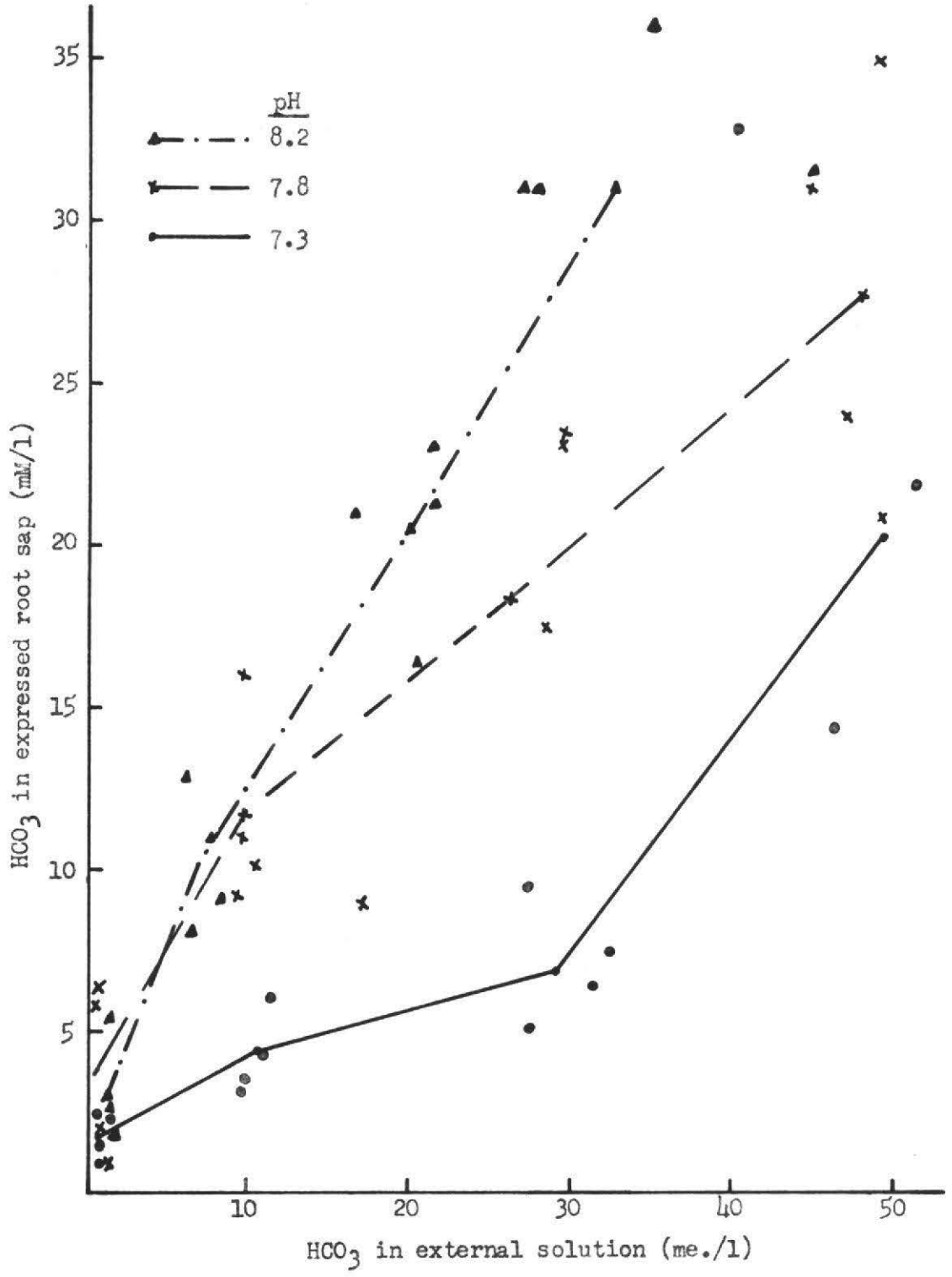

Figure 6. The bicarbonate concentration of expressed root sap of beans compared to the bicarbonate level of the external solution at various pH levels

8.2 (Table 13). The internal HCO_3 of pH 7.3 compared to pH 7.8 and 8.2 at 10 me./l HCO_3 in the external solution was highly significant, but there was no significance between the internal HCO_3 at pH 7.8 or 8.2 at the same HCO_3 level (Table 14). Above 10 me./l HCO_3 in the external solution there was a significant difference in internal HCO_3 between samples at pH 7.3, 7.8, and 8.2, and this difference became greater with increased HCO_3 in the nutrient solution. The increase in internal HCO_3 with increasing external HCO_3 was greatest at pH 8.2, next at pH 7.8, and lowest at pH 7.3. The external pH, however, did not seem to affect the internal HCO_3 concentration (Table 13) to the extent as did the increase in the external HCO_3 concentration, even though the analysis of variance showed the pH levels to be significant (Table 14).

Greater changes in internal HCO_3 might suggest a greater increase in cations or production of organic acids; thus the pH of the roots would not be expected to change appreciably. This might then suggest that beans, a chlorosis-susceptible variety, may have a higher production of organic acids or at least a higher potential to produce them, whereas corn would have a low tendency to produce them. Jacobson (1955) indicated that the production of organic acids resulted from an excess cation uptake, and Jackson (1957) showed that in all his treatments increased organic acid production also showed increased cation uptake. The results of this experiment would seem to bear this out since an excess cation uptake would tend increase the sap pH (Jacobson, 1955, and Jackson, 1957). If only small amounts of CO_2 from the external solution are fixed (Jacobson, 1957), then a buildup of CO_2 from respiratory and metabolic processes would be expected if the mechanism is blocked that normally removed this CO_2 from the plant. The external

Table 13. The pH, bicarbonate, and carbon dioxide of bean root sap (mean of four replications)

Proposed treatments		Root sap			Nutrient solution		
$NaHCO_3$	pH	pH	$HCO_3 + CO_2$	HCO_3	pH	$HCO_3 + CO_2$	HCO_3
me./l			mM/l	mM/l		mM/l	mM/l
0	7.3	6.3	3.4	1.6	6.6	0.6	0.4
10		6.6	7.0	4.3	7.4	11.4	10.4
30		6.8	9.9	7.0	7.6	28.5	26.8
50		7.1	24.1	20.2	7.6	52.5	48.8
0	7.8	6.3	7.3	3.8	6.8	0.8	0.6
10		6.9	15.2	11.7	7.7	9.8	9.4
30		6.8	25.3	18.3	7.9	26.2	25.4
50		6.9	36.2	27.7	8.0	47.7	46.6
0	8.2	6.4	5.1	3.3	7.5	1.4	1.3
10		7.0	12.6	10.2	8.1	7.3	7.1
30		7.4	22.5	20.4	8.4	20.1	19.9
50		7.7	32.9	31.1	8.2	34.4	33.7

Table 14. Analysis of variance of bicarbonate and pH of bean roots

Source	D. F.	S. sqs.	M. sqs.	F	Sig.
		HCO_3			
Total	47	5248.12			
pH	2	633.27	316.64	17.21	.005
HCO_3	3	3749.45	1249.82	67.91	.005
pH x HCO_3	6	202.90	33.82	1.84	N.S.
Error	36	662.50	18.40		
		pH			
Total	47	22.30			
pH	2	1.19	0.60	17.15	.005
HCO_3	3	18.45	6.15	177.25	.005
pH x HCO_3	6	1.41	0.24	6.92	.01
Error	36	1.25	0.04		

HCO_3 may be this inhibiting factor. Ordin (1954) showed evidence from his work with barley roots, that the consumption of oxygen is adequate to provide more than the required amount of CO_2 from respiration for the production of organic acids found by Jacobson (1955). It may be possible that some plants cannot use or fix this metabolically produced CO_2 and hence it would accumulate if not allowed to pass from the plant.

The pH of the internal root sap increased very slightly although significantly (Table 14) compared to the increase in internal HCO_3 with an increase in external HCO_3 (Figure 7). The largest change in root pH was when the nutrient solution was held at 8.2. There seemed to be little, if any, difference between samples that were maintained at pH 7.3 and 7.8.

Results and Discussion of the HA Soybean Experiment

With HA soybeans grown at pH 7.3 there was essentially no increase in internal HCO_3 (Figure 8) with increased HCO_3 in the nutrient solution. However, at pH 7.8 and 8.2 the increase in internal HCO_3 was high. This increase was from fourfold to eightfold compared to 7.3. At pH 7.8 there tended to be a steady increase in internal HCO_3 as the external HCO_3 was increased compared to samples at pH 8.2. At pH 8.2 there was a very high increment increase in internal HCO_3 between 0 and 10 me./l HCO_3 in the nutrient solution. This tapered off and gradually leveled off at 30 me./l with possibly a decrease as the external HCO_3 solution approached 50 me./l. No explanation is given for the extremely sharp increase of HCO_3 between 0 and 10 me./l other than it may suggest a high inhibitory effect of HCO_3 at the high pH.

The internal HCO_3 increased significantly with increasing external pH (Tables 15 and 16). In this respect they react like bean roots but

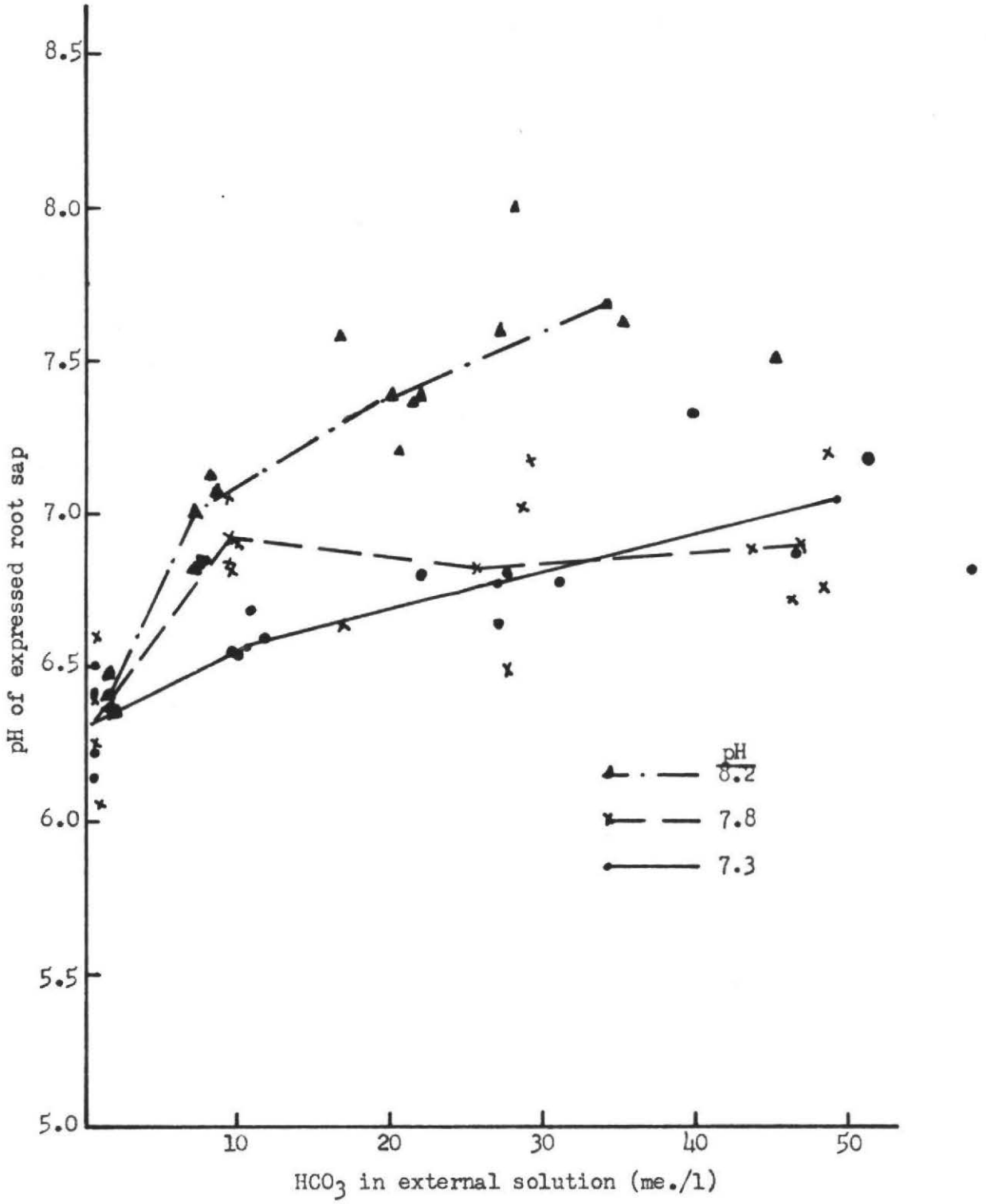

Figure 7. The pH of expressed root sap of beans compared to the bicarbonate level of the external solution at various pH levels

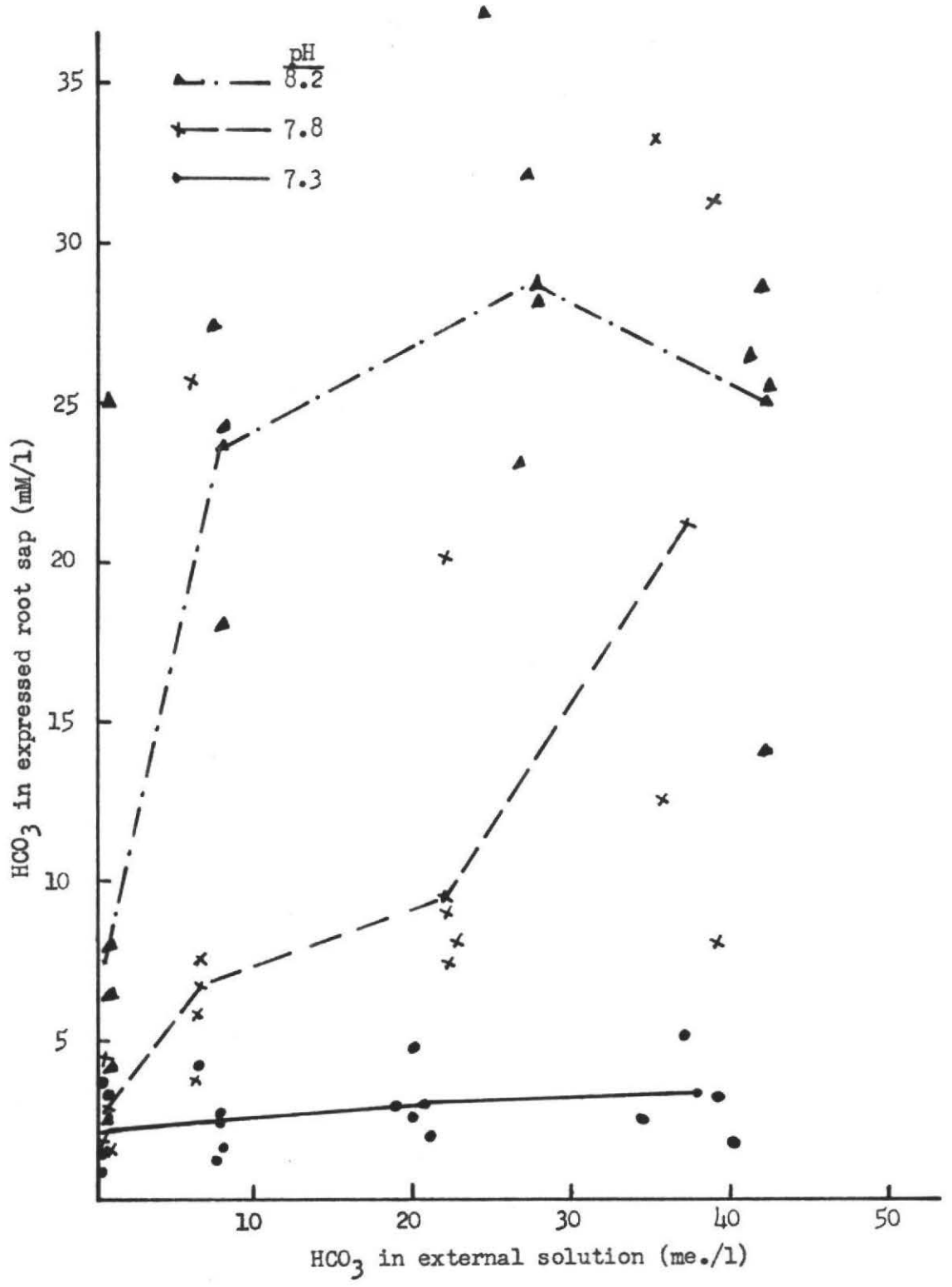

Figure 8. The bicarbonate concentration of expressed root sap of HA soybean compared to the bicarbonate level of the external solution at various pH levels

Table 15. The pH, bicarbonate, and carbon dioxide of hawkeye soybean roots (mean of four replications)

Proposed treatments		Root sap			Nutrient solution		
$NaHCO_3$	pH	pH	$HCO_3 + CO_2$	HCO_3	pH	$HCO_3 + CO_2$	HCO_3
me./l			mM/l	mM/l		mM/l	mM/l
0	7.3	6.4	4.8	2.4	5.9	1.1	0.3
10		6.5	4.2	2.5	7.2	8.9	7.6
30		6.6	5.3	3.1	7.6	21.7	20.4
50		6.6	5.1	3.4	7.6	40.1	37.7
0	7.8	6.2	5.0	2.8	5.9	1.1	0.3
10		6.9	13.8	10.8	7.1	8.2	6.8
30		7.0	13.8	11.2	7.5	24.2	22.7
50		7.2	24.4	21.2	7.8	39.0	37.3
0	8.2	6.8	14.9	11.1	6.6	1.4	0.7
10		7.1	27.8	23.6	7.7	8.6	8.0
30		7.1	34.1	28.0	8.4	28.9	28.6
50		7.3	26.6	23.5	8.3	42.9	42.4

Table 16. Analysis of variance of bicarbonate and pH of hawkeye soybean roots

Source	D. F.	S. sqs.	M. sqs.	F	Sig.
		HCO_3			
Total	47	5221.58			
pH	2	989.01	494.50	12.45	.005
HCO_3	3	2312.85	770.95	19.42	.005
pH x HCO_3	6	490.24	81.71	2.06	N.S.
Error	36	1429.48	39.71		
		pH			
Total	47	11.34			
pH	2	4.75	2.38	39.38	.005
HCO_3	3	3.86	1.29	21.34	.005
pH x HCO_3	6	0.55	0.09	1.52	N.S.
Error	36	2.17	0.06		

not like corn. Between 0 and 10 me./l HCO_3 in the external solution there was a sharp increase in sap pH compared to the increase after 10 me./l HCO_3 (Figure 9).

Results and Discussion of the PI Soybean Experiments

In the case of PI soybeans, the internal HCO_3 increased with an increase in external HCO_3 at each pH level (Figure 10 and Table 17). There was, however, no significance between the separate pH levels (Table 18). It should nevertheless be noted that there was a difference between the internal HCO_3 at the pH 8.2 and 7.8 levels after 30 me./l HCO_3 in the external solution. It is surprising how parallel the values for the internal HCO_3 were at the three pH levels.

The HCO_3 did not seem to have much effect on root pH until after 30 me./l (Figure 11). The increment increase in pH with respect to the external HCO_3 increase was nearly linear with a gradual slope up to 30 me./l. After 30 me./l the pH leveled off.

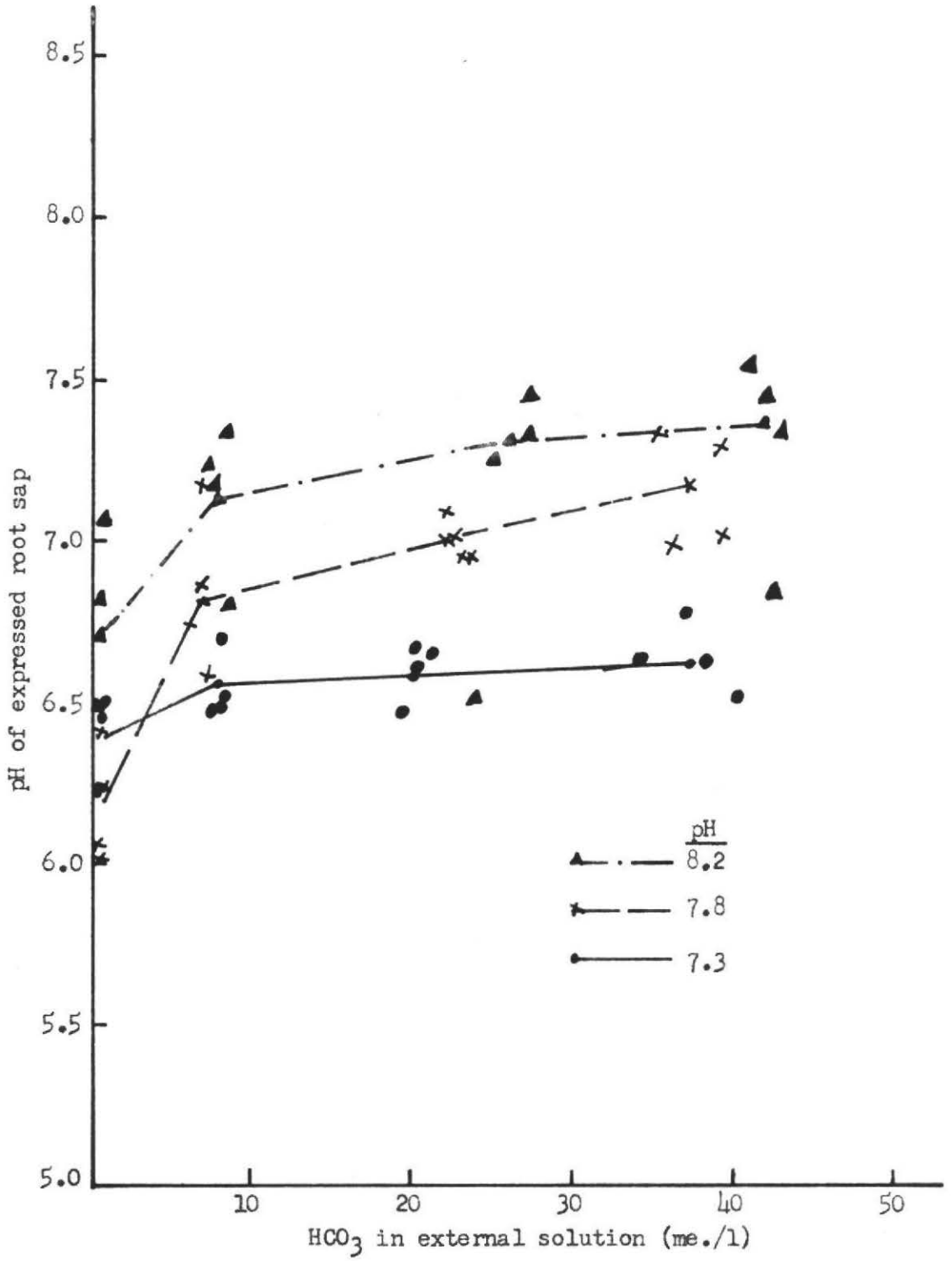

Figure 9. The pH of expressed root sap of HA soybeans compared to the bicarbonate level of the external solution at various pH levels

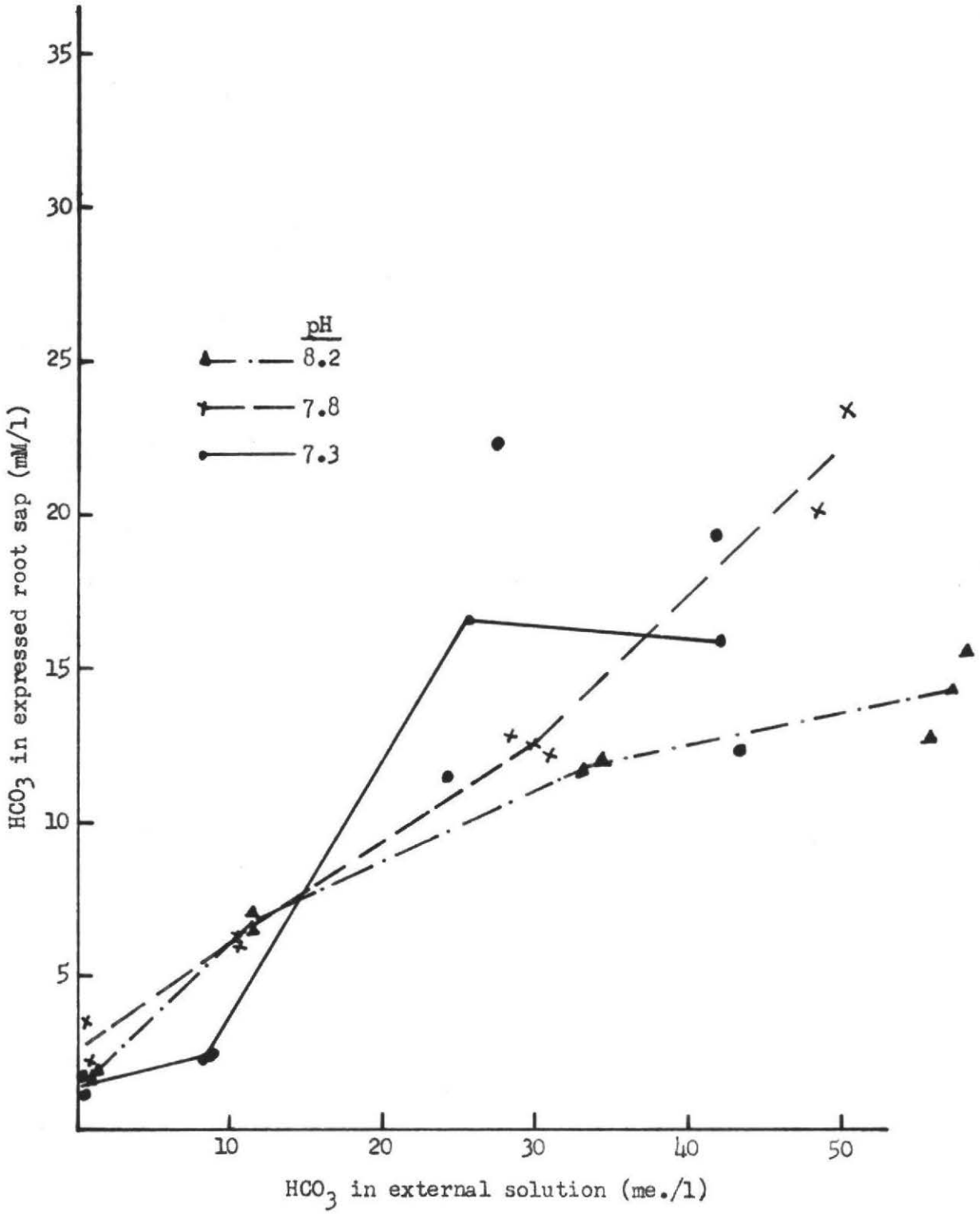

Figure 10. The bicarbonate concentration of expressed root sap of PI soybeans compared to the bicarbonate level of the external solution at various pH levels

Table 17. The pH, bicarbonate, and carbon dioxide of PI soybean roots (mean of two replications)

Proposed treatment		Root sap			Nutrient solution		
$NaHCO_3$	pH	pH	$HCO_3 + CO_2$	HCO_3	pH	$HCO_3 + CO_2$	HCO_3
me./l			mM/l	mM/l		mM/l	mM/l
0	7.3	6.4	3.1	1.5	6.9	0.3	0.2
10		6.3	4.9	2.2	7.6	9.2	8.6
30		6.8	23.1	16.7	7.7	28.8	25.6
50		6.8	21.4	15.7	7.5	45.3	42.2
0	7.8	6.4	5.5	2.9	7.5	0.5	0.4
10		6.7	9.3	6.1	7.6	10.4	9.8
30		6.9	16.6	12.5	7.7	30.6	29.1
50		7.2	25.7	21.8	7.5	51.6	48.9
0	8.2	6.4	3.5	1.8	7.3	0.8	0.7
10		6.7	10.5	6.8	7.6	12.6	11.8
30		6.8	19.8	13.8	7.9	34.8	33.7
50		6.5	25.9	14.1	7.8	59.0	56.7

Table 18. Analysis of variance of bicarbonate and pH of PI soybean roots

Source	D. F.	S. sqs.	M. sqs.	F	Sig.
		HCO_3			
Total	23	1163.94			
pH	2	16.48	8.24	0.92	N.S.
HCO_3	3	945.49	315.16	35.37	.005
pH x HCO_3	6	95.03	15.84	1.78	N.S.
Error	12	106.94	8.91		
		pH			
Total	23	1.69			
pH	2	0.30	0.15	11.20	.005
HCO_3	3	0.77	0.26	18.90	.005
pH x HCO_3	6	0.45	0.08	5.50	.01
Error	12	0.16	0.01		

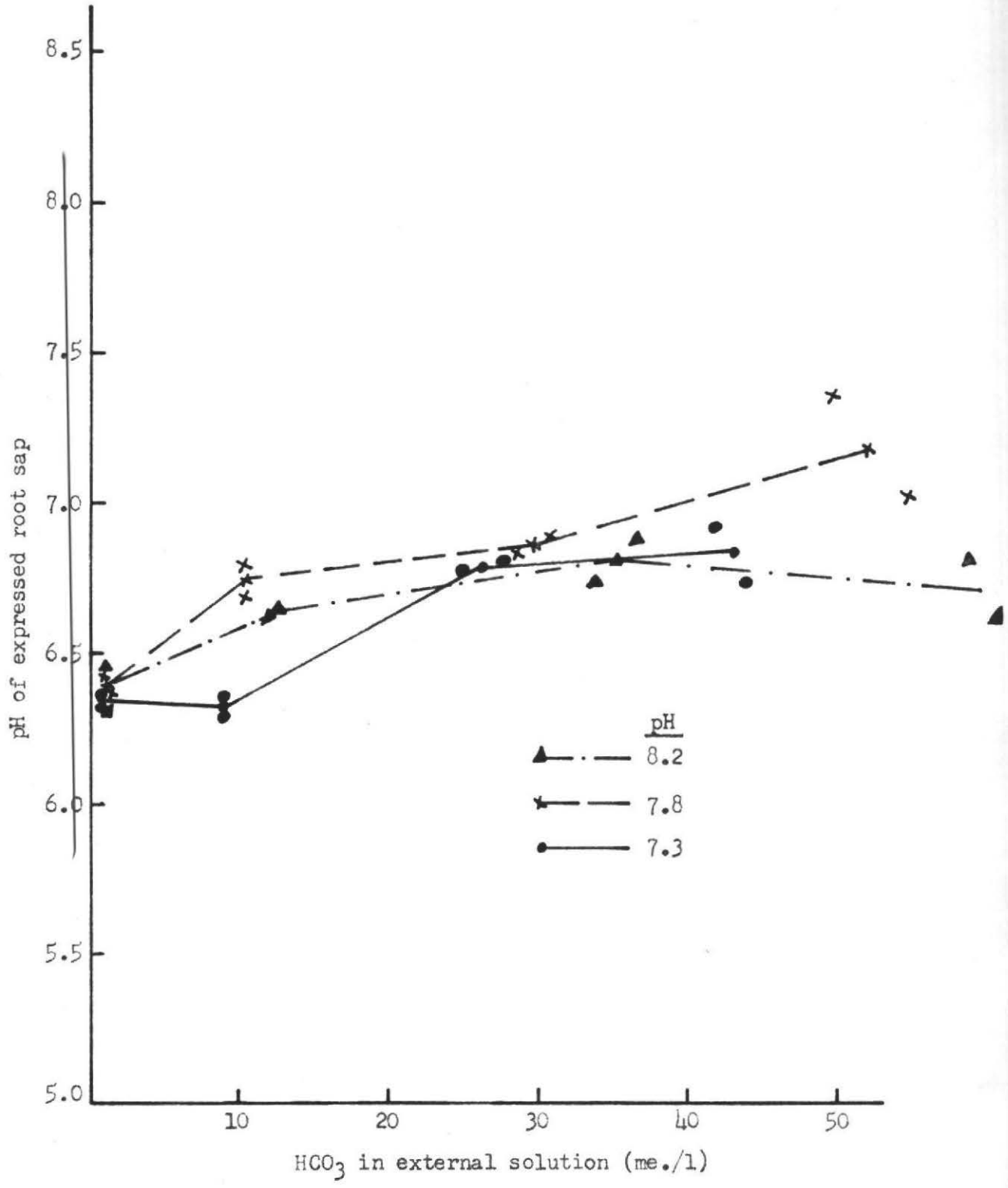

Figure 11. The pH of expressed root sap of PI soybeans compared to the bicarbonate level of the external solution at various pH levels

GENERAL DISCUSSION AND CONCLUSIONS

The objective of this study was to determine the HCO_3 content of plant species which are known to show resistance or susceptibility to chlorosis. A plant said to be chlorosis-susceptible is one which does not show the capacity to absorb and keep iron mobile in an environment conducive to the inactivation of iron. Corn and HA soybeans are known to be able to continue metabolizing iron and not show signs of chlorosis when the environment is such that iron would be inactivated. Red Kidney beans and PI soybeans have shown signs just oppostie to corn and HA soybeans. These soybean varieties were chosen for this study because they exhibited these differences but were of the same genus and species.

It is known that HCO_3 makes up a large part of the anions in the soil solution on a calcareous soil. Lunt et al. (1958) found as much as 20 me./l HCO_3 after 20 days incubation and 23 me./l after 40 days with Millville loam, a highly calcareous soil. In well aerated, non-sodic soils, they indicate the HCO_3 concentration is limited to about 8 to 10 me./l by the calcium in the soil solution, but with higher CO_2 pressure higher concentrations of HCO_3 are feasible.

A high HCO_3 concentration in the external solution does not necessarily mean that this HCO_3 is taken up into the plant when there is a high concentration of internal HCO_3. It may indicate that the HCO_3 has a definite effect upon the process of respiration, whereby the CO_2 produced by this process may not be passed to the external solution. CO_2 has been shown to be taken up and fixed by plant roots (Poel, 1953;

Jacobson, 1955; Goss, 1957; and Jackson, 1957). The fixation of CO_2 has been associated with an increased production of organic acids by the plant. With the over production of one or few organic acids, the plant tends to upset the cation absorption and translocation, thus upsetting the metabolic processes. The results of this study indicate this.

Of the species studied, red kidney beans had the highest internal HCO_3 concentration. PI soybean, being a chlorosis-susceptible variety, might be expected to follow the same trend as beans. This was apparent at the lower pH levels even though the values never reached those of beans (Figures 12 and 13). However, at pH 8.2 (Figure 14) the internal HCO_3 of PI soybean was depressed and the curve followed more closely the curve characteristic of corn, which yielded the least internal HCO_3 of the species studied. HA soybeans showed relatively no change in internal HCO_3 at pH 7.3 but did show an increase at each of the higher pH levels. The high internal HCO_3 at pH 8.2 might indicate that the metabolic processes were upset.

When comparing the pH of root sap of corn and beans it can be seen that the pH of beans increased much more than that of corn (Figures 15, 16, and 17) with increased external HCO_3. At pH 7.3 and pH 7.8 the PI soybeans tended to follow the pH increase of beans. However, at pH 8.2 they again followed the curve characteristic of corn. The pH of HA soybean tended to follow the curve characteristic of the beans. These increases were similar to those of the HCO_3 increases. The results of this study would suggest that an increase in internal HCO_3 is paralleled with an increase in pH. With a high increment change in internal HCO_3 there was a larger increment change in pH.

The phenomena suggested, however, doesn't seem to be followed by each specie of plant, especially at the higher pH levels. This may

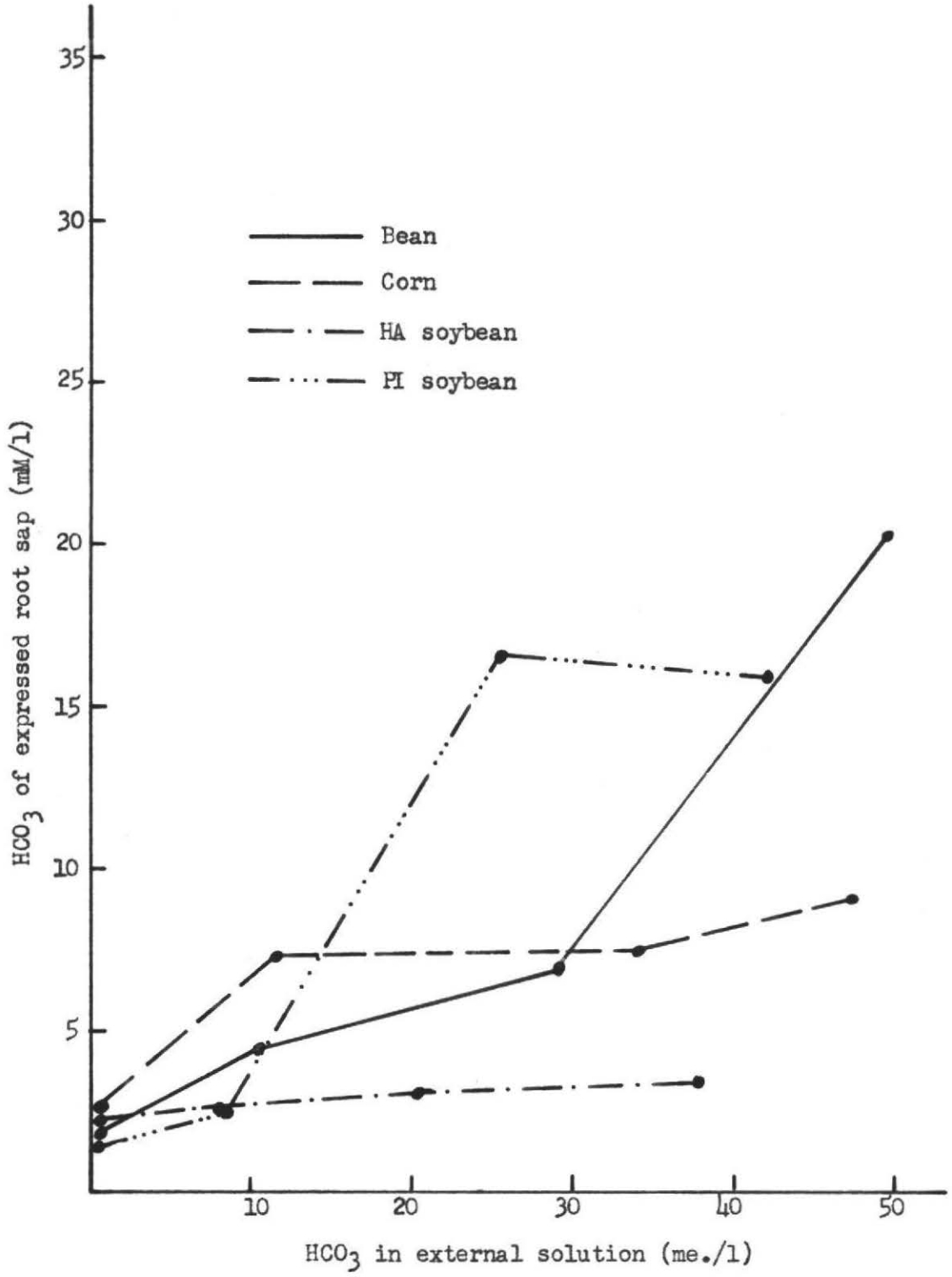

Figure 12. The bicarbonate concentration of expressed root sap of four varieties of plants compared to the bicarbonate level of the external solution at pH 7.3

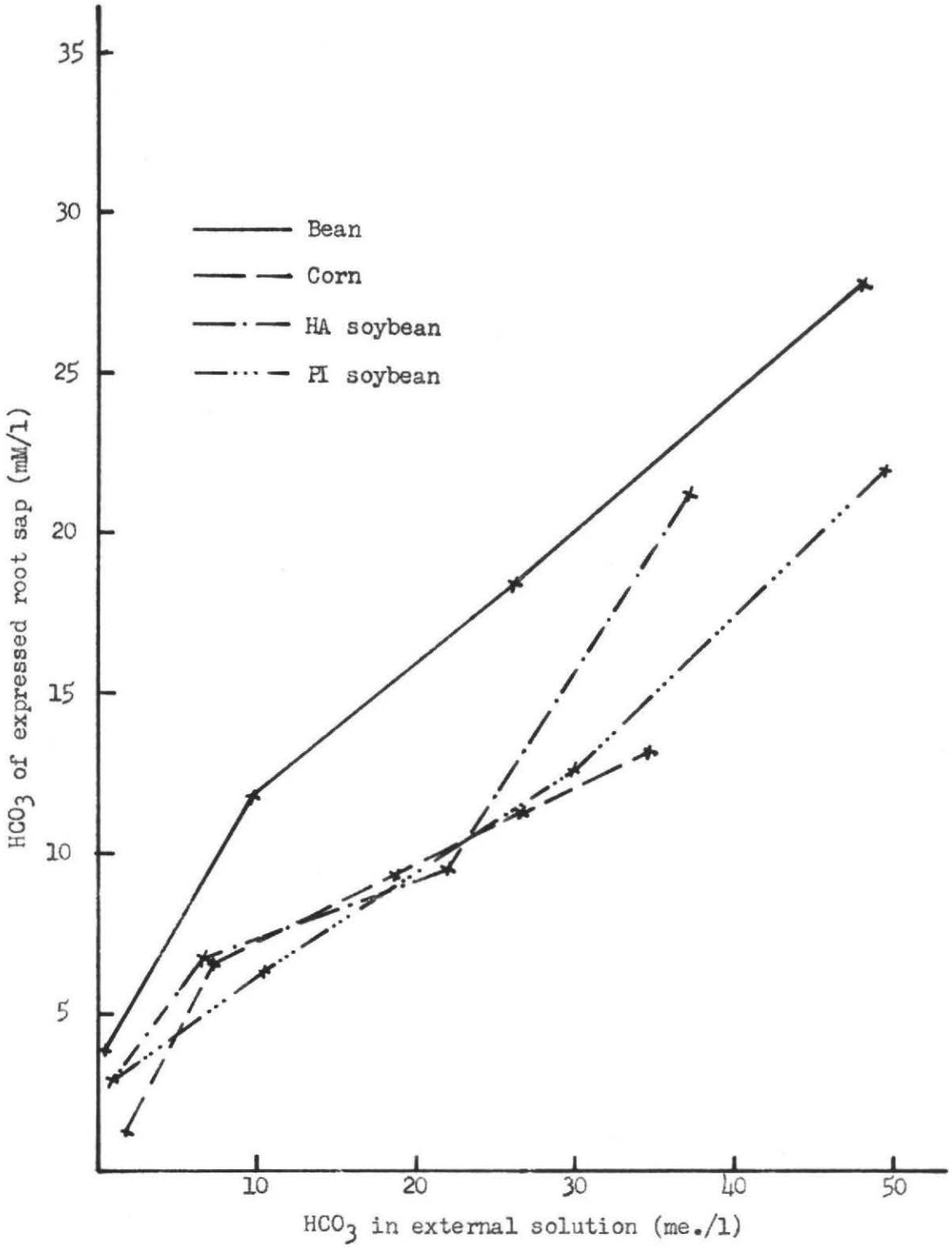

Figure 13. The bicarbonate concentration of expressed root sap of four varieties of plants compared to the bicarbonate level of the external solution at pH 7.8

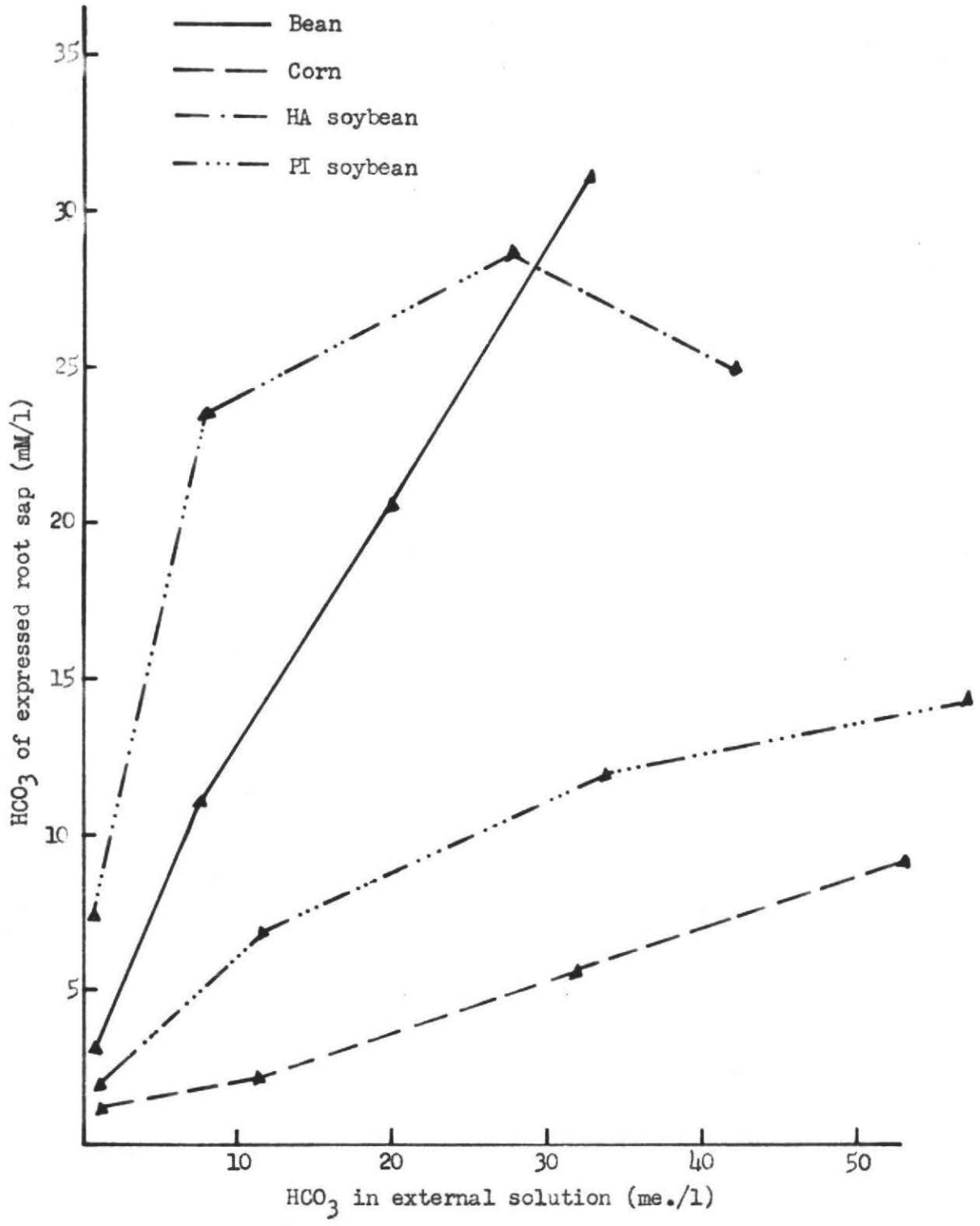

Figure 14. The bicarbonate concentration of expressed root sap of four varieties of plants compared to the bicarbonate level of the external solution at pH 8.2

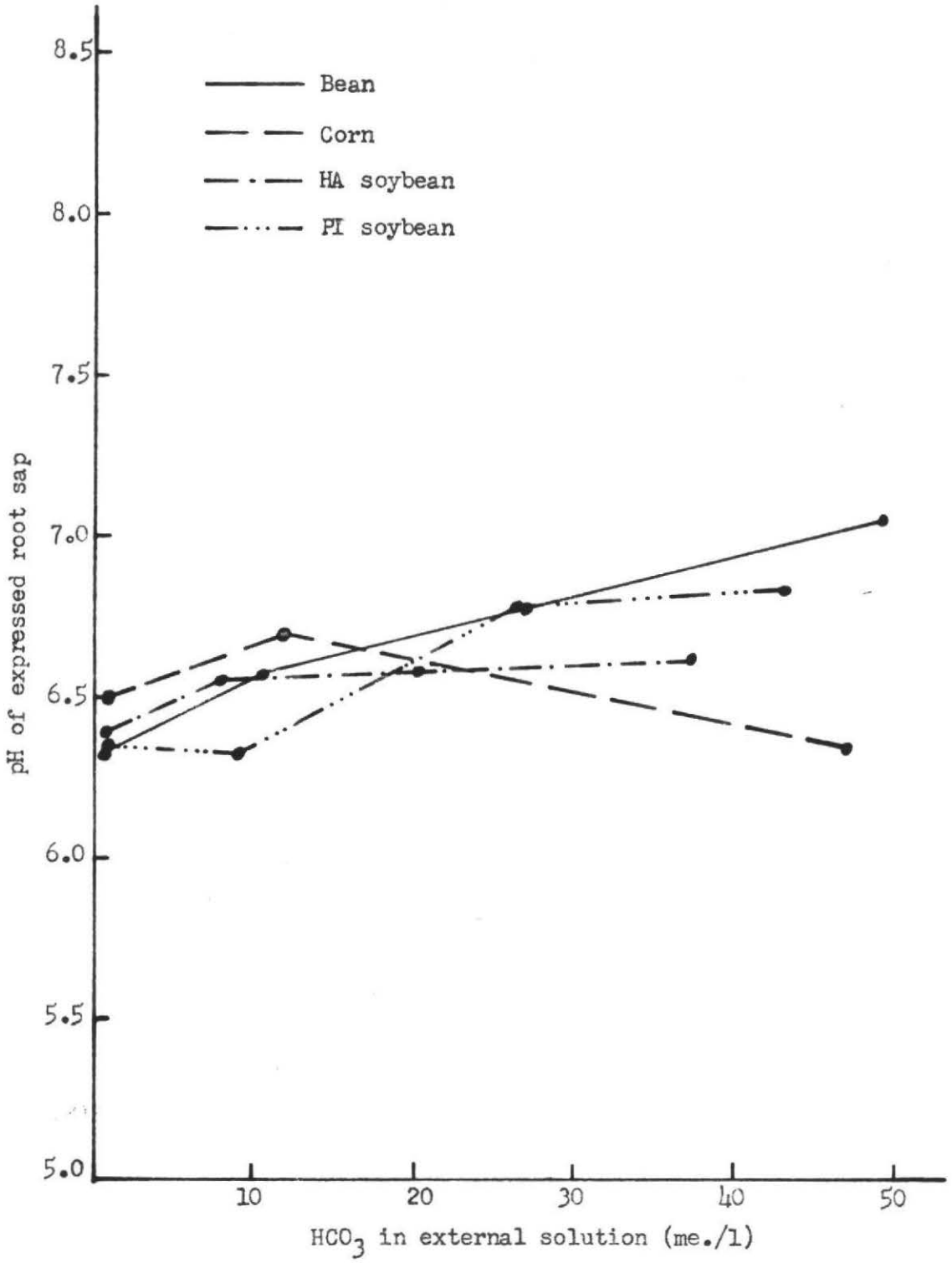

Figure 15. The pH of expressed root sap of four varieties of plants compared to the bicarbonate level of the external solution at pH 7.3

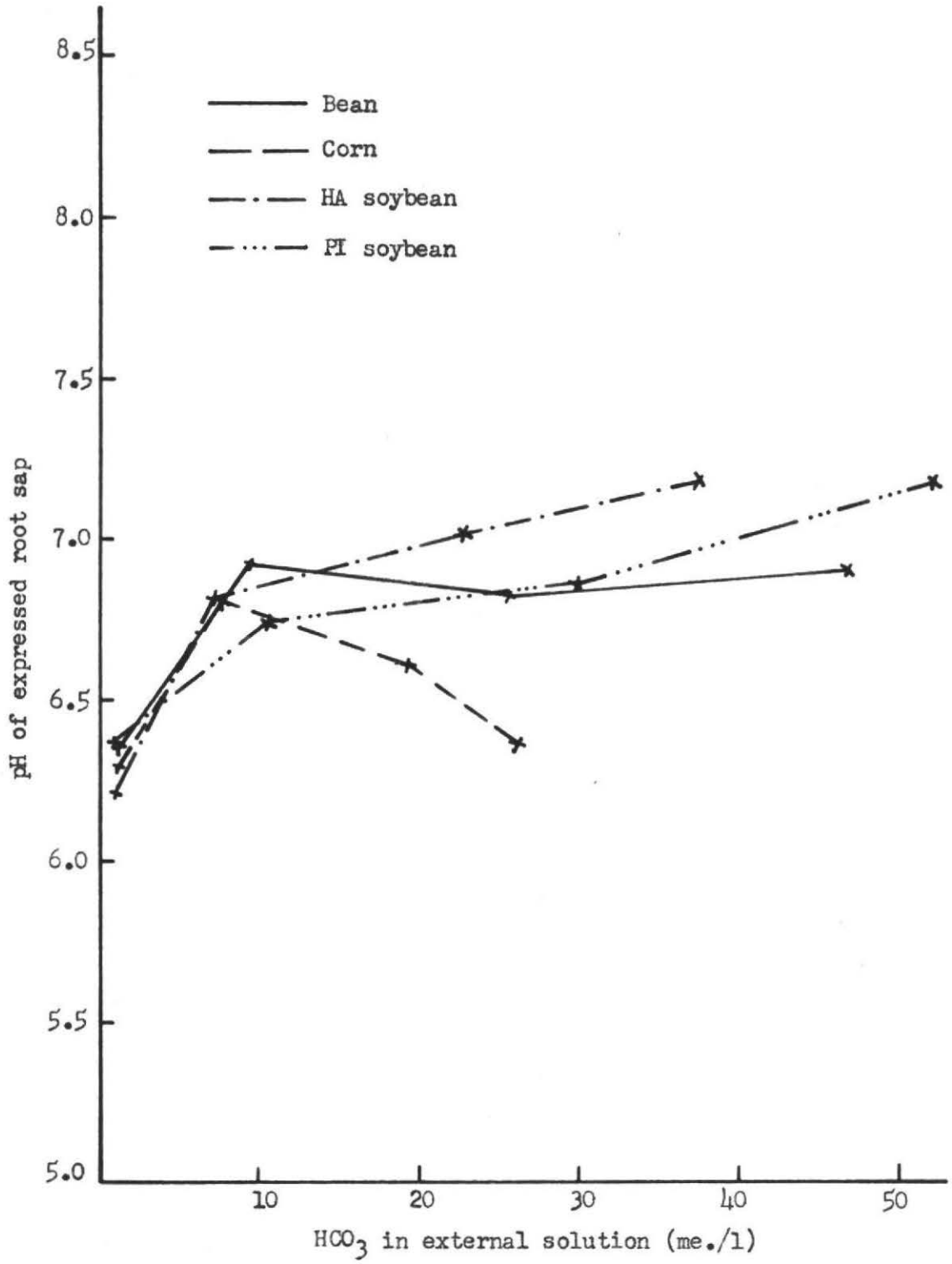

Figure 16. The pH of expressed root sap of four varieties of plants compared to the bicarbonate level of the external solution at pH 7.8

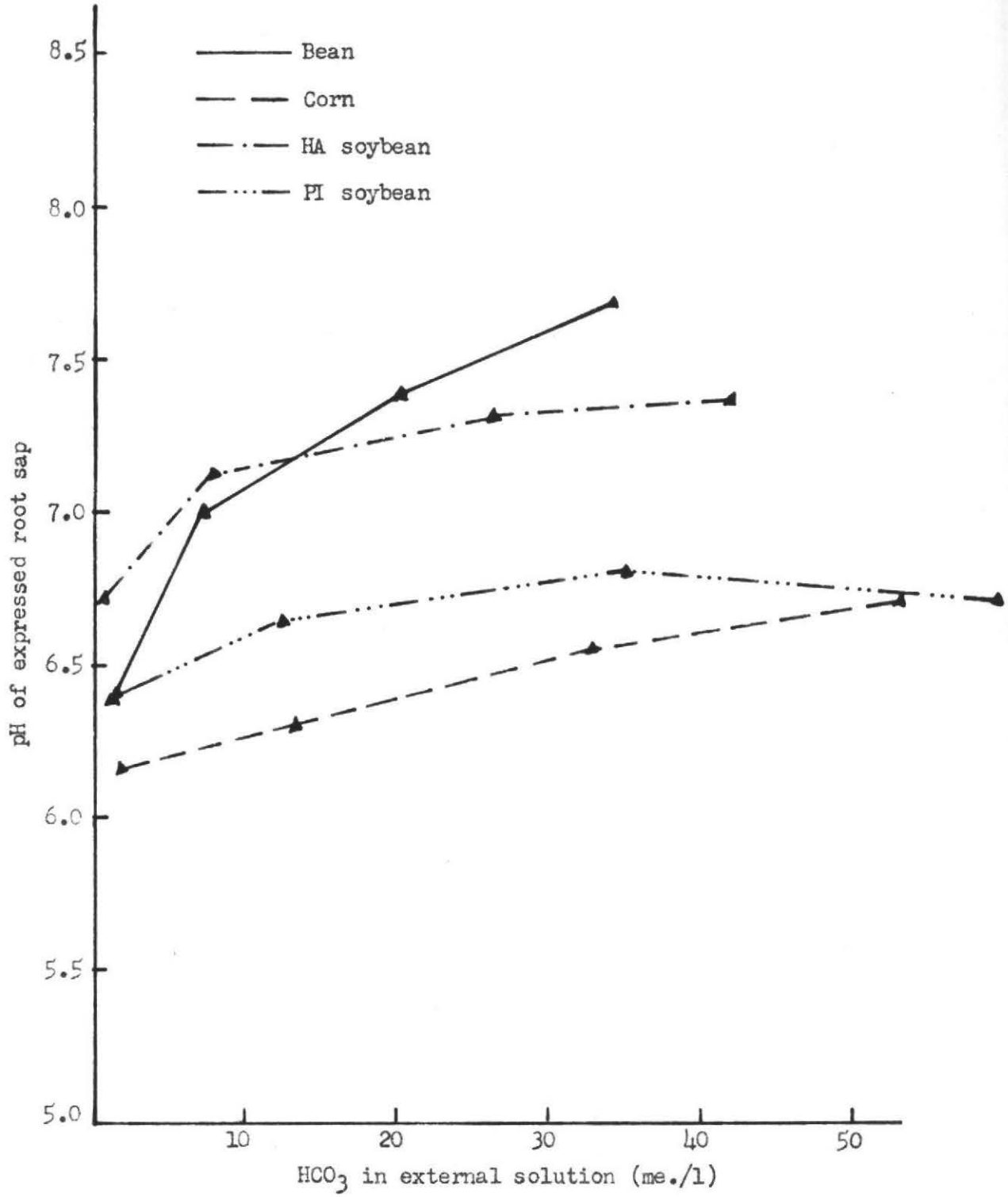

Figure 17. The pH of expressed root sap of four varieties of plants compared to the bicarbonate level of the external solution at pH 8.2

suggest the possibility that certain species may have the ability to pass from its system the CO_2 being produced within. It would be suggested that corn would have a greater ability to pass this CO_2 from its system than the other species at a high pH. It might then be suggested that corn would not be as affected by severe conditions of high HCO_3 and high pH as would other varieties of plants. HA soybeans, a chlorosis-resistant variety, may be resistant to chlorosis at the lower pH levels but might be more easily injured or have its metabolism system upset easier than corn at the higher pH levels.

With the increase in sap pH the possibility for an inactivation of iron would be greater than at a lower or stable pH. This might be accomplished by the precipitation of iron, rendering it inactive, with other substances at these higher pH ranges.

From the results of this study some correlation might be made between the internal HCO_3 concentration of plant sap and the susceptibility of the plant to chlorosis. If a high concentration of internal HCO_3 is an indication of a high organic acid content and an increased cation uptake, then the results of this work tend to verify this. With an increased internal HCO_3 concentration there is also an increase in root pH. The increase in root pH might then be due to an excess cation absorption. Although there are deviations from what might be expected, definite trends seem to be present. The theory of whether a plant is susceptible to chlorosis if it has a high internal HCO_3 would then seem to be upheld. These results point this out, especially at the pH levels of 7.8 and 7.3.

It is felt that the harvest method used in this study does not measure exactly the internal HCO_3 concentration, although it does give

a very good approximation. After considering the problem of contamination from adhering solution and diffusion outward of internal HCO_3 or CO_2 after the plant is removed from the solution, it was felt that a method for determining the HCO_3 of the intact roots would be better.

SUMMARY

The internal HCO_3 concentration of expressed root sap of chlorosis-resistant plants (kingcrost Ky 7 corn and hawkeye soybean) and chlorosis-susceptible plants (red kidney bean and P.I. 64519-5-1 soybean) were investigated. The plants were grown in nutrient solution in the greenhouse and treated at 18 and 21 days of age with 0, 10, 30, and 50 me./l $NaHCO_3$ at pH levels of 7.3, 7.8, and 8.2. The roots were harvested for the sap and the HCO_3 measured in a Van Slyke blood gas apparatus and the pH with a model GS pH meter.

Five different harvest methods were studied to determine which method of harvest gave the best value of the internal HCO_3 and pH of root sap. The pH of corn roots was determined by pH paper and indicators at low and high HCO_3 treatments with different CO_2 pressures in the aeration stream. Experiments were also conducted on corn roots to determine the uptake of HCO_3 when the intact roots were treated with a high HCO_3 solution for different lengths of time. Studies were conducted to determine the loss of HCO_3 from the roots when they were exposed to the atmosphere for different lengths of time. A study was also conducted to find the change in HCO_3 concentration when the sap samples were analyzed immediately or stored for different lengths of time in a refrigerator or in a deep freeze. The solubility of CO_2 in the mineral oil used to cover the sap samples was also determined.

Results obtained showed that of the species studied corn had the smallest change in internal pH and HCO_3 with increased external pH and HCO_3 concentration. Beans showed the greatest internal HCO_3 increase.

The soybean varieties showed increases that were within the ranges of the beans and the corn. The hawkeye variety tended to follow the increases of the corn and the PI variety tended to follow the increases characteristic of beans at the pH levels of 7.3 and 7.8 whereas at the higher pH level they showed variable results. The results suggest that an increase in internal HCO_3 is paralleled with an increase in pH. A high increment change in internal HCO_3 was associated with a higher increment change in pH. The external HCO_3 tended to have a greater effect on the internal HCO_3 of plants than the external pH.

Some correlation was pointed out between the HCO_3 concentration and a plant's resistance or susceptibility to chlorosis. Of the plant species studied, chlorosis-resistant plants tended to have a lower internal HCO_3 concentrations and chlorosis-susceptible plants tended to have a higher internal HCO_3 concentration.

LITERATURE CITED

Arnon, D. I., and Johnson, C. M. Influence of hydrogen ion concentration on the growth of higher plants and controlled conditions. Plant Physiol. 17:538-539. 1942.

Baxter, P., and Belcher, R. Role of bicarbonate ion in lime induced chlorosis. Australian Inst. Agr. Sci. Jour. 21:32-34. 1955.

Biddulph, O. The translocation of minerals in plants. In E. Troug, ed. Mineral Nutrition of Plants. University of Wisconsin Press, Madison. pp. 261-275. 1951.

Bonner, J. Plant Biochemistry. Academic Press, Inc., New York. p. 477. 1950.

Brown, J. C. The effect of the dominance of a metabolic system requiring iron or copper on the development of lime-induced chlorosis. Plant Physiol. 28:495-502. 1953.

Brown, J. C., and Hendricks, S. B. Enzymic activities as indication of iron deficiency. Plant Physiol. 27:651-660. 1952.

Brown, J. C., and Holmes, R. S. Iron, the limiting element in a chlorosis: Part I. Availability and utilization of iron dependent upon nutrition and plant species. Plant Physiol. 30:451-457. 1955.

Broyer, T. C. The nature of the process of inorganic solute accumulation in roots. In E. Troug, ed. Mineral Nutrition of Plants. University of Wisconsin Press, Madison. pp. 228-229. 1951.

Burgess, P. S., and Pohlman, G. G. Citrus chlorosis as affected by irrigation and fertilizer treatments. Arizona Agr. Sta. Bul. 124:183-232. 1928.

Carlson, G. H. Relationship of bicarbonate concentration of plant tissue to that of the growth media as a factor in chlorosis. (M. S. Thesis. Dept. of Agronomy) Utah State University, Logan. 1957.

Chapman, G. W. The relation of iron and manganese to chlorosis in plants. New Phytol. 30:266-283. 1931.

Chapman, H. D., Liebig, G. F., and Vaneslow, A. P. Some nutritional relationships as revealed by a study of mineral deficiency and excess systems on citrus. Soil Sci. Soc. Amer. Proc. 4:196-200. 1939.

DeKock, P. C. Iron nutrition of plants at high pH. Soil Sci. 79:172-173. 1955.

Epstein, E., and Stout, P. R. The micronutrient cations iron, manganese, zinc, and copper. Their uptake by plants from the absorbed state. Soil Sci. 72:47-65. 1951.

France, C. J., and Loomis, W. E. The absorption of phosphorus and iron from nutrient solutions. Plant Physiol. 22:633-634. 1947.

Fritz, F., and Beevers, H. Cytochrome oxidase content and respiratory rates of etiolated wheat and barley seedlings. Plant Physiol. 30: 309-317. 1955.

Gauch, H. G., and Wadleigh, C. H. The salt tolerance and chemical composition of Rhoades and Dallis grasses grown in sand culture. Bot. Gaz. 122:259-271. 1941.

Goss, J. A. A comparison of the effects of the bicarbonate ion with other anions on the uptake of some radioisotopes by Phaseolus vulgaris and Hordium vulgare plants. (Ph. D. Thesis. Dept. of Botany) University of California, Los Angeles. 1957.

Harley, C. P., and Lindner, R. C. Observed responses of apple and pear trees to some irrigation waters of North Central Washington. Proc. Amer. Soc. Hort. Sci. 45:35-44. 1945.

Hawk, P. B., Oser, B. L., and Summerson, W. H. Practical Physiological Chemistry. Blakiston Co., Philadelphia. pp. 642-649. 1947.

Heller, V. G., Hangeman, R. H., and Hartman, E. T. Sand culture studies of the use of saline and alkali waters in greenhouses. Plant Physiol. 15:727-773. 1940.

Hoagland, D. R., and Arnon, D. I. The water culture method for growing plants without soil. Circ. No. 347. University of California, Berkley. p. 32. 1950.

Honda, S. I. Ascorbic acid oxidase in barley roots. Plant Physiol. 30:174-181. 1955.

Hurd-Karrer, A. M. Hydrogen-ion concentration of leaf juice in relation to environment and plant species. Amer. Jour. Bot. 26:834-845. 1939.

Iljin, W. S. Bichemische vorgange wahrend der kalkchlorose der weissen lupine. Ber. Deut. Bot. Gaz. 61:138-148. 1943. (Translation: original not seen.)

Iljin, W. S. Metabolism of plants affected with lime-induced chlorosis (Calciose): II. Organic acids and carbohydrates. Plant and Soil 3:339-350. 1951.

Iljin, W. S. Metabolism of plants affected with lime-induced chlorosis (Calciose): III. Mineral elements. Plant and Soil 4:11-28. 1952.

Ingalls, R. A., and Shive, J. W. Relation of H-ion concentration of tissue fluids to the distribution of iron in plants. Plant Physiol. 6:339-350. 1931.

Jackson, W. A. Carbon dioxide fixation by plant roots and its influence on cation absorption. (Ph. D. Thesis. Dept. of Soils) North Carolina State College, Raleigh. 1957.

Jacobs, J. H. The effects of carbon dioxide on the consistency of protoplasm. Biol. Bul. 42:14-19. 1922.

Jacobs, M. H. The production of intracellular acidity by neutral and alkaline solutions containing carbon dioxide. Amer. Jour. Physiol. 53:547-563. 1920.

Jacobson, L. Carbon dioxide fixation and ion absorption in barley roots. Plant Physiol. 30:264-269. 1955.

Johnson, M. O. Manganese as a cause of the depression of the assimilation of iron by pineapple plants. Indus. Eng. Chem. 9:47-49. 1917.

Kearney, T. A., and Cameron, F. K. Some mutual relations between alkali soils and vegetation. U. S. D. A. Report 71. p. 78. 1902.

Kock, F. C., and Hanke, M. E. Practical Methods in Biochemistry. Williams and Wilkins Co., Baltimore. pp. 234-242. 1948.

Kubie, L. S. The solubility of O_2, CO_2, and N_2 in mineral oil and the transfer of carbon dioxide from oil to air. Jour. Biol. Chem. 72:545-548. 1927.

Lindner, R. C., and Harley, C. P. Nutritional interrelations in lime-induced chlorosis. Plant Physiol. 19:436-437. 1944.

Lindsay, W. L. Effect of bicarbonate ion and root aeration on lime-induced chlorosis. (M. S. Thesis. Dept. of Agronomy) Utah State University, Logan. 1953.

Lindsay, W. L., and Thorne, D. W. Bicarbonate ion and oxygen level as related to chlorosis. Soil Sci. 77:270-279. 1954.

Loewing, W. F. Effect of insulation and soil characteristics on tissue-fluid reaction in wheat. Plant Physiol. 5:293. 1930.

Lunt, O. R., Holmes, R. S., Brown, J. C., and Tiffin, L. O. Inconsistencies of the bicarbonate ion as a direct cause of chlorosis. 1958. (In preparation for publication.)

Mann, H. G. Availability of manganese and iron as affected by applications of calcium and magnesium carbonates to the soil. Soil Sci. 30:117-141. 1930.

Marcour, M. The uptake of iron by bean plants as influenced by age, and calcium and bicarbonate ions in solution cultures. (M. S. Thesis. Dept. of Agronomy) Utah State University, Logan. 1952.

Marsh, R. P., and Shive, J. W. Adjustment of iron supply to requirements of soybean in solution culture. Bot. Gas. 79:1-27. 1925.

McGeorge, W. T. Soil properties contributing to citrus chlorosis as revealed by seedling tests and field experiments. Arizona Agr. Exp. Sta. Tech. Bul. 112. 1946.

McGeorge, W. T. Nutrient interrelations in lime-induced chlorosis as revealed by seedling tests and field experiments. Arizona Agr. Exp. Sta. Tech. Bul. 116. 1948.

McGeorge, W. T. Lime-induced chlorosis on western crops. Better Crops with Plant Food. 35(5):17. 1951.

Miad, Y. The distribution of iron chlorotic pear trees. Amer. Soc. Hort. Sci. Proc. 21:93-98. 1924.

Miller, G. W. The effect of the bicarbonate ion on the respiration of excised roots. (M. S. Thesis. Dept. of Agronomy) Utah State University, Logan. 1954.

Miller, G. W., and Thorne, D. W. Effect of bicarbonate ions on the respiration of excised roots. Plant Physiol. 31:151-155. 1956.

Miller, G. W., Brown, J. C., and Holmes, R. S. Chlorosis in soybean as related to iron, phosphorus, bicarbonate, and cytochrome oxidase activity. 1959. (In preparation for publication.)

Olson, R. V., and Carlson, C. W. Iron chlorosis of sorghums and trees as related to extractable soil iron and manganese. Soil Sci. Soc. Amer. Proc. 14:109-112. 1949.

Ordin, L. Inhibition of barley root metabolism in relation to ion absorption. (Ph. D. Thesis. Dept. of Soils and Plant Nutrition) University of California, Berkley. 1954.

Poel, L. W. Carbon dioxide fixation by barley roots. Jour. Exp. Bot. 4:157-163. 1953.

Porter, L. K., and Thorne, D. W. Interrelation of carbon dioxide and bicarbonate ions in causing plant chlorosis. Soil Sci. 79:373-382. 1955.

Quinn, E. L., and Jones, C. L. Carbon Dioxide. Reinhold Publishing Corp., New York. p. 105. 1936.

Rediske, J. H., and Biddulph, O. The absorption and translocation of iron. Plant Physiol. 28:576-593. 1953.

Rhoads, W. A., Wallace, A., and Romney, E. M. Lime-induced chlorosis studied. California Agr. 13(3):6. 1959.

Ridenour, W. P., Weatherford, W. D., Jr., and Capell, R. G. Solubility of gases in molten paraffin and microcrystalline waxes. Ind. Eng. Chem. 46(2):2376-2381. 1946.

Rogers, C. H., and Shive, J. W. Factors affecting the distribution of iron in plants. Plant Physiol. 7:227-251. 1932.

Scheflon, L., and Jacobs, M. B. The Handbook of Solvents. D. Van Nostrand Co., New York. p. 586. 1953.

Seidell, A. Solubilities of Inorganic and Organic Compounds. D. Van Nostrand Co., New York. p. 812. 1952.

Shedlovsky, T., and MacInnes, D. A. First ionization constant of carbonic acid, 0° to 38° C., from conductance measurements. Jour. Amer. Chem. Soc. 57:1705-1710. 1935.

Small, J. pH and Plants. D. Van Nostrand Co., New York. pp. 25-63. 1946.

Sommers, I. I., and Shive, J. W. The iron-manganese relation in plant metabolism. Plant Physiol. 17:582-602. 1942.

Steward, F. C., and Preston, C. Effects of pH and the components of bicarbonate and phosphate buffered solutions on the metabolism of potato discs and their ability to absorb ions. Plant Physiol. 16:481-519. 1941.

Stewart, E., and Leonard, C. D. Chelates as sources of iron for plants growing in the field. Science 116:564-566. 1952.

Stolwijk, J. A. J., and Thimann, K. V. On the uptake of carbon dioxide and bicarbonate by roots, and its influence on growth. Plant Physiol. 32:513-520. 1957.

Thorne, D. W., and Wallace, A. Some factors affecting chlorosis on high-lime soils: 1. Ferrous and ferric iron. Soil Sci. 57:299-312. 1944.

Thorne, D. W., and Wann, F. B. Nutrient deficiencies in Utah orchards. Utah Agr. Exp. Sta. Bul. 338. 1950.

Thorne, D. W., Wann, F. B., and Robinson, W. Hypotheses concerning lime-induced chlorosis. Soil Sci. Soc. Amer. Proc. 15:256-258. 1950.

Thronton, N. C. The influence of CO_2 on the acidity of plant tissue. Contrib. Boyce Thompson Inst. 5:403-418. 1933.

Ulrich, A. Metabolism of non-violatile organic acids in excised barley roots as related to a cation-anion balance during salt accumulation. Amer. Jour. Bot. 28:526-536. 1941.

Van Slyke, D. D. Notes on a portable form of the manometric gas apparatus and on certain points in the technique of its use. Jour. Biol. Chem. 73:121-126. 1927.

Van Slyke, D. D., and Neill, J. M. The determination of gases in blood and other solutions by vacuum extraction and manometric measurements. Jour. Biol. Chem. 61:523-573. 1924.

Van Slyke, D. D., and Sendroy, J., Jr. Carbon dioxide factors for the manometric blood gas apparatus. Jour. Biol. Chem. 73:127-144. 1927.

Wadleigh, C. H., and Brown, J. W. The chemical status of bean plants afflicted with bicarbonate-induced chlorosis. Bot. Gaz. 113(4): 373-392. 1952.

Wall, R. F., and Cross, F. B. Greenhouse studies of the toxicities of Oklahoma salt contaminated waters. Oklahoma Agr. Exp. Sta. Tech. Bul. T-20. 1943.

Wann, F. B. Control of chlorosis in American grapes. Utah Agr. Exp. Sta. Bul. 299. 1941.

Warnock, R. E. The iron content of some plants as influenced by conditions associated with lime-induced chlorosis. (M. S. Thesis. Dept. of Agronomy) Utah State University, Logan. 1952.

APPENDIX

Table 19. Bicarbonate, pH, and carbon dioxide of expressed sap of corn roots grown in nutrient solution at different levels of pH and bicarbonate

Proposed	pH 7.3			pH 7.8			pH 8.2		
$NaHCO_3$	pH	HCO_3	CO_2	pH	HCO_3	CO_2	pH	HCO_3	CO_2
me./l		mM/l	mM/l		mM/l	mM/l		mM/l	mM/l
				Root Sap					
0	5.9	1.5	4.3	6.5	1.3	1.7	6.3	1.4	1.6
	6.6	2.0	1.1	6.9	4.4	1.4	6.2	1.4	2.3
	6.4	2.5	2.2	6.9	1.5	6.0	5.7	0.7	2.8
	6.8	4.7	1.6	6.4	1.4	1.2	6.3	1.6	2.7
10	6.9	7.7	2.4	6.2	3.4	4.7	6.3	2.5	3.4
	6.7	6.3	3.0	6.9	8.9	2.8	6.3	2.8	3.0
	6.2	4.4	7.6	6.9	6.6	2.1	6.4	3.1	3.3
	7.0	11.1	2.8	6.6	4.6	3.1	6.1	1.4	2.7
30	-	-	-	6.1	6.5	12.0	6.5	3.8	2.7
	7.0	20.1	5.2	7.2	14.3	2.1	6.2	5.6	5.8
	6.1	10.1	17.8	6.6	7.4	4.7	6.7	3.0	1.3
	6.1	7.6	15.6	6.5	8.8	6.3	6.7	10.3	4.8
50	6.6	22.7	12.2	6.9	19.4	5.5	6.7	7.4	3.1
	6.0	7.3	19.8	6.3	11.4	13.1	6.6	9.7	6.7
	6.5	8.5	6.2	6.0	5.2	12.8	6.4	10.8	6.7
	6.0	6.1	15.8	6.1	8.7	18.0	6.9	8.9	2.8
				Nutrient Solution					
0	6.0	0.3	0.7	7.8	1.5	0.1	7.3	5.6	0.6
	5.9	1.3	0.1	7.6	7.3	0.5	7.0	1.2	0.3
	7.1	1.3	0.2	6.2	0.5	0.7	6.6	0.4	0.3
	6.2	0.6	0.8	7.2	1.2	0.1	6.6	1.2	0.8
10	7.4	10.3	0.9	5.8	0.4	1.4	-	-	-
	7.8	15.7	0.7	7.8	7.3	0.3	7.3	13.2	1.6
	7.6	11.2	0.6	7.5	7.3	0.6	7.4	10.4	0.9
	7.1	10.2	0.8	7.7	6.6	0.3	6.7	15.0	6.6
30	7.1	41.3	8.0	7.1	16.3	3.4	8.2	40.5	0.7
	7.8	3.6	0.2	7.6	19.1	1.2	7.8	30.4	1.1
	6.9	32.2	9.0	7.6	19.5	1.3	7.6	30.8	2.0
	6.2	15.9	24.9	6.7	20.8	10.4	7.7	29.6	1.6
50	7.4	47.2	4.9	7.7	34.3	1.6	7.6	57.4	3.2
	7.4	47.4	4.3	6.0	16.4	27.0	7.8	48.0	1.7
	7.8	46.6	1.6	7.3	30.6	3.8	7.8	48.3	1.9
	6.8	48.4	1.7	7.1	27.0	5.4	8.2	58.4	0.9

Table 20. Bicarbonate, pH, and carbon dioxide of expressed sap of bean roots grown in nutrient solution at different levels of pH and bicarbonate

Proposed $NaHCO_3$	pH 7.3			pH 7.8			pH 8.2		
	pH	HCO_3	CO_2	pH	HCO_3	CO_2	pH	HCO_3	CO_2
me./l		mM/l	mM/l		mM/l	mM/l		mM/l	mM/l
				Root Sap					
0	6.5	2.0	1.5	6.6	6.4	3.8	6.5	2.1	1.7
	6.4	2.2	2.2	6.4	5.9	5.8	6.4	3.0	2.7
	6.1	0.8	1.5	6.2	2.0	2.6	6.4	5.4	0.6
	6.2	1.3	1.8	6.0	0.8	1.8	6.3	2.5	2.8
10	6.7	4.3	2.2	7.0	9.3	2.0	7.1	9.0	0.2
	6.6	6.0	3.7	6.9	10.3	3.0	6.8	12.8	4.6
	6.5	3.5	2.4	6.8	11.0	3.8	6.8	7.9	2.8
	6.5	3.4	2.4	6.8	16.0	5.3	7.1	11.0	2.0
30	6.8	9.6	3.5	6.6	9.0	5.5	7.4	23.1	2.4
	6.6	5.0	2.8	7.2	23.2	3.7	7.2	16.3	2.5
	6.8	6.3	2.6	6.5	17.5	13.4	7.6	20.9	1.3
	6.8	7.2	2.7	7.0	23.6	5.2	7.4	21.4	2.3
50	6.9	14.4	4.4	7.2	34.9	5.2	8.0	30.9	0.8
	7.3	32.8	3.7	6.8	20.7	8.5	7.7	36.2	2.0
	6.8	12.0	4.3	6.9	31.1	9.7	7.5	30.7	2.5
	7.2	21.8	3.4	6.7	24.0	10.7	7.6	30.7	1.9
				Nutrient Solution					
0	6.7	0.3	0.2	7.0	0.4	0.1	7.4	1.4	0.1
	7.0	0.6	0.0	6.6	0.3	0.2	7.6	1.3	0.1
	6.8	0.3	0.1	6.8	0.6	0.2	7.4	1.4	0.1
	6.1	0.3	0.5	6.9	1.0	0.3	7.5	1.2	0.1
10	7.5	10.7	0.8	7.8	9.1	0.4	8.0	8.4	0.2
	7.3	11.5	1.4	7.8	10.0	0.4	8.2	6.1	0.1
	7.5	9.8	0.7	7.8	9.3	0.4	7.8	6.4	0.2
	7.4	9.7	0.9	7.5	9.0	0.7	8.1	7.7	0.1
30	7.3	27.3	3.2	7.8	16.6	0.6	8.4	21.4	0.2
	7.8	26.9	0.9	8.0	28.7	0.7	8.5	20.3	0.2
	7.6	31.1	1.8	7.8	27.6	1.0	8.4	16.4	0.2
	7.8	21.8	0.9	7.9	28.5	0.9	8.3	21.5	0.2
50	7.4	46.2	4.3	8.0	48.6	1.2	8.5	27.9	0.2
	7.9	39.8	1.1	8.0	48.1	1.2	7.6	35.0	1.9
	7.4	58.0	6.0	7.9	43.6	1.2	8.3	45.0	0.6
	7.6	51.0	3.5	8.1	46.3	0.8	8.4	27.1	0.3

Table 21. Bicarbonate, pH, and carbon dioxide of expressed sap of Hawkeye soybean roots grown in nutrient solution at different levels of pH and bicarbonate

Proposed	pH 7.3			pH 7.8			pH 8.2		
$NaHCO_3$	pH	HCO_3	CO_2	pH	HCO_3	CO_2	pH	HCO_3	CO_2
me./l		mM/l	mM/l		mM/l	mM/l		mM/l	mM/l
				Root Sap					
0	6.5	3.9	3.0	6.2	1.7	2.2	6.8	8.2	3.1
	6.2	1.1	1.6	6.4	2.9	2.7	7.1	25.0	5.2
	6.2	1.8	2.6	6.2	1.8	3.0	6.7	6.8	3.5
	6.5	2.7	2.2	6.0	4.6	1.0	6.5	4.3	3.6
10	6.5	1.6	1.3	6.9	7.6	2.6	6.8	18.2	7.1
	6.5	1.5	1.3	7.3	25.7	3.6	7.3	23.9	2.7
	6.5	2.8	2.1	6.5	3.8	2.7	7.2	27.6	3.8
	6.7	4.2	2.0	6.7	6.2	2.7	7.2	24.1	3.8
30	6.6	2.0	2.5	7.0	9.0	2.5	6.5	19.2	14.8
	6.6	2.6	1.6	6.9	7.4	2.1	7.4	37.2	3.3
	6.7	4.8	2.5	7.1	20.1	4.0	7.3	23.0	3.0
	6.4	2.8	2.4	7.0	8.2	1.9	7.4	32.4	3.5
50	6.6	3.2	1.9	7.0	8.0	1.9	6.8	14.1	4.9
	6.6	2.5	1.4	7.0	12.7	3.2	7.3	25.4	2.8
	6.5	1.7	1.3	7.3	32.7	3.7	7.4	26.1	2.3
	6.8	6.1	2.5	7.3	31.4	4.0	7.5	28.5	2.0
				Nutrient Solution					
0	5.8	0.2	0.8	6.1	0.3	0.7	6.5	0.6	0.5
	6.0	0.3	0.7	5.7	0.2	1.3	6.3	0.5	1.8
	5.8	0.3	1.0	6.0	0.4	1.0	6.8	0.7	0.3
	5.8	0.3	1.0	5.9	0.2	0.5	6.7	0.8	0.4
10	7.2	8.0	1.4	7.2	7.1	1.2	7.7	8.4	0.4
	7.2	2.9	1.3	7.0	6.8	1.8	8.1	8.4	0.2
	7.1	7.9	1.5	7.2	7.7	1.1	7.6	7.7	0.4
	7.2	6.5	1.1	6.9	5.7	1.6	7.2	7.7	1.2
30	7.7	22.0	1.2	7.6	22.6	1.4	8.3	33.9	0.4
	7.6	20.2	1.2	7.5	23.4	1.2	8.2	27.0	0.4
	7.7	20.0	1.0	7.5	22.3	1.6	8.7	25.0	0.1
	7.5	19.6	1.6	7.5	22.7	1.6	-	-	-
50	7.6	38.7	2.5	7.9	39.3	1.1	8.2	42.4	0.7
	7.4	34.8	3.4	7.5	35.9	2.7	8.3	43.0	0.6
	7.7	40.2	1.9	7.7	35.3	1.7	8.2	42.2	0.7
	7.7	37.0	1.8	7.9	38.8	1.3	8.7	41.8	0.2

Table 22. Bicarbonate, pH, and carbon dioxide of expressed sap of PI soybean roots grown in nutrient solution at different levels of pH and bicarbonate

Proposed	pH 7.3			pH 7.8			pH 8.2		
$NaHCO_3$	pH	HCO_3	CO_2	pH	HCO_3	CO_2	pH	HCO_3	CO_2
me./l		mM/l	mM/l		mM/l	mM/l		mM/l	mM/l
				Root Sap					
0	6.4	1.2	1.3	6.4	3.6	3.0	6.4	1.9	1.6
	6.3	1.7	2.0	6.4	2.2	2.2	6.3	1.6	1.9
10	6.3	2.2	2.5	6.8	6.3	2.4	6.6	6.5	3.5
	6.3	2.3	2.8	6.7	5.9	3.9	6.6	7.1	3.8
30	6.8	22.2	8.4	6.8	12.7	4.5	6.6	11.7	6.7
	6.8	11.1	4.5	6.9	12.2	3.8	6.8	15.8	5.5
50	6.9	19.3	5.7	7.0	23.5	5.6	6.6	15.5	9.2
	6.7	12.1	5.8	7.4	20.1	2.2	6.3	12.6	14.5
				Nutrient Solution					
0	6.9	0.3	0.1	7.0	0.4	0.1	7.2	0.7	0.1
	7.0	0.2	0.1	7.0	0.4	0.1	7.3	0.7	0.1
10	7.5	8.5	0.7	7.6	9.8	0.7	7.8	11.6	0.4
	7.6	8.8	0.5	7.6	9.8	0.6	7.4	12.0	1.1
30	7.7	26.9	1.3	7.6	27.8	1.7	8.1	32.8	0.7
	7.7	24.2	5.2	7.8	30.3	1.3	7.7	34.6	1.6
50	7.5	41.3	3.0	7.6	49.9	4.1	7.8	57.7	2.3
	7.5	43.0	3.3	7.5	47.9	1.2	7.8	55.8	2.3