FACTORS AFFECTING THE DISTRIBUTION OF IRON IN PLANTS[1]

C. H. ROGERS AND J. W. SHIVE

(WITH EIGHT FIGURES)

I. Introduction

The influence of acid and alkaline reactions of the growth medium on iron relationships in plants has been known, or at least suspected, for over a century. The internal conditions of the plant in their relation to iron metabolism and chlorophyll production are not at all well understood. A number of investigations (**22, 23, 20, 18, 16, 14, 10, 3, 1**) have been carried out in which the general conclusion is that the reaction of the growth medium governs, to a large extent, the quantities of iron available to the plant. These observations are in keeping with the results obtained by PATTEN and MAINS (**24**). Working with HCl solutions containing definite concentrations of iron, they found that, beginning with a pH of 3.5, iron was precipitated in increasing amounts up to pH 6.0, above which point practically no iron remained in solution. This was accomplished by adding, in separate experiments, ammonium hydroxide, sodium hydroxide, and hydrogen sulphide to the hydrochloric acid solutions containing definite quantities of iron. The range of iron precipitation under these conditions thus extends from pH 3.5 as the lower limit to pH 6.0 as the upper limit.

All of this work deals with the influence of the reaction of the growth medium on the availability of iron. Very little has been said regarding the possible influence of internal reactions on the availability and mobility of iron within the plant. Investigations (**21, 12, 13, 17**) in which actual quantitative measurements were employed in determining iron in the plant tissues generally indicate that plants chlorotic from lack of available iron contain as much, or more, total iron per unit weight of tissue than do normal green plants of the same species. From this it follows that certain internal conditions may render unavailable to the chlorophyllous cells the iron already in the plant.

INGALLS and SHIVE (**15**) found that not only does the H-ion concentration of plant tissue fluids fluctuate over a 24-hour day and night period, but that the soluble (filterable) iron content also fluctuates, and, in most cases, is directly proportional to the H-ion concentration of the respective tissue fluids. The pH of fleshy succulents fluctuates much more than does that of thin-leaved or non-succulent forms, the H-ion concentration in all

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cases being higher at night and decreasing with increased light intensity. Plants with a pH of tissue fluids lying near or at the upper limits of iron precipitation show a much greater fluctuation of filterable iron content following variation in pH of tissue fluids than do plants with a pH near the lower limits of iron precipitation.

GUSTAFSON (**11**), using *Bryophyllum calycinum,* found a fluctuation of approximately two pH units over a 24-hour period. On the other hand, plants kept in darkness (all other factors being similar) showed very little or no fluctuation over the same 24-hour period.

AUBERT (**4**) was one of the pioneers who attempted to explain why plants were more acid at night than in the daytime. His work was done with cacti,—fleshy succulents in which there is considerable fluctuation in pH from day to night. He concluded that increased acidity at night is due to the formation of organic acids, chief of which is malic acid. The acid formation is the result of incomplete or partial oxidation of the more complex compounds. In the daytime, these acids are broken down by the sunlight: a photolytic process. SPOEHR (**29**), working with the same plant, arrived at conclusions similar to those of AUBERT. By the action of sunlight and a mercury arc lamp, he found that malic acid solutions are decomposed and the acidity of expressed cactus sap is greatly decreased. RICHARDS (**26**), also using the cactus, arrived at conclusions similar to those of AUBERT and SPOEHR. He thinks, however, that this partial oxidation, acid-forming process, goes on in the daytime as a part of natural processes, but is hidden, for the most part, by photolytic action, which breaks down the acids formed. WARMING (**30**) finds it generally true that in succulent xerophytic land plants the air-containing intercellular spaces are very narrow. In this connection he cites measurements by ALTENKIRCH. From these facts and the researches of AUBERT (**4**), WARMING thinks that this limited air passage does not allow for sufficient oxygen supply; hence the formation of acids due to partial oxidations.

II. Experimentation

A. INTRODUCTORY

It has frequently been found that certain plants become chlorotic, apparently from lack of iron in the chlorophyllous cells of the leaves, although excessive quantities of iron are present in the tissues of the stems and leaves of these plants. The problem here investigated is an attempt to find in just what tissues these excessive accumulations of iron occur, and to determine why such a phenomenon occurs in some plants and not in others.

Experiments were planned whereby quantitative determinations might be made for total and soluble iron content of plants having juices with very high pH values and others having tissue fluids with very low pH values, the

plants being grown both in artificial media and in the open field. For determinations of soluble iron, the juices of the stems and leaves were extracted and filtered and the filtrates analyzed. Total iron was determined by analyses of the dried plant tissues. A study was then made of the relation between soluble and total iron content of these plants and the pH values of the extracted tissue fluids, to determine to what extent pH values of the extracted juices influenced distribution and availability of iron in the plants for chlorophyll production.

An investigation was also undertaken to determine the H-ion concentrations of the various tissues and of cells in sectional areas of the different organs; and to study the influence of pH values of the different tissues upon iron accumulations in them, and upon translocation and precipitation of iron in these plants. This investigation was carried out by means of microchemical methods and microscopical observation of sectional areas, which are described in the following pages.

B. Treatment of samples preparatory to analysis

In order to obtain comparative results, plant samples for pH and iron determinations must be representative, and must be taken at definite intervals over a period of 24 hours, since it has been shown that the pH of plant sap varies with light intensity (**11, 15**) and the soluble iron content varies with the pH (**15**). In the present series of experiments, it was decided to collect samples at 4-hour intervals, namely, at 10 A. M., 2 P. M., 6 P. M., 2 A. M., and 6 A. M. The collection of samples was always made on clear days, so that maximum differences in light intensity between day and night could be obtained.

The samples were broken or cut with a highly polished, stainless steel knife into small pieces and thoroughly mixed, so that representative samples for analyses could be obtained. This was the only time during the procedure that the material was allowed to come in contact with anything but glass surfaces. Part of the material collected at each interval was put into small glass dishes suitable for weighing. From this, dry weight and total iron determinations were made. The other part of each sample was put into glass vials, paraffined cork stoppers were inserted, and the whole plunged into an ice-salt mixture and frozen as soon as possible. After being thoroughly frozen, the material was allowed to thaw in warm water and the sap was extracted by means of a small handscrew press. Two or 3 cc. of the sap were poured into a short Pyrex tube, and electrometric pH determinations were made immediately. The remainder of the sap (at least 4 or 5 cc.) was filtered through a high-grade quantitative filter-paper. One cc. duplicate or triplicate samples of the filtered juices were measured into Pyrex digestion tubes immediately after filtration was completed. Iron

analyses of these samples were later made. Green weights were quickly obtained and recorded, using the material set aside for this determination. These procedures were accomplished as quickly as possible after sampling, in order to avoid any appreciable physical or chemical changes before determinations were completed. After weighing the green tissue, it was dried in an electric oven at 85° C. for 48 hours, and then six to eight hours at 102°. The dry weights were recorded and this material was preserved for total iron determinations. In order to calculate the amount of filterable (soluble) iron per gram of dry tissue, the number of grams of water per gram dry weight was determined, and this figure multiplied by the figure representing the amount of iron found in the 1-cc. sample of filtered juice; the resulting figure was taken to represent the amount of soluble iron per gram of dry tissue.

The material to be collected was thoroughly washed by spraying with distilled water once the day before and again three or four hours before collecting of samples began. This was done to rid the tissue, as much as possible, of dust and dirt that might have accumulated during growth of the plants.

III. Analytical methods

A. Microchemical

pH DETERMINATIONS.—In making pH determinations of cells and tissues, a series of indicator dyes recommended by CLARK (**5**) and SMALL (**27**) was used. The series consisted of the following eight dyes, listed with their color and pH ranges:

Meta Cresol Purple	Red 1.2–2.8 Yellow
LaMotte Yellow	Red 2.6–4.2 Yellow
Bromphenol Blue	Yellow 3.0–4.6 Blue
Bromcresol Green	Yellow 3.8–5.4 Blue
Methyl Red	Red 4.4–6.0 Yellow
Chlorphenol Red	Yellow 5.2–6.8 Red
Bromthymol Blue	Yellow 6.0–7.6 Blue
Phenol Red	Yellow 6.8–8.4 Red

Aqueous solutions of the alkali salts were prepared according to directions given by CLARK (**5**).

For making the actual pH determinations, free-hand sections of the tissues were made with a sectioning razor, the sections quickly rinsed in distilled water, and placed in spot plate depressions containing the dye solutions. A large spot plate with eight depressions was used, so that different sections of the same tissue could be put in all of the eight dyes at the same time. After standing half an hour or more, observations could be made. This was accomplished by quickly rinsing the sections in distilled water,

placing on a slide in a drop of distilled water, and immediately observing with the microscope.

IRON TESTS.—Tests for iron were made by making free-hand sections with a high quality, highly polished, stainless steel sectioning razor, quickly rinsing in distilled water, treating with a dilute (1–15) solution of hydrochloric acid, and then from 15 to 30 minutes with a 1.5 per cent. solution of potassium ferrocyanide. In the presence of iron, a blue precipitate is formed. This iron complex, commonly called Prussian blue or Berlin blue, is ferric ferrocyanide, and is formed in the presence of ferric iron. Ferrous iron may be determined by using potassium ferricyanide, but for total iron (ferrous and ferric iron) this is unnecessary, since the ferrous ferrocyanide formed in the presence of ferrocyanide and ferrous iron soon oxidizes to the ferric ferrocyanide. These tests may also be made either directly on the micro slide, or in watch glasses and then transferred to the slide. In all of the microchemical tests, the sections were handled with glass-tipped forceps.

B. MACROCHEMICAL

pH DETERMINATIONS.—pH determinations were made on about 3 cc. of extracted sap, by means of a hydrogen electrode and Type K Leeds and Northrup potentiometer. Hydrogen was bubbled through the solution until a constant potential was obtained, a procedure which usually required about 40 seconds. The electrode was cleaned and blacked frequently and checked against a standard buffer solution at frequent intervals throughout the work.

IRON ANALYSES.—Iron analyses were made according to the WONG (**31**) method, somewhat modified, which consisted of thoroughly digesting the sample with sulphuric acid, oxidizing with sodium chlorate, and adding potassium thiocyanate; after which the contents were made to a known volume and colorimetrically compared with a known standard solution. The red coloration obtained is due to the formation of ferric thiocyanate. The analyses were made in Pyrex digestion tubes, graduated to 12.5 cc. and 25 cc. Details of the method are given by INGALLS and SHIVE (**15**), and may therefore be omitted here.

The material for total iron was furnished by that used in making moisture and dry weight determinations. This material was ground to a powder with a mortar and pestle and allowed to dry for a day, first in the oven at 75° C. for six hours, and then in a desiccator. One-tenth gram samples were accurately weighed and transferred to the previously mentioned graduated Pyrex digestion tubes.

IV. Influence of H-ion concentration on soluble and total iron content

The purpose of these experiments was to study the relations of H-ion concentration to total iron and soluble iron in plants with juices having very high and very low pH reactions, and to determine whether the filterable (soluble) iron content of such plants remains fairly constant over a 24-hour day and night period under these conditions of very high and very low pH reactions. In accordance with the work of PATTEN and MAINS (**24**), in which they found that all iron was precipitated from a hydrochloric acid solution at and above a pH of 6.0, while all iron remained in solution below a pH of 3.5, it might be inferred that plant juices with a pH above 6.0 contain no soluble iron, and those with a pH below 3.5 contain iron in the soluble form only. From the data presented in these experiments, it becomes apparent that the results obtained by PATTEN and MAINS with hydrochloric acid solutions cannot be applied to extracted plant juices.

By determining the pH values of the expressed juices of a number of species, it was found that the juices of *Oxalis repens* and *Rumex acetosella* had the lowest pH values of any species tested, while *Solanum tuberosum* and *Glycine max* juices had rather high pH values. These plants were grown in ordinary wooden flats containing a well composted and thoroughly mixed soil.

Samples of the plants were collected at 4-hour intervals over a 24-hour (day and night) period, and the juices extracted as previously described. The results of the pH and iron determinations of the juices of these plants are given in tables I and II, and the averages of these over the 24-hour period are given in table III. The data from tables I and II are illustrated graphically in figures 1 and 2 respectively.

In preparing the graphs illustrating the relations of light intensity, H-ion concentration, and soluble iron content, both iron and pH values were plotted on the same graph, using a common abscissa but different ordinates. For purposes of clearer illustration, the values for soluble iron were arranged in the inverse or descending order, since these values decrease with increasing pH values.

It has been shown by INGALLS and SHIVE (**15**), and it might well be expected, that in such a plant as *Bryophyllum,* where the pH of the plant juice fluctuates widely from near the lower limits to the upper limits of iron precipitation (pH 3.5–6.0), according to PATTEN and MAINS, the soluble iron content might also vary over a wide range during consecutive periods of high and low light intensity; and it has indeed been found that the higher the light intensity and the higher the pH of the plant tissue

fluids, the lower is the soluble iron content. If the results of PATTEN and MAINS could apply to plant juices, it might then be expected that in plants which show fluctuating pH values, either entirely above the extreme upper limit (pH 6.0) of iron precipitation or entirely below the extreme lower limit (pH 3.5), there should be little or no variation in the soluble iron content with variation in light intensity and with resulting variations in pH values of tissue fluids. Again, from the experimental results presented here, it will be seen that such values do not hold true in the case of extracted plant juices.

INGALLS and SHIVE (**15**), working with nine different species with pH values ranging from 4.04 to 6.1, found the soluble iron content to vary in an inverse relation with the pH values; that is, decrease in pH values of plant juices is followed by a corresponding increase in soluble iron content, and *vice versa*. Some of the species showed much greater fluctuations in pH values and soluble iron content of the tissue fluids than did others, the fluctuation increasing with increased succulency of the plants.

OXALIS AND RUMEX

It will be observed from table I that fluctuations in the pH of the plant juices of *Oxalis* and *Rumex* do not vary through wide ranges with variation in light intensity from day to night. *Oxalis* shows a range in pH

TABLE I

pH, FILTERABLE AND TOTAL IRON IN *Rumex* AND *Oxalis* OVER A 24-HOUR PERIOD

SPECIES	TIME OF COLLECTING	pH	IRON (MG. PER GM. DRY WEIGHT)	
			FILTERABLE	TOTAL
			mg.	*mg.*
Oxalis (leaf)	10 A. M.	1.995	0.063	0.167
	2 P. M.	1.893	0.053	0.158
	6 P. M.	1.961	0.047	0.183
	10 P. M.	2.198	0.050	0.189
	2 A. M.	2.164	0.072	0.165
	6 A. M.	1.927	0.061	0.160
Rumex (leaf)	10 A. M.	2.502	0.092	0.194
	2 P. M.	2.603	0.072	0.194
	6 P. M.	2.671	0.075	0.196
	10 P. M.	2.570	0.077	0.206
	2 A. M.	2.520	0.092	0.191
	6 A. M.	2.468	0.093	0.200

variation from 2.198 to 1.893, and the juices of *Rumex* show a variation between 2.671 and 2.468 from day to night. The filterable iron content of the *Oxalis* plant shows a corresponding range of variation, but in this plant there appears to be no relation between the variation in pH and the varia-

tion in soluble iron content; while *Rumex* shows the definite inverse relation between pH and filterable iron content of plant juices which has been found for many other species. It is to be emphasized that although these fluctuations are no doubt significant, they vary over a rather narrow range.

While the juices of *Rumex* at all times show pH values considerably below the lower limit (pH 3.5) of iron precipitation as indicated by PATTEN and MAINS (**24**), this plant nevertheless shows the same correlation between pH and soluble iron content of tissue fluids as do the species investigated by INGALLS and SHIVE (**15**). On the other hand, *Oxalis,* with extremely low pH values of tissue fluids, shows no such correlation, nor does there appear to be any direct relation between light intensity and pH of tissue fluids in this plant, although both pH values and soluble iron content fluctuate quite as much during a 24-hour period as do these values in *Rumex.* The relations of pH values and soluble iron content for *Rumex* are clearly indicated by the graphs of figure 1, which were plotted from the data of table I.

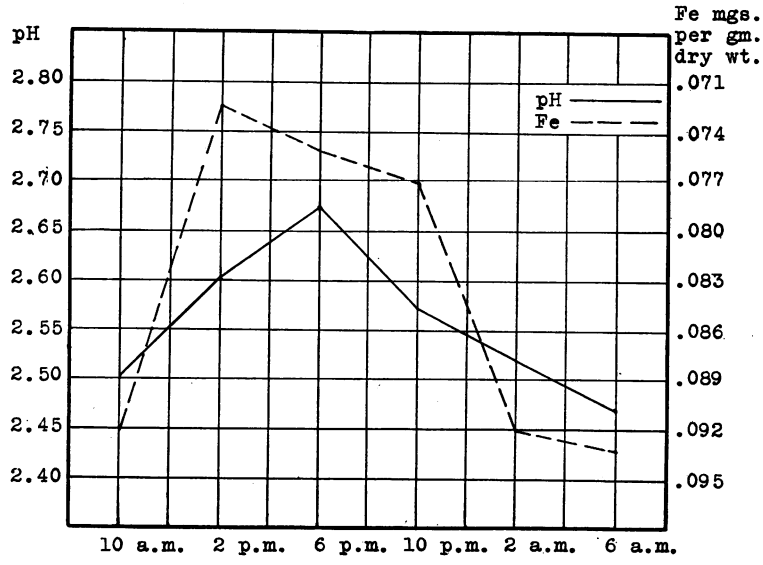

FIG. 1. Graph showing relation of pH to soluble iron content of *Rumex* leaves during 24-hour experimental period.

From the evidence obtained in the experiments with *Rumex,* it may safely be concluded that, although the pH values during a 24-hour period never reach the lowest point (pH 3.5) in the iron precipitation range, as found by PATTEN and MAINS (**24**), the soluble iron content is nevertheless directly correlated with fluctuations in the H-ion concentration.

Soy bean and potato

As may be observed from the data presented in table II and the graphs of figure 2, which were plotted from these data, the soluble iron contents of both the potato and the soy bean plant show, during a 24-hour period,

TABLE II

pH, filterable and total iron content in potato and soy bean plants at different intervals over a 24-hour period

Species	Time of sampling	pH	Iron (mg. per gm. dry weight)	
			Filterable	Total
			mg.	*mg.*
Potato (leaf)	10 A. M.	6.188	0.026	0.612
	2 P. M.	6.576	0.028	0.522
	6 P. M.	6.424	0.029	0.580
	10 P. M.	6.306	0.029	0.498
	2 A. M.	6.272	0.031	0.538
	6 A. M.	6.238	0.034	0.652
Soy bean (whole plant)	10 A. M.	6.002	0.010	0.314
	2 P. M.	6.086	0.010	0.444
	6 P. M.	5.934	0.012	0.484
	10 P. M.	6.103	0.011	0.594
	2 A. M.	6.019	0.016	0.619
	6 A. M.	5.833	0.019	0.682

fluctuations which are closely correlated, in an inverse relation, with the fluctuations in pH values of the tissue fluids. The average pH of the potato is 6.334 and that of the soy bean is 5.996, as indicated in table III.

TABLE III

Average soluble iron, total iron, and pH values for *Oxalis*, *Rumex*, potato and soy bean, over a 24-hour period

Species	pH	Iron	
		Soluble	Total
		mg.	*mg.*
Oxalis (leaf)	2.023	0.058	0.170
Rumex (leaf)	2.563	0.084	0.197
Potato (leaf)	6.334	0.029	0.567
Soy bean (whole plant)	5.996	0.013	0.522

Soluble iron content of the soy bean fluctuates very strikingly following fluctuations in the pH values of tissue fluids. In the juices of these plants, the pH varies from just above the point of complete iron precipitation (as

found by PATTEN and MAINS) to just below this point. The same close relation holds for fluctuations in the potato, with the exception of the first daylight period, although no pH value is as low as the extreme upper limit of iron precipitation. From the foregoing results, it may be concluded that although the pH values of tissue fluids of certain plants may lie consider-

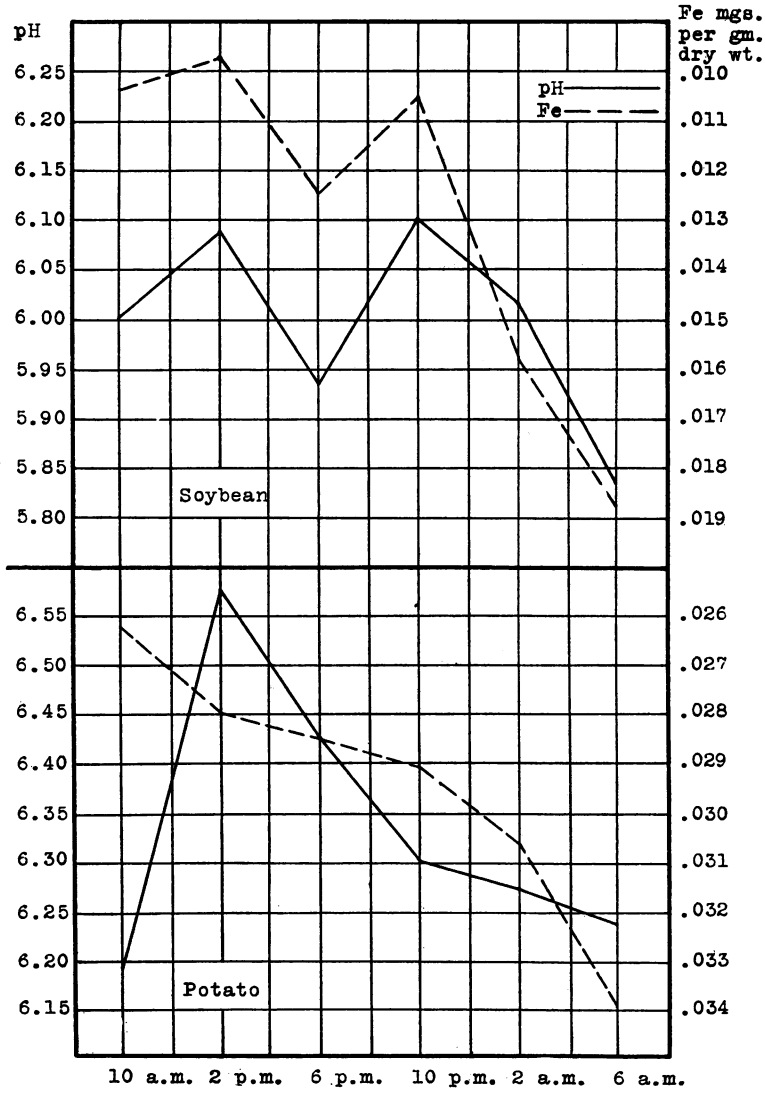

FIG. 2. Graphs showing relation of pH to soluble iron content of soy bean and of potato plants during 24-hour experimental period.

ably above the upper limit of iron precipitation in inorganic solutions, the soluble iron content still rises and falls within a narrow range with the fluctuating pH values resulting from variations in light intensity. This feature of the problem will receive more detailed consideration in a later section, but it might not be out of place here to emphasize several important points.

From the results obtained in this investigation, it is apparent that the range in pH values over which iron precipitation may proceed in simple inorganic solutions, according to the work of PATTEN and MAINS, cannot hold for complex organic systems such as are represented by the tissue fluids in plants. Plants yielding composite tissue fluids with pH values considerably above 6.0 still contain soluble iron, although in relatively low concentrations, yet in sufficient concentration to supply the needs of the plant under favorable conditions. Furthermore, plants yielding composite tissue fluids with pH values considerably below the lower limit of iron precipitation in simple inorganic solutions still contain insoluble iron, or at least iron which does not appear in the filtered plant juices with the methods here employed; although INGALLS and SHIVE (**15**) have shown that in some high-acid plants, practically all of the iron is present in the filterable form, and may be recovered in the filtrate.

It will be seen from tables I, II, and III that plants with juices having high pH values have high total iron and low soluble iron content, while plants with juices having low pH values have low total iron and high soluble iron content. This means that in plants with relatively high acid reaction, most of the iron is in solution; while in plants with relatively low acid reaction, most of the iron is in the insoluble form and accumulates as such. It is more than probable that if, for some reason, all of the iron in the soy bean or potato plant should suddenly become soluble, it would be extremely toxic to the respective plants. Even chlorotic plants, as has already been stated, may have large accumulations of iron present, even in the chlorotic leaves; but this iron may still be unavailable for the processes requiring it in the chlorophyllous cells. Two important questions arise: Where and why do such accumulations of iron occur? Consideration of these questions will be attempted in the following section.

V. H-ion concentration and distribution of iron as determined by microchemical examination of sectional areas

In carrying out this investigation, it was assumed that a microchemical study of sectional areas of plant tissues might bring out some facts with regard to H-ion concentration of tissues, and its relation to distribution of iron, which could not be obtained by making chemical analyses of tissues of different plant organs and determining the pH values of their juices. This proved to be the case.

In these studies, fresh tissue sections were prepared, microchemical tests were made for iron, and pH values of the different tissues over these sectional areas were determined as previously described. Plants were purposely chosen which yielded composite juices having very low and very high pH values. Among the former were *Oxalis repens* and *Rumex acetosella,* and among the latter were such plants as *Zea mays, Solanum tuberosum, Trifolium repens,* and *Glycine max.*

Sections were prepared from plants grown in the field, and also from those grown under controlled experimental conditions in the greenhouse. Great numbers of these tissue sections were prepared and studied, but only a few photographs (made from camera lucida drawings) of representative sectional areas are here presented and discussed. In the diagrams of these sectional areas, iron, as determined by the microchemical tests employed, is indicated in the cells of the different tissues by stippling, and the attempt is thus made to show approximately the relative quantities of iron present in the cells of the various tissues as these were made apparent by microscopical examinations of the sections after chemical treatment. The methods are strictly qualitative, however, and must be so regarded; they do not differentiate between iron in the soluble form and as a precipitate.

The methods employed in the determination of pH values of the different tissues and cells in sectional areas may not be regarded as being particularly accurate, nor has any method yet been devised by means of which great accuracy may be attained in determining pH values of tissues or cells in cross-section. An attempt has been made, however, to determine two values between which it may be regarded that the true pH of the tissues or cells in question is located. In the following consideration of data relating to sectional areas of plant tissues, two such values are always presented.

OXALIS.—The data representing the pH values of the different tissues in a cross-section of the stem of *Oxalis* are presented in table IV. These data indicate that the pH of all the tissues is relatively very low, the values

TABLE IV

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF THE STEM OF *Oxalis* STUDIED IN CROSS-SECTION

TISSUES	pH VALUES	TISSUES	pH VALUES
Cortex	4.2–3.8	Cambium	
Epidermis	2.8–2.6	Xylem	3.0–2.4
Endodermis	2.8–2.2	Pith	4.2–3.8
Phloem	4.4–3.8		

lying between pH 2.8 and 2.2 for endodermis cells, and between pH 4.4 and 3.8 for phloem. The average pH of the composite tissue fluid extracted from the stem of this plant, as previously given, is pH 2.023.

Examination of the diagram of figure 3, showing a cross-section of the stem of *Oxalis,* brings out the fact that the iron in this stem, as determined by microchemical methods, is rather uniformly scattered in all the tissues, and that the iron content of all the tissues is apparently very low, as this is determined by the methods here employed. This uniform distribution and low iron content are characteristic of the conditions throughout the plant. Accumulations of iron were not found anywhere in the tissues of this plant, except for slight accumulations which were sometimes observed in rather large isodiametric cells in the outer cortex.

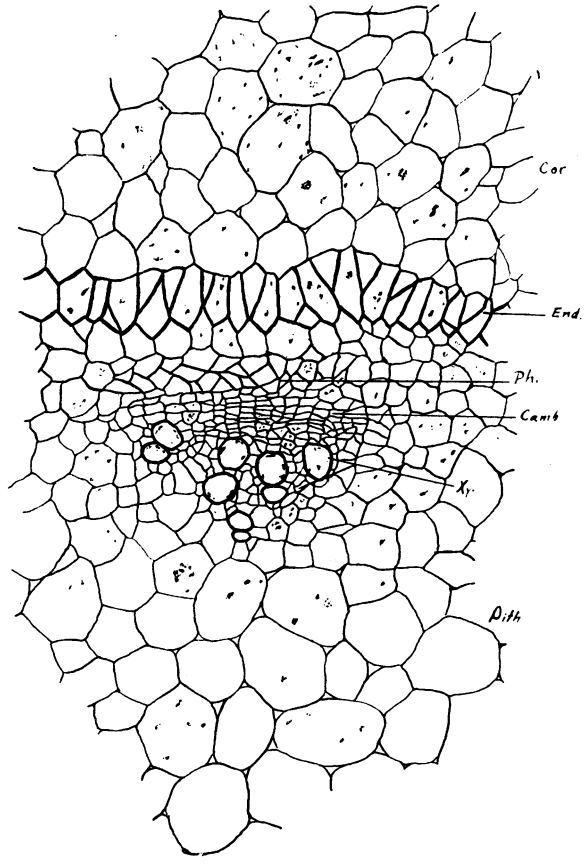

FIG. 3. Section of *Oxalis* stem showing low iron content but uniform distribution of iron in practically all tissues.

While the results obtained by chemical analyses of the plant tissues and of the extracted tissue fluids are in agreement with the results obtained by microchemical and microscopical studies of sectional areas, as here described, the former method does not, by any manner of means, give as detailed or as accurate a picture of the conditions with respect to iron and pH relations as does the latter method.

RUMEX.—As will be observed from the data of table V, the pH values of the tissues across a sectional area of *Rumex* petiole are rather low, ranging from 4.4 to 4.0 for phloem to 2.8–2.4 for sclerenchyma cells. These

TABLE V

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF *Rumex* IN CROSS-SECTION OF LEAF PETIOLE

TISSUES	pH VALUES	TISSUES	pH VALUES
Cortex	4.0–3.6	Xylem	3.0–2.6
Phloem	4.4–4.0	Pith	3.8–3.4
Sclerenchyma	2.8–2.4		

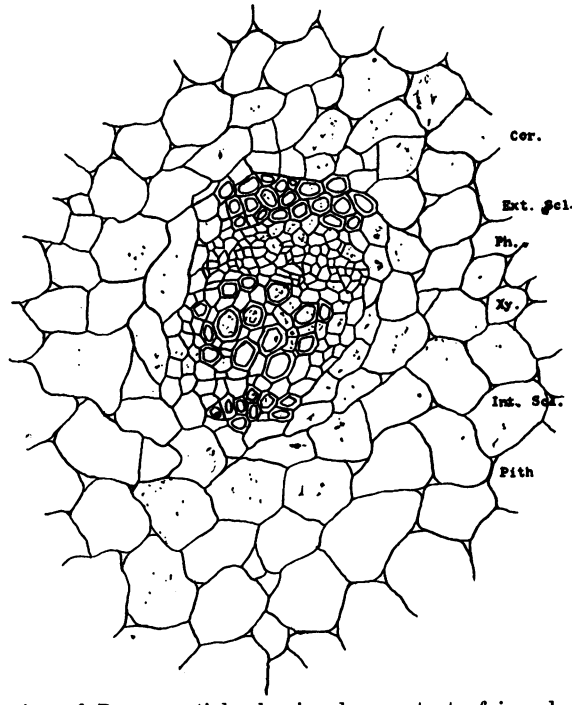

FIG. 4. Section of *Rumex* petiole showing low content of iron but somewhat uniform distribution in practically all tissues.

data are typical of those for sections taken from any part of the plant. The diagram of figure 4, like that of figure 3 for *Oxalis*, shows no accumulation of iron in any of the tissues, but indicates a fairly uniform distribution of iron in relatively small quantities throughout the cross-section.

It is thus apparent that in plants in which all the tissues show relatively low pH values, iron does not accumulate in any one tissue to a greater extent than it does in another. Furthermore, chemical analyses of the filtered tissue fluids of such plants indicate that most of the iron is in the soluble form. It is therefore to be concluded that iron migrates freely in these plants, and that there is no serious obstruction by any of their tissues to its movement from channels of translocation to the chlorophyllous cells of the leaves. Plants of this type have shown no tendency to become chlorotic from lack of iron when grown in artificial culture media containing mere traces of iron, even under experimental conditions definitely unfavorable for growth. These plants present no difficult iron problems, such as are frequently encountered in attempting to grow in artificial growth media plants in which the pH values of the tissues lie close to those representing the upper limit in the range for iron precipitation.

CORN.—A thorough study was made of sectional areas of the corn plant, for the reason that, when growing this plant in culture solutions, some difficulty was always experienced in preventing chlorosis of the leaves caused by the apparent absence of sufficient iron in the leaf mesophyll. The pH values of the composite tissue fluids extracted are relatively high. Table VI, however, shows the xylem and the cells of the bundle sheath to have relatively low pH values; while the phloem, cortex, and leaf mesophyll have high pH values.

TABLE VI

APPROXIMATE PH VALUES OF VARIOUS TISSUES OF STEM AND LEAF OF CORN PLANT STUDIED IN CROSS-SECTION

TISSUES	PH	TISSUES	PH
Xylem (parenchyma)	4.8–4.6	Phloem	6.2–5.8
Xylem (vessels)	4.4–4.0	Cortex	6.0–5.6
Bundle sheath	4.6–4.4	Leaf mesophyll	6.0–5.6
Sclerenchyma	4.8–4.6		

In direct contrast to the conditions found in *Oxalis* and *Rumex*, the corn plant shows very heavy accumulations of iron, primarily in the bundle sheath and in cortical cells immediately surrounding it. These conditions are shown in the diagram of figure 5, which is typical of many sectional

areas prepared from stems and leaves. It is significant that in both the stem and leaf of corn, small amounts of iron were always found in the vessels. This was usually observed in all the plants studied. No iron was found in cortical cells of the stem, nor in mesophyllous cells of the leaf slightly removed from the bundle region. No trace of iron was found in the phloem of the stem or leaf. Apparently iron penetrates the phloem of the leaf and stem with difficulty or not at all. The pH of this tissue is the highest of any found in corn.

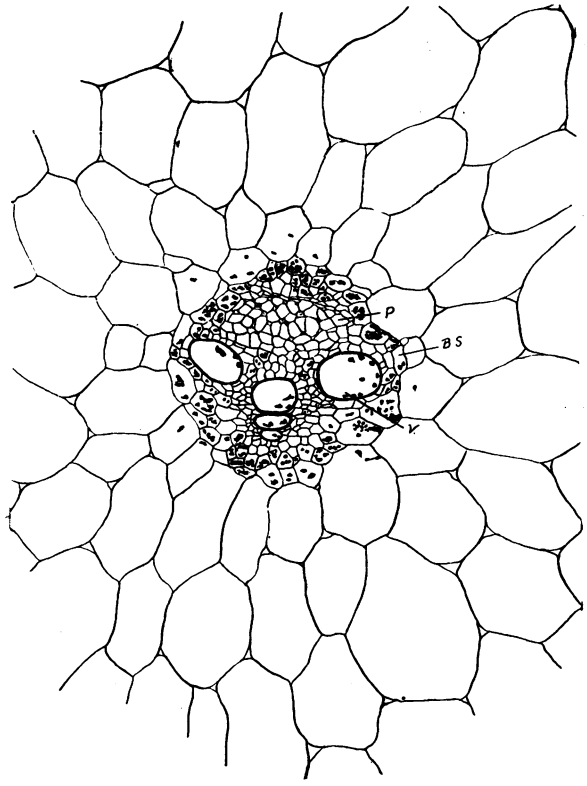

FIG. 5. Section of corn stem showing very heavy iron accumulations in the tissues of the bundle sheath.

It is remarkable to note that in a chlorotic corn leaf, chlorophyll was present only in the mesophyllous cells surrounding the bundles and those near the strip of sclerenchymatous tissue near the upper surface opposite each large vascular bundle. In the leaf blade, beginning a short distance from the midrib, the bundle and this strip of mechanical tissue approach each other rather closely, so that the spongy tissue between the two is only

two or three cells in thickness. In a chlorotic leaf, these cells, as well as those near the bundle and sclerenchymatous tissue, possess chlorophyll, while those between the bundles are devoid of it. Such a condition is externally apparent to the naked eye. The course of the veins is marked by dark green lines, while the space between them is yellow or greenish yellow, depending upon the degree of chlorosis.

A study of sectional areas of chlorotic corn leaves and of green leaves brings out the fact that chlorotic leaves contain as much or more iron than do green leaves, in so far as this can be detected by microchemical methods. This is indicated also by chemical analyses of the tissues in question. In the chlorotic leaves, however, the iron is confined to the bundle regions; and from these regions it penetrates, if at all, only to the cells lying in close proximity to them or to sclerenchyma tissue. This is undoubtedly due, in large measure, to the steep pH gradient existing between the cells of the bundle sheath and those immediately to the exterior.

The root tissues of corn, as a whole, are somewhat more acid than are the corresponding stem tissues. In the roots of corn, iron in very small quantities was found somewhat well distributed, except that small accumulations were sometimes observed in the cells between the endodermis and the xylem, and in the phloem. In the prop roots, larger accumulations were observed in the phloem.

From the foregoing considerations, it is evident that in the corn plant certain tissues may offer considerable resistance to the passage of iron from the channels of translocation to the chlorophyllous cells, and such obstructions may become rather serious under slightly unfavorable growth conditions. In this plant, the morphological structure is such that the channels of iron translocation are completely surrounded by tissues with very high pH values, through which iron migrates with difficulty.

TABLE VII

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF LEAF PETIOLE OF CLOVER AND SOY BEAN STEM STUDIED IN CROSS-SECTION

PLANT	TISSUES	pH	TISSUES	pH
Clover	Xylem (vessels)	4.8–4.6	Phloem	7.2–6.8
	Xylem (parenchyma)	5.6–5.4	Cortex	6.8–6.4
	Sclerenchyma	4.8–4.6	Pith	6.8–6.4
Soy bean	Xylem (vessels)	5.0–4.6	Phloem	7.4–6.8
	Xylem (parenchyma)	6.6–6.2	Cortex	7.0–6.6
	Sclerenchyma	5.0–4.6	Pith	7.2–6.6

CLOVER AND SOY BEAN.—The pH data for clover and soy bean are given in table VII. It will be observed that the tissues of these two legumes, except the xylem and sclerenchyma, show relatively high pH values. In these two plants there is always a steep pH gradient between xylem and phloem, between sclerenchyma and phloem, and between sclerenchyma and cortex; so that from the xylem outward there are regions of relatively low pH which alternate with regions of high pH. The diagrams of figures 6 and 7, which represent respectively cross-sectional areas of petiole of clover and stem of soy bean, show that while iron occurs generally in the xylem tissues, it accumulates to a marked degree in the border regions between xylem and phloem and between sclerenchyma and phloem. This is particu-

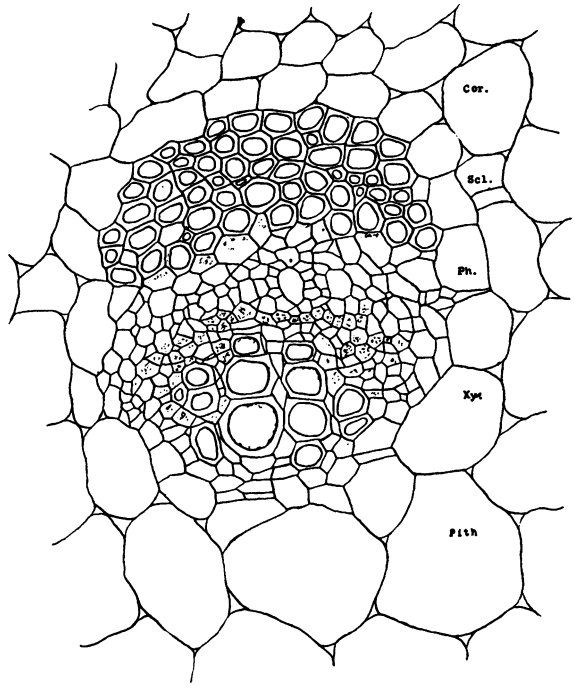

FIG. 6. Section of clover petiole showing iron accumulation in the phloem.

larly true of the soy bean, whose tissues show somewhat higher pH values than do the corresponding tissues of clover. Much of this accumulated iron is without doubt in the precipitated form, since chemical analyses show very high total iron for these plants and exceedingly low total iron for the filtrates of the extracted tissue fluids. Small amounts of iron were generally found also in the cortical tissues of these two species, just outside of low pH sclerenchyma. While iron was not found in this mechanical tissue,

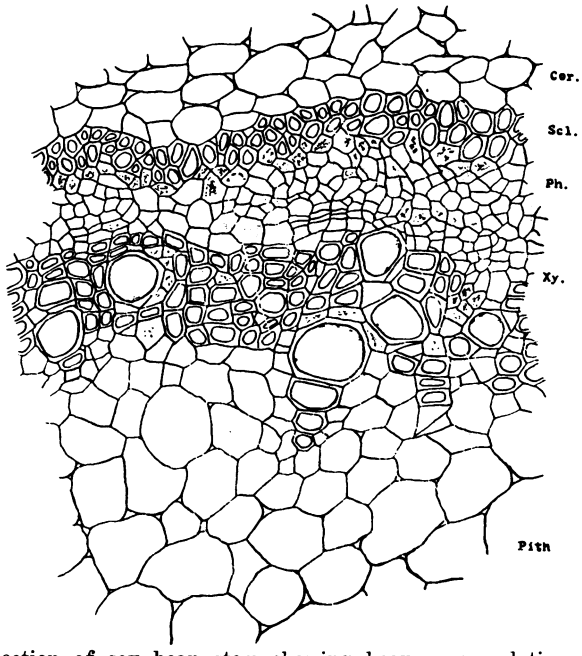

FIG. 7. Section of soy bean stem showing heavy accumulations of iron in the phloem.

except an occasional trace, it appears to have some influence in the translocation of iron into bordering tissues having much higher pH values.

POTATO.—As indicated in table VIII, the pH values of the tissues of the potato plant are very high, ranging from pH 7.6–6.6 for phloem to pH 5.0–4.6 for xylem. There is thus a steep pH gradient between xylem and

TABLE VIII

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF STEM AND PETIOLE OF POTATO PLANT STUDIED IN CROSS-SECTION

TISSUES	pH	TISSUES	pH
Xylem (parenchyma)	6.6–6.2	Cortex	6.8–6.4
Xylem (vessels)	5.0–4.6	Pith	7.0–6.4
Phloem	7.6–6.8		

phloem, with heavy accumulations of iron in the phloem, as indicated in the diagram of figure 8, representing a cross-sectional area of a leaf petiole. Small quantities of iron are also found in the vessels and scattered throughout the cells of the xylem parenchyma.

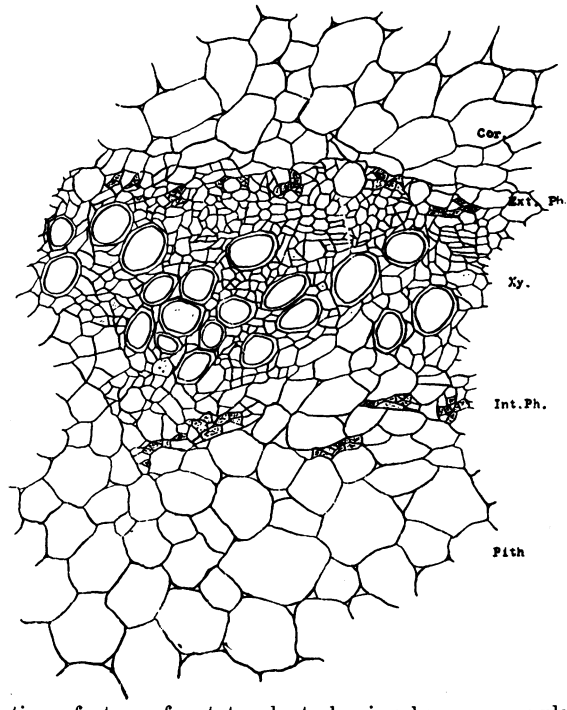

FIG. 8. Section of stem of potato plant showing heavy accumulations of iron in the external and internal phloem.

As has previously been shown, the total iron content of this plant is very high, while the iron content in the filtrate of its extracted juices is very low. It appears, therefore, that the iron accumulation in the internal and external phloem largely represents precipitated or non-functioning iron.

The potato plant, in marked contrast to the corn plant, may be grown in artificial culture media without showing such a strong tendency toward becoming chlorotic as is exhibited by the corn plant in the same medium, although the tissues of the former show considerably higher pH values than do the corresponding tissues of the latter, and in spite of the fact that the tissue fluids of the corn contain a much higher filterable iron content per gram of dry tissue than do those of the potato. It is apparent, therefore, that there is an inherent difference between these two plants with respect to the economy of iron utilization and its transfer from channels of translocation to chlorophyllous cells, for which at the present time no adequate explanation can be offered.

Discussion

A study of the tissues of different plants, and the determination of their approximate pH values, bring out the fact that, in all the plants studied,

the xylem tissues always showed the lowest pH values and phloem the highest. Sclerenchymatous tissues, whenever these occurred, showed pH values which were about the same or slightly higher than those of the xylem.

In the study of iron distribution in the plants, as determined by the microchemical methods here employed, it is significant that small amounts of iron were always present in the vessels and in the xylem parenchyma, but in no instance did pronounced iron accumulation occur in these tissues. On the other hand, marked accumulations of iron, whenever these were present, usually occurred in high pH tissues, such as phloem where this bordered on the xylem or other low pH tissue. As already explained, much or all of the iron in these heavy accumulations may be regarded as being in the precipitated form, not mobile, and therefore cannot function in the metabolic processes of the plant. It is logical to conclude also that the xylem furnishes the tissues in which the upward translocation of iron occurs.

The root tissues of the plants here studied, as a whole, appeared somewhat more acid than did the corresponding tissues of the stem. From the standpoint of pH values, plants such as corn should experience no great difficulty in absorbing iron from the surrounding medium, provided that an available supply is present, since epidermis and subepidermal tissues of the roots of all the corn plants studied were relatively quite acid, and no cortical tissue of roots was found which showed pH values at or above the extreme upper limit (pH 6.0) in the range of iron precipitation as determined by PATTEN and MAINS.

The only tissues in which iron was found to accumulate in the corn roots were the cells between the endodermis and xylem and in the phloem, and these accumulations were found only occasionally and were not particularly pronounced. In all the species investigated, iron was found in small quantities in the young roots, but no accumulations were observed in these organs, the iron being rather uniformly distributed throughout the tissues.

Although iron is directly or indirectly necessary for chlorophyll production, it is apparent that if the chlorophyllous cells are comprised in a tissue with a pH reaction near or above the upper limit in the range of iron precipitation, the iron may be prevented from entering or penetrating such tissue because of precipitation, as a result of which a chlorotic condition may occur. As already pointed out, however, the range in pH values over which iron precipitation occurs in some plants is wider than that indicated by PATTEN and MAINS for inorganic systems. Hence in certain plants it is possible that iron in small quantities may penetrate tissues having pH values considerably above the upper limit (6.0) of this range, while in others it may not be possible for iron to penetrate such tissues. The former

type of plant may show no tendency toward chlorosis, when subjected to a given set of conditions under which the latter may suffer severely from chlorosis owing to lack of iron in chlorophyllous cells. Two species which showed this pronounced contrast under like experimental conditions are corn and potato, the former showing a marked tendency toward chlorosis from lack of iron in chlorophyllous tissues under certain experimental conditions, while the latter showed no such tendencies under the same conditions, in spite of the fact that the potato plant contains a much lower percentage of soluble (filterable) iron than does the corn plant. The plants of both these species contain a large excess of total iron, most of which is in the insoluble (non-filterable) form.

Many agricultural plants with tissues having high pH values show these same inherent differences, for which, as has previously been pointed out, no adequate explanation can be offered at the present time on the basis of experimental evidence. It may be suggested, however, that certain organic iron compounds are able to resist the precipitating action of alkaline media, and that iron in the presence of certain organic compounds is retained in solution form in alkaline media. MARSH and SHIVE (**19**) have shown that, of the four iron compounds (ferric glycerophosphate, soluble ferric phosphate, ferric tartrate, and ferrous sulphate) in solution in a culture medium at pH 6.2, no ferrous sulphate and no soluble ferric phosphate remained in solution at the end of a given experimental period; while 40 per cent. of the iron present in the same medium as ferric tartrate and 20 per cent. as ferric glycerophosphate remained in solution at the end of the period under the same experimental conditions.

REED and HAAS (**25**), in a series of qualitative tests, found that tartrates and citrates were effective in holding iron in solution in an alkaline medium. Citrates were somewhat more effective in this respect than tartrates. By testing for iron with potassium thiocyanate, they found abundant quantities in solution in a medium at pH 7.6, with sodium citrate present. FISCHER (**7**) obtained similar results using glycerol in an alkaline medium. DAKIN (**6**) mentions that this also holds true for hydroxyaspartic acid, although he gives no experimental evidence. GILE and CARRERO (**8, 9, 10**) found that, of a number of iron sources, only ferric tartrate furnished sufficient iron for rice grown in alkaline solutions. When judged by the growth of rice plants, ferrous sulphate, ferric citrate, and ferric tartrate afforded sufficient iron, when used in proper quantities in acid and neutral solutions. Many other workers have found similar results with certain organic compounds.

SMYTHE and SCHMIDT (**28**) state that those substances which possess a certain particular grouping within the molecule will hold iron as an undissociated compound. Such a combination in most cases is rather resistant

to the action of alkaline solutions. They found the following classes of substances to possess the necessary grouping: hydroxymono-carboxylic acids (lactic, gluconic); dicarboxylic acids (oxalic, malonic); hydroxydicarboxylic and hydroxytricarboxylic acids (tartaric, citric); amino acids which are also hydroxy- or dicarboxylic acids (aspartic acid, serine); certain inorganic acids (phosphoric, arsenic); certain phosphorus-containing compounds (nucleic acid, glycerophosphoric acid); and certain proteins (casein, gelatin). They suggest an explanation, based on the residual charge of ions, for the manner in which the iron may be united.

It appears logical to assume that, if such compounds as those just mentioned serve to hold iron in solution in an external alkaline medium, they should serve the same purpose inside the plant, provided that they are present there. Many such compounds are known to be present in some plants, and certain ones may be entirely lacking in others. It may then be suggested as a possibility that some plants with high pH tissues may show little tendency toward chlorosis, even under somewhat unfavorable growth conditions, because they may contain certain organic solvents, in the presence of which iron in the mobile form resists the precipitating influence of high pH reactions. On the other hand, certain plants with high pH tissues may show a strong tendency toward chlorosis, because they may not contain the particular organic solvents which hold iron in solution in the tissues against the precipitating influence of high pH reactions.

It seems probable that the precipitated iron which is present in certain tissues of high pH plants may serve as a reserve iron supply, in that certain of these organic solvents may move across such tissues containing the precipitated iron, and may become effective factors in translocating some of it to tissues more remote.

Summary

1. Plants which yield composite tissue fluids having high pH values in general show very low soluble (filterable) iron content and very high total iron.

2. Plants which yield composite tissue fluids having low pH values in general show relatively high soluble iron and low total iron. In some cases nearly all of the iron in the plant samples appears in the filtered extracts.

3. The range of pH values over which iron appears to precipitate in plants of different species is wider than the corresponding range for inorganic systems.

4. Plants whose extracted tissue fluids show very high pH values, and those with tissue fluids having very low pH values, still show slight fluctuations in soluble iron content over a day and night period, which corresponds in the inverse relation to fluctuations in pH values of the tissue fluids due to variation in light intensity over the same period.

5. The highest pH values of specific tissues for each species investigated occurred in the phloem, with cortex only slightly lower; and the lowest pH in the xylem. Steep pH gradients always occurred between xylem and phloem.

6. Iron accumulations usually occurred in high pH tissues lying adjacent to relatively low pH tissues with a steep pH gradient between. It is concluded from chemical analyses that iron in these accumulations is in a precipitated form and not available for plant processes.

7. No iron accumulations were found in plants with low pH tissues throughout. In these plants the iron content is low and uniformly distributed in practically all of the tissues.

8. The xylem furnishes the main channels of iron translocation from the roots to the leaves.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION,
NEW BRUNSWICK, NEW JERSEY.

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