RELATION OF H-ION CONCENTRATION OF TISSUE FLUIDS TO THE DISTRIBUTION OF IRON IN PLANTS

R. A. INGALLS AND J. W. SHIVE

(WITH TEN FIGURES)

Although iron is an essential element for plant growth, it is not always present in an available form for assimilation by the plant. Factors influencing the availability of iron are not well understood. Previous investigations of the iron problem have dealt mainly with a study of the external medium in which the plants were grown, with less emphasis attached to the study of the conditions existing within the plants themselves.

PATTEN and MAIN (**18**) found that iron was precipitated from solution in varying degrees from pH 3.5 to 6.0, practically all being precipitated at 6.0 and above, thus rendering it unavailable for absorption by the plant. This was also brought out by HOPKINS and WANN (**10**). They found difficulty in growing plants in a medium of pH 6.0, due to the fact that iron was removed by adsorption on calcium phosphate which gradually precipitated as the solution became alkaline, a physical chemical effect capable of influencing iron availability within the plant as well as in culture media.

The fact that lack of available iron is not entirely due to the conditions existing in the culture media may be shown by reference to the work of APPEL (**2**). He found that buckwheat plants were less sensitive to changes in reaction of culture media than were corn plants, corn requiring much more iron than buckwheat. Little difficulty, therefore, was experienced in growing buckwheat without chlorosis in solution cultures in which corn suffered from lack of available iron. After a study of the internal conditions existing within the plant, presented in the following pages, an explanation of this phenomenon may be attempted.

LOEHWING (**13**) has reported that plants grown in a medium high in lime display peculiar iron immobility characterized by copious precipitation in the roots. He states that the lime reduces the sap acidity to the point of interference with internal iron mobility. Lime injury involving chlorosis has been reported for corn by MAZÉ (**16**), for pineapples by GILE (**5**), for rice by GILE and CARRERO (**6**), for pears by MILAD (**17**), and for citrus fruits by LIPMAN (**22**).

In addition to the work cited, some work has been reported on the H-ion concentration of tissue fluids. HAAS (**8**) made studies of actual and total acidities and of the total alkalinity of a number of plants of agricultural importance, together with a study of the influence of liming the soil upon

these acidities. In this connection he reports that in ten out of fourteen cases the addition of lime was followed by a decrease in actual acidity of the plant juice, which seems to point to the fact that plant juices are influenced to some degree by the nature of the medium in which they are grown. Other points of interest in his study of plant juices were as follows: (1) the presence of a hydrogen-ion concentration gradient for tissue fluids which is not always in the same direction in different species or in the stems and leaves of the same species; (2) an increase in acidity with age increase in the plant, and (3) that illumination tends to decrease the acidity of the plant. This point was further brought out by GUSTAFSON (7). He worked with *Bryophyllum calycinum,* determining the pH of its juice at various intervals throughout the day and the early part of the evening. He found that the acidity of the juice decreased during the day and increased again at night, but if the plant was kept in the dark continuously for 24 hours or more, the acidity did not continue to increase but began gradually to decrease, as the food supply was used up in respiration. The highest acidity was reached at 10 A. M. while the lowest was obtained at 4 P. M. The experiment was carried out during both clear and cloudy days, and it was found that the same general results were obtained on both types of days but to a different degree.

CLEVENGER (**4**) found a similar nocturnal change in pH of the tissue extract of cowpeas due to change in light intensity. He also pointed out that the pH varied with temperature, being higher at high temperatures than at low temperatures. ATKINS (**3**) made a study of the variation in the juices of different plants and found a range in pH from 1.4 to 8.0, but only in rare cases did he find any above 7.0.

The purpose of this investigation has been to determine first, the influence of day to night variations in light intensity upon the hydrogen-ion concentration of the plant tissue fluids of a number of different species; second, to study the relation between hydrogen-ion concentration of the plant tissue fluids, as influenced by variation in light intensity, and the "filterable" or soluble iron content of the plant tissues; third, to study the relation between hydrogen-ion concentration of the plant tissue fluids and the total iron content of the plant tissues; and finally, to study the influence of these internal factors upon the iron mobility, its distribution, and ability to function in the plant tissues.

Experimental methods

Plants used in the first part of this investigation were grown in sand and solution cultures to insure as uniform a medium of growth as possible. It was later found, however, that quite similar results were obtained with

plants grown in soil plots, provided that care was taken to select plants of the same age which had been grown under similar conditions. The species here used may be classed into two groups according to the purpose for which they were grown. Group 1 comprised buckwheat (*Fagopyrum esculentum*), clover (*Trifolium repens*), sedum (*Sedum reflexum*), and bryophyllum (*Bryophyllum calycinum*). These were grown for the express purpose of studying the influence of light intensity on the pH of the tissue fluids. The hydrogen-ion concentration of the juices of both stems and leaves of the plants of this group were determined at intervals of two hours during the day and night. Care was taken to select the various experimental periods at times when the sky was cloudless during the day in order that the plants might be subjected to the maximum variation in light intensity during a day and night period.

Group 2 comprised buckwheat (*Fagopyrum esculentum*), bryophyllum (*Bryophyllum calycinum*), rumex (*Rumex patientia*), sedum (*Sedum reflexum*), tobacco (*Nicotiana rustica*) *tomato* (*Lycopersicum esculentum*), asparagus (*Asparagus officinalis*), soybean (*Glycine max*), and clover (*Trifolium repens*), and were grown for the purpose of determining the filterable or soluble iron content of tissue fluids of plants having different pH values, and also to determine the filterable iron content of the tissue fluids of plants of the same species at various intervals throughout a day and night period.

Sand cultures were grown in clay percolators similar to those described by ALLISON AND SHIVE (**1**). They were approximately 31 cm. in height with a diameter of 15 cm. at the top and 5 cm. at the base. The opening through the bottom of each percolator was closed with a cork carrying a short glass tube through which the percolating solution could escape. The upper end of the tube was closed by a small plug of glass wool. Each percolator contained about 5 kg. of quartz sand which had been thoroughly washed in an agate pan until the overflowing water was free from sediment. TOTTINGHAM'S (**25**) solution modified by JONES and SHIVE (**11**) was supplied to each culture. This solution was continuously renewed by means of a drip and drain system (**22**) which allowed one liter of new solution to flow into each culture at a constant rate during a period of 24 hours. Each culture was flushed once a week with distilled water to avoid any excessive salt concentration which might have resulted from evaporation at the surface of the sand, or from water loss through transpiration. Buckwheat and soybeans were grown in solution cultures in two-quart colorless glass jars. They also were supplied with the constant solution renewal system described by SHIVE and STAHL (**22**). In both sand and solution

cultures, iron was supplied to the plants in the form of a freshly prepared ferrous sulphate solution containing 0.1 mg. of iron per cc. of solution.

In the early stages of growth, 0.1 cc. of the iron solution containing 0.1 mg. of iron per cc. was added to 1000 cc. of nutrient solution. This amount, however, was increased and sometimes reduced during the later stages of growth, as the external conditions and the requirements of the plants made this necessary.

Seeds used in these experiments were germinated between moist filter-papers and then transferred to a germinating net as described by SHIVE (**21**) When the seedlings were 4 cm. tall they were carefully selected for uniformity of size and vigor and transferred to their respective media, ten plants being used in each sand culture and three in each solution culture.

To prepare the material for the extraction of the tissue, it was cut into small pieces and placed in test tubes. These were then plugged with paraffined cork stoppers plunged into a salt-ice mixture and frozen as quickly as possible in order to prevent any appreciable chemical change before freezing. In all cases duplicate samples were used. Preparatory to expressing the tissue fluids, the test tubes containing the samples were placed in tepid water and the material allowed to thaw. This usually required from five to ten minutes. At this stage, the material was removed from the tubes and the juice extracted by means of a small screw press. Precautions were taken to prevent the tissue fluids from coming in contact with anything except glass surfaces.

pH determinations were made electrometrically by means of the hydrogen electrode and a type K Leeds and Northrup potentiometer. About 5 cc. of juice were used at each determination. It was placed in a short Pyrex tube and hydrogen was allowed to bubble through until a constant potential was attained. Electrodes were cleaned and platinized before making determinations and again frequently throughout the experiments. They were also checked against a standard acetate solution of known pH value.

Samples of green plant tissues of the various species studied, from which the moisture content and total iron content of the plant were determined, were taken at the end of each experimental interval (usually at the end of two hour intervals) throughout a day and night period. For total iron determinations, the plant tissues were dried in an oven at about 85° C. for 48 hours and at 100° C.–102° C. for 24 hours. They were then ground to a powder with a pestle and mortar in order that uniform samples might be obtained. The material was then placed in a desiccator over night, after which duplicate samples of 0.1 gm. each were weighed out and placed in Pyrex test tubes. Iron analyses were made according to a method described

by WONG (**26**). It consisted in completely digesting the weighed sample with 1 cc. of concentrated iron-free sulphuric acid, allowing the contents of the tube to cool for about 20 seconds and then adding to it a ten per cent. solution of sodium chlorate. This solution was added carefully drop by drop and allowed to run down the side of the tube to prevent excess spattering when the solutions came in contact with one another. Boiling was then continued until white fumes appeared. The content of the tube was clear and colorless when oxidation was completed.

Potassium sulphocyanate, 5 cc. per sample, was used as an indicator, and the contents made to a known volume with distilled water. The red color produced by the indicator varied in depth according to the amount of iron present. Each sample containing the unknown was compared in a Duboscq colorimeter against a standard iron solution. This solution was prepared by dissolving 0.7 gm. of recrystallized ferrous ammonium sulphate in about 50 cc. of distilled water. To this was added 20 cc. of 10 per cent. sulphuric acid and then sufficient one-tenth normal potassium permanganate solution was added to just oxidize the ferrous salt completely. It was then diluted to 1 liter. This solution contained 0.1 mg. of iron per cc.

To both the standard and the unknown iron solution to be determined was added 0.25 cc. of dilute (30 per cent.) nitric acid. It was found that the addition of this solution prevented the reduction of iron from the ferric to the ferrous form, thus preserving the color of the test and standard solutions without change for a sufficient length of time to compare with accuracy at least twelve unknowns against the same sample of the standard.

Since it was impossible to remove all the moisture by pressure from the plant tissue, some means had to be adopted by which total filterable iron could be determined on the basis of dry plant tissue. This was accomplished as follows:

The tissue for which iron determinations were to be made was cut into rather small pieces and thoroughly mixed. One sample was taken from the mixed tissue and frozen in order to extract the tissue fluid. Iron analyses were made on 1 cc. of the extracted filtered fluid according to the method previously described. The remainder of the plant tissue was weighed, dried, and the moisture content determined. From this, the amount of moisture per gram of dried plant tissue was calculated.

The quantitative iron analyses on 1 cc. samples of the filtered tissue fluid, together with the determinations of moisture and the content of solid material in the filtrates, furnished the necessary basis for the calculation of the iron per gram of dry material in the plant tissues in question.

Experimental results

Introductory

The practice of supplying iron to certain species grown in artificial media in suitable proportions and in such a manner as to prevent the appearance of chlorosis from lack of this element and produce healthy green plants is attended with considerable difficulty. To accomplish this requires a knowledge of the internal nature of the particular species in question as well as the chemical nature of the culture media.

It has long been observed that not only do plants of different species require different amounts of iron, but also that plants of the same species require different amounts of iron from time to time, depending upon the degree of light intensity: that is, the iron requirement of the plants varies with the light intensity. However, this is much more pronounced in some species than in others. Among the species exhibiting the characteristic of marked variation in iron requirement with variation in light intensity are those in which the pH of the tissue fluids lies close to or above the precipitation point of iron; and such plants are frequently employed in experimental work. In this type of plant, chlorosis may occur under certain conditions from lack of iron in the chlorophyllous cells with an adequate concentration of available iron in the culture media. It was found that the proper concentrations of available iron required in the culture medium to prevent chlorosis in these plants during a period of low light intensity, such as a period of cloudy weather, was entirely inadequate to maintain the normal green color in the plants during periods of high light intensity, such as a series of successive clear days during the summer months.

On the other hand, those species in which the pH of the tissue fluids is considerably below the precipitation point of iron do not exhibit any marked variation in the iron requirement with variation in light intensity, nor is the iron concentration in the medium required to maintain the healthy green color in these plants ever so great as it must be for the type of plant in which the pH of the tissue fluids lies near or above the precipitation point of iron. It is thus evident that the cause of chlorosis due to the lack of iron is largely dependent upon factors existing within the plant, through the agency of which the iron may be precipitated at certain points along the paths of translocation in stems or leaves, thus preventing the migration of iron into the chlorophyllous cells.

It is well known that under most field conditions where the soil solution is slightly acid in reaction, plants can usually obtain a sufficient supply of iron during all phases of growth. In certain alkaline soils, however, some important agricultural plants are unable to obtain a sufficient amount of

available iron, thus causing considerable loss in production, while under the same conditions other plants appear to suffer no injury from lack of available iron. It is reasonable to assume, therefore, that an internal mechanism renders small quantities of iron mobile in the plant and available to the chlorophyllous cells. It was with these points in mind that the present investigation was undertaken.

The influence of light intensity on the H-ion concentration of plant tissue fluids

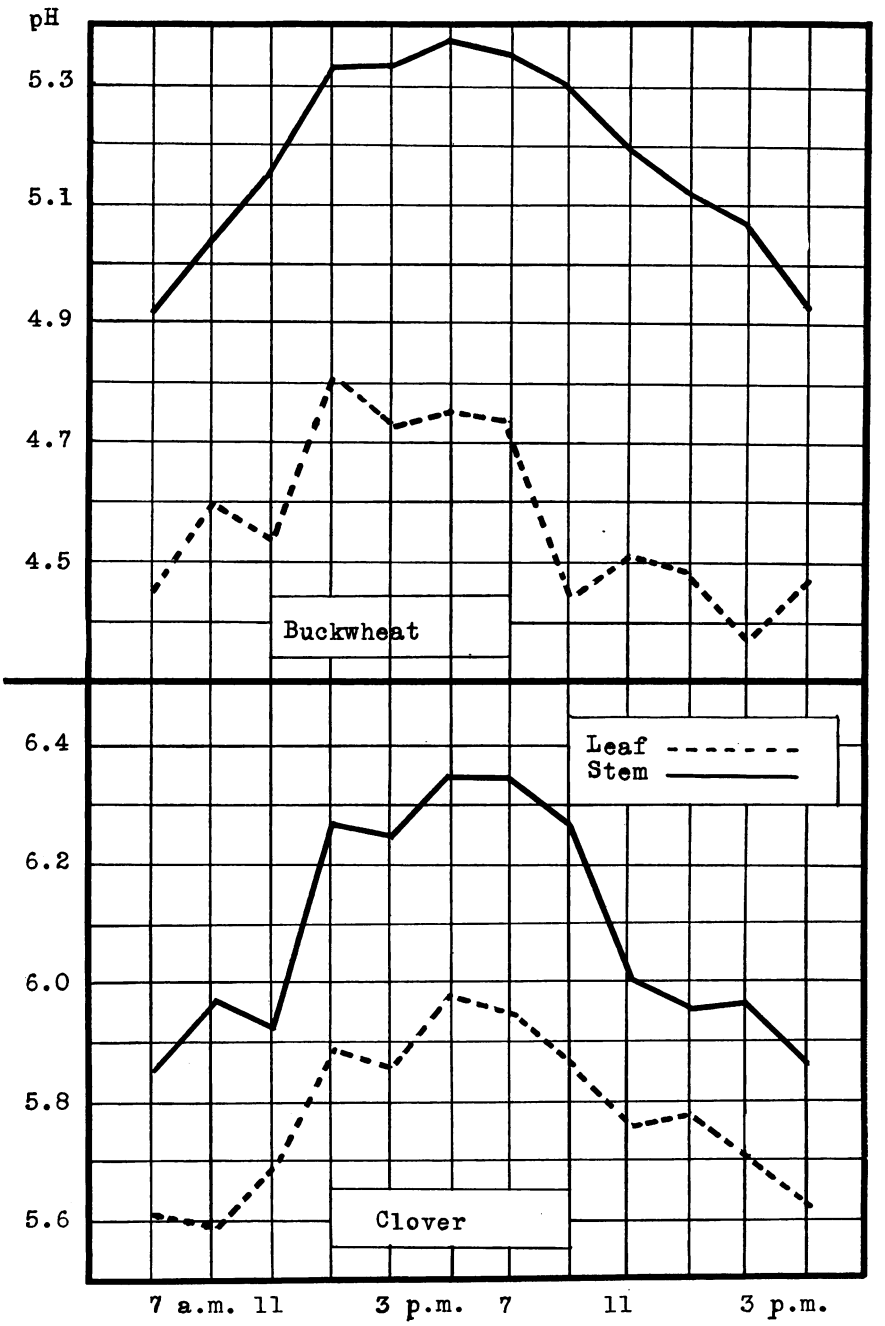
The data obtained from the experiments conducted for the purpose of showing the effect of light intensity on the hydrogen-ion concentration of plant tissue fluids are presented in tables I and II. In the first column of each table is given the time at which pH determinations of tissue fluids were made during a period of 24 hours. In the succeeding columns are given the average pH values of juices of stems and leaves of the plants indicated at the head of the respective columns. Each value represents the average of two or more determinations. The results given in table I are all from plants with thin leaves, while those of table II deal with thick-leaved, fleshy plants.

Figure 1 shows the graphs plotted from the data as given in table I, representing the course of change in the pH values of the stem and leaf juices of buckwheat plants during a period of 24 hours. The experiment from which these data were obtained was carried out in the spring of the year, the

TABLE I

pH VALUES OF TISSUE FLUIDS OF STEMS AND LEAVES OF BUCKWHEAT, CLOVER AND *Rumex* PLANTS AT TWO HOUR INTERVALS

TIME	BUCKWHEAT		CLOVER		*Rumex*
	STEMS	LEAVES	STEMS	LEAVES	LEAVES
9:00 A. M.	4.615	5.047	5.596	5.968	4.210
11:00 A. M.	4.531	5.139	5.697	5.934	4.193
1:00 P. M.	4.804	5.342	5.883	6.272	4.328
3:00 P. M.	4.726	5.333	5.868	6,238	4.480
5:00 P. M.	4.767	5.376	5.985	6.342	4.514
7:00 P. M.	4.757	5.351	5.951	6.340	4.311
9:00 P. M.	4.446	5.300	5.866	6.255	4.277
11:00 P. M.	4.548	5.190	5.765	6.002	4.176
1:00 A. M.	4.497	5.139	5.783	5.951	4.193
3:00 A. M.	4.353	5.089	5.714	5.968	4.108
5:00 A. M.	4.447	5.021	5.630	5.850	4.032
7:00 A. M.	4.454	4.920	5.613	5.850	4.057



FIGS. 1 (upper) and 2 (lower). Graphs representing the course of change in pH values of leaf and stem juices of buckwheat and clover plants during a 24-hour experimental interval.

sky being perfectly clear during the experimental interval, so that the plants were exposed to approximately maximum variation of light intensity from day to night for this period of the year. These graphs clearly bring out the fact that there is considerable change in hydrogen-ion concentration of the juices of both stems and leaves which decreases during the day with increase in light intensity and increases during the night. It will be observed that the juices of buckwheat stems are much more acid than are those of the leaves, the stems having a maximum pH of 4.79 and a minimum of 4.45, while the corresponding pH values for leaves are 5.37 and 4.92, respectively. While there is a marked difference in pH values between stems and leaves, as indicated by the graphs, the range of variation in these values over a 24 hour period is about the same for the juices of both stems and leaves. Not only are they alike in this respect, but also there is a pronounced similarity in the general trend of the graphs representing the course of change in pH values. It will be observed, however, that the graph representing pH values of stem juices is somewhat less regular in its general outline than is that representing pH values of leaf juices. Highest H-ion concentration for stems is indicated at 3 A. M. and for leaves at 5 A. M., while minimum H-ion concentrations (maximum pH value) are indicated for stem and leaf tissue fluids at 1 P. M. and 5 P. M., respectively. Although the pH of the stem juices appears to fall after 1 P. M., there is no immediate pronounced decrease until 5 P. M., when the values decrease quite rapidly following the rapid decrease in light intensity.

In figure 2 are given the data for clover as taken from table I and plotted in the same manner as are those for buckwheat in figure 1. The experiment from which these data were obtained was carried out on a clear day similar to that on which the data for buckwheat were obtained. It will be observed that the pH values for the juices of clover are much higher than for those of buckwheat, these values for the juices of clover being above the precipitation point of iron during the day and slightly below this point during the night period. Nevertheless, the range in daily variation appears to be much the same in both species. Here again, the juices of stems are much more acid than are those of the leaves, the stems having a maximum pH value of 5.98 and a minimum of pH 5.59, while the leaves have a maximum of 6.34 and a minimum of 5.83. Highest acidity is indicated at 7:00 A. M. and lowest acidity at 5:00 P. M.

The next plant to be considered is *Sedum,* which is a thick leaved, fleshy succulent. The data for this species are presented in table II and are graphically shown in figure 3. It will be observed that this plant shows the daily change in hydrogen-ion concentration to a much more marked degree than do those already considered. In stems, the range is from pH 4.78 at 7:00 A. M. to 5.49 at 7:00 P. M., while the leaves show a corresponding range

TABLE II

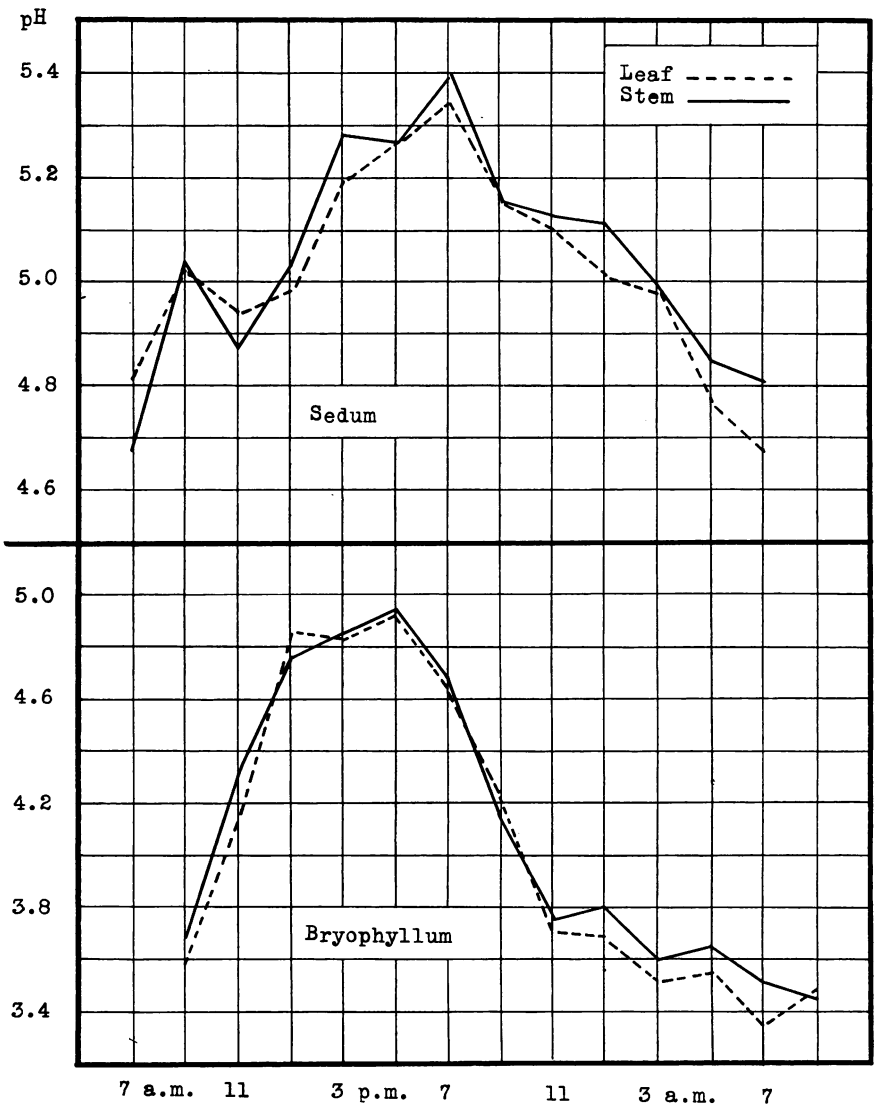
pH VALUES OF TISSUE FLUIDS OF STEMS AND LEAVES OF *Sedum*, *Bryophyllum* AND *Tradescantia* PLANTS AT TWO HOUR INTERVALS

TIME	*Sedum*		*Bryophyllum*		*Tradescantia*	
	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES
9:00 A. M.	5.131	5.122	3.685	3.584	4.641	4.395
11:00 A. M.	4.970	5.038	4.311	4.125	5.241	5.207
1:00 P. M.	5.122	5.081	4.750	4.852	5.528	5.453
3:00 P. M.	5.376	5.292	4.835	4.818	5.461	5.384
5:00 P. M.	5.359	5.359	4.936	4.920	5.511	5.495
7:00 P. M.	5.494	5.440	4.666	4.649	5.545	5.494
9:00 P. M.	5.249	5.249	4.159	4.243	5.148	5.173
11:00 P. M.	5.224	5.198	3.753	3.719	5.351	5.300
1:00 A. M.	5.207	5.114	3.821	3.685	5.275	5.266
3:00 A. M.	5.087	5.080	3.618	3.516	4.666	4.556
5:00 A. M.	4.869	4.945	3.652	3.550	4.548	4.717
7:00 A. M.	4.784	4.911	3.516	3.347	4.599	4.646
9:00 A. M.	.....	.....	3.449	3.483	4.954	4.903

from 4.91 at 7:00 A. M. to 5.46 at 5:00 P. M. In this plant the pH values of stem and leaf juices show no significant differences and the graphs representing these values follow somewhat the same course throughout. This appears to be characteristic of fleshy, thick leaved plants such as were here used.

Bryophyllum, another of the thick leaved, fleshy plants, shows an extreme range in the pH values of its tissue fluids from day to night, as is indicated by the graphs of figure 4. This range is more than double that indicated for *Sedum.* The maximum pH 4.92 for leaf juices occurred at 5:00 P. M. and the minimum pH 3.34 at 7:00 A. M., while the corresponding maximum and minimum values for stems, pH 4.93 and pH 3.44 are shown for 5:00 P. M. and 7:00 A. M., respectively. The graphs representing the courses of pH values for stems and leaves throughout the experimental period run quite closely together, but again there are no significant differences between stem and leaf values such as are indicated for the thin-leaved plants.

In table I and table II are presented also data for *Rumex* and *Tradescantia,* dealing with the influence of light intensity on the H-ion concentration of plant tissue fluid. These data are not here discussed, and are presented merely to emphasize the fact that acidity change with variation in light intensity is a phenomenon common to many types of plants and occurs



FIGS. 3 (upper) and 4 (lower). Graphs representing the course of change in pH values of leaf and stem juices of *Sedum* and *Bryophyllum* plants during a 24-hour experimental interval.

in proportion to the degree of succulency. This is further emphasized by tests of many species, the data for which are not here presented.

It has long been known that the succulent plants exhibit periodic rise and fall of the acid content of their juices, and the relation of this phenomenon to the respiratory processes has been the subject of extensive and thor-

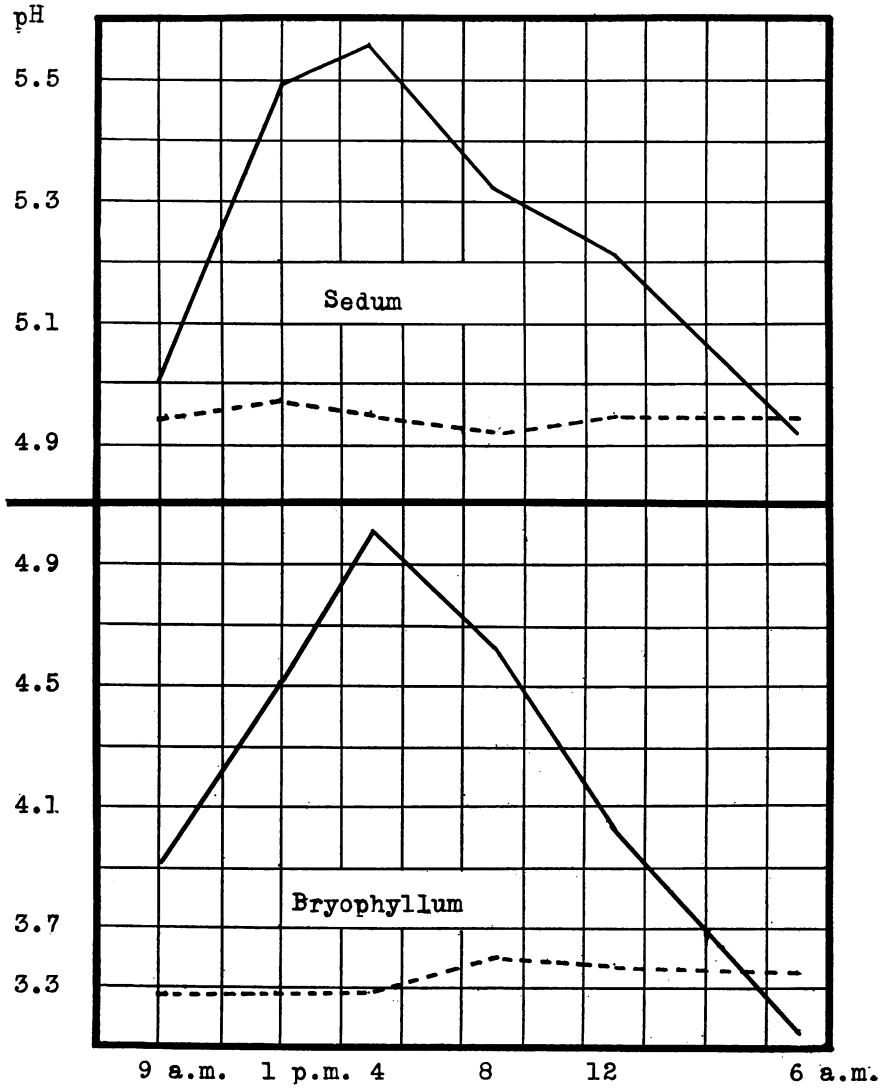
ough investigation. Both RICHARDS (**19**) and SPOEHR (**23**) have shown that the most important single factor which leads to the formation of acids in fleshy succulents is a low or insufficient oxygen supply, while their disappearance is mainly due to the photolytic action of light which breaks them down into simpler substances. But whether the low pH values of plant tissue fluids in the absence of light are the result of the formation and accumulation, under these conditions, of titratable organic acids, or whether they are due to the formation of carbonic acid from the accumulation and solution of carbon dioxide in the plant juices at night, as appears to be indicated by the work of MAQUENNE and DEMOUSSY (**14**) are questions which have not been experimentally investigated.

The present investigation, however, is not concerned with the causes underlying the accumulation and disappearance of organic acids in plants, but only with the fact that pH fluctuations of plant tissue fluids do occur, not only in fleshy succulents but also, to a lesser extent, in plants exhibiting a low degree of succulency, and that these fluctuations are directly related to variations in light intensity. To demonstrate that light is the important factor in the diurnal pH fluctuations of plant tissue fluids, a comparison was made of the juices of the plants of *Bryophyllum* and *Sedum,* exposed in the greenhouse to alternating day and night during 24 hour periods, with the juices of plants of the same age grown under similar conditions, but kept in dark chambers in the greenhouse during corresponding 24 hour periods. Care was taken to keep the temperatures approximately equal around the plants inside and outside of the dark chambers

TABLE III

pH VALUES OF THE TISSUE FLUIDS OF *Sedum* AND *Bryophyllum* PLANTS IN CONTINUOUS DARKNESS AS COMPARED WITH THOSE EXPOSED TO INTERMITTENT PERIODS OF LIGHT AND DARKNESS

TIME	*Sedum*		*Bryophyllum*	
	LIGHT	DARK	LIGHT	DARK
	pH	*pH*	*pH*	*pH*
9:00 A. M.	5.004	4.936	3.922	3.483
1:00 P. M.	5.495	4.970	4.514	3.483
4:00 P. M.	5.562	4.953	5.004	3.483
8:00 P. M.	5.325	4.920	4.632	3.615
12:00 P. M.	5.207	4.953	4.023	3.584
6:00 A. M.	4.920	4.936	3.347	3.550



FIGS. 5 (upper) and 6 (lower). Graphs representing the pH values of tissue fluids of *Sedum* and *Bryophyllum* plants which were exposed to intermittent (unbroken line) and continuous (broken line) periods of darkness.

during the experimental periods. Data for such a comparison are given in table III and are shown graphically in figures 5 and 6.

It will be observed that the juices of the plants exposed to alternate light and dark show the usual wide range in pH values, while the juices of the plants kept in continuous darkness show only very slight fluctuations which are not at all related to the light factor.

It may be of interest here to emphasize the point that comparisons of plant tissues or tissue fluids, particularly with respect to pH values and also, as will be brought out later, with respect to soluble iron content, can be of little value unless the samples upon which quantitative measurements are made are collected at the same time during the day or night. External conditions, particularly light intensity, which is subject to almost continuous fluctuation, have a pronounced influence upon these internal factors and may render any set of measurements of them useless for purposes of comparison unless careful attention is given to the collection and preparation of experimental material.

Relation of pH values to soluble iron content of plant tissue fluids

It has been suggested by HOFFER and CARR (**9**) that the mobility of iron and aluminum salts in plants is associated with high sap acidity, and they have shown that under certain conditions relatively large quantities of iron and aluminum will accumulate in different parts of corn plants. It has also been shown by MARSH and SHIVE (**15**) that plants under certain conditions may become chlorotic from lack of iron in the leaves when the total iron content of the plants is excessively high. Furthermore, in view of the fact that the iron requirement of plants is relatively high during periods of high light intensity and low during periods of diminished light, it is of interest to determine whether or not the soluble or *filterable* iron in plants bears any relation to the periodic fluctuation in the pH values of the tissue fluids resulting from variations in light intensity.

Accordingly, the soluble (filterable) iron content of tissue extracts taken at regular intervals throughout 24-hour experimental periods was determined for a number of species. The manner of taking samples, preparing the extracts, and the technique employed in making the pH tests and the chemical analyses have already been described. The data are presented in table IV. This experiment, like those previously described, was carried out on clear days so that the plants were exposed to approximately maximum variation in light intensity from day to night.

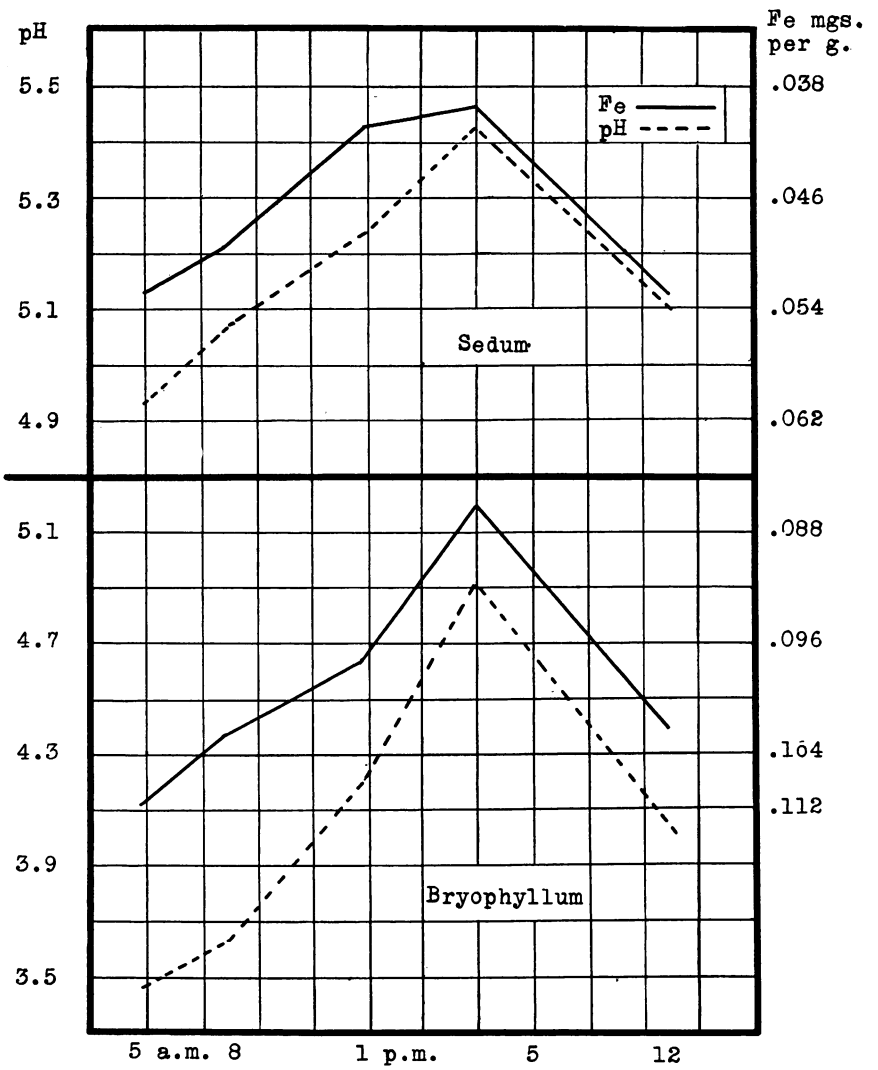
Examination of the data of table IV brings out the fact that there is a direct and very exact relation between the H-ion concentration of the tissue fluids and the soluble iron content of all the species investigated. In each species, the fluctuation in pH values of the plant juices with variation in light intensity is followed, in the inverse order, by a corresponding fluctuation in the soluble iron content. That is, for each species, low pH values correspond with high soluble iron content and high pH values with low soluble iron content.

To bring out the exactness of this relation and to show the course of fluctuation of the soluble iron content in the plants during a 24-hour period,

TABLE IV

pH VALUES AND SOLUBLE (FILTERABLE) IRON CONTENT, EXPRESSED AS MG. PER GRAM OF DRY TISSUE, AT INTERVALS DURING 24-HOUR EXPERIMENTAL PERIODS

PLANT	TIME	STEMS		LEAVES	
		ACIDITY	FE	ACIDITY	FE
		pH	*mg.*	*pH*	*mg.*
Asparagus	6:00 A. M.	5.478	0.0342	5.687	0.0299
	12:00 Noon	5.528	0.0277	5.850	0.0239
	5:00 P. M.	5.664	0.0245	6.086	0.0223
	12:00 Night	5.511	0.0302	5.816	0.0267
Clover	6:00 A. M.	5.630	0.0327	5.934	0.0316
	12:00 Noon	5.866	0.0202	6.137	0.0275
	5:00 P. M.	5.968	0.0241	6.357	0.0247
	12:00 Night	5.731	0.0329	5.985	0.0289
Soybean	6:00 A. M.	5.731	0.0354	5.883	0.0330
	12:00 Noon	5.782	0.0300	5.923	0.0299
	5:00 P. M.	5.830	0.0247	6.230	0.0212
	12:00 Night	5.799	0.0333	5.960	0.0244
Tobacco	6:00 A. M.			5.579	0.0438
	12:00 Noon			5.765	0.0315
	5:00 P. M.			5.833	0.0364
	12:00 Night			5.664	0.0445
Tomato	5:00 A. M.	5.190	0.0392	5.579	0.0314
	9:00 A. M.	5.207	0.0373	5.718	0.0299
	1:00 A. M.	5.461	0.0331	5.985	0.0256
	5:00 P. M.	5.562	0.0298	6.154	0.0237
	9:00 P. M.	5.528	0.0361	6.036	0.0266
	1:00 A. M.	5.308	0.0345	5.799	0.0295
Buckwheat	5:00 A. M.	4.395	0.0751	4.903	0.0588
	9:00 A. M.	4.427	0.0762	4.970	0.0566
	1:00 P. M.	4.598	0.0668	5.038	0.0489
	5:00 P. M.	4.804	0.0626	5.359	0.0445
	9:00 P. M.	4.767	0.0676	5.258	0.0518
	1:00 A. M.	4.362	0.0768	5.089	0.0588
Sedum, whole plant	5:00 A. M.	4.936	0.0535		
	8:00 A. M.	5.055	0.0502		
	1:00 P. M.	5.241	0.0429		
	5:00 P. M.	5.427	0.0399		
	12:00 Night	5.122	0.0535		
Bryophyllum	5:00 A. M.			3.483	0.1071
	8:00 A. M.			3.612	.....
	1:00 P. M.			4.210	0.0972
	5:00 P. M.			4.896	0.0861
	12:00 Night			4.023	0.1020
Dock, whole plant	5:00 A. M.	4.091	0.0762		
	8:00 A. M.	4.193	0.0640		
	1:00 P. M.	4.226	0.0557		
	5:00 P. M.	4.480	0.0513		
	12:00 Night	4.362	0.0640		



FIGS. 7 (upper) and 8 (lower). Graphs representing the course of variation in pH values and soluble iron content of *Sedum* and *Bryophyllum* plants during a 24-hour experimental interval.

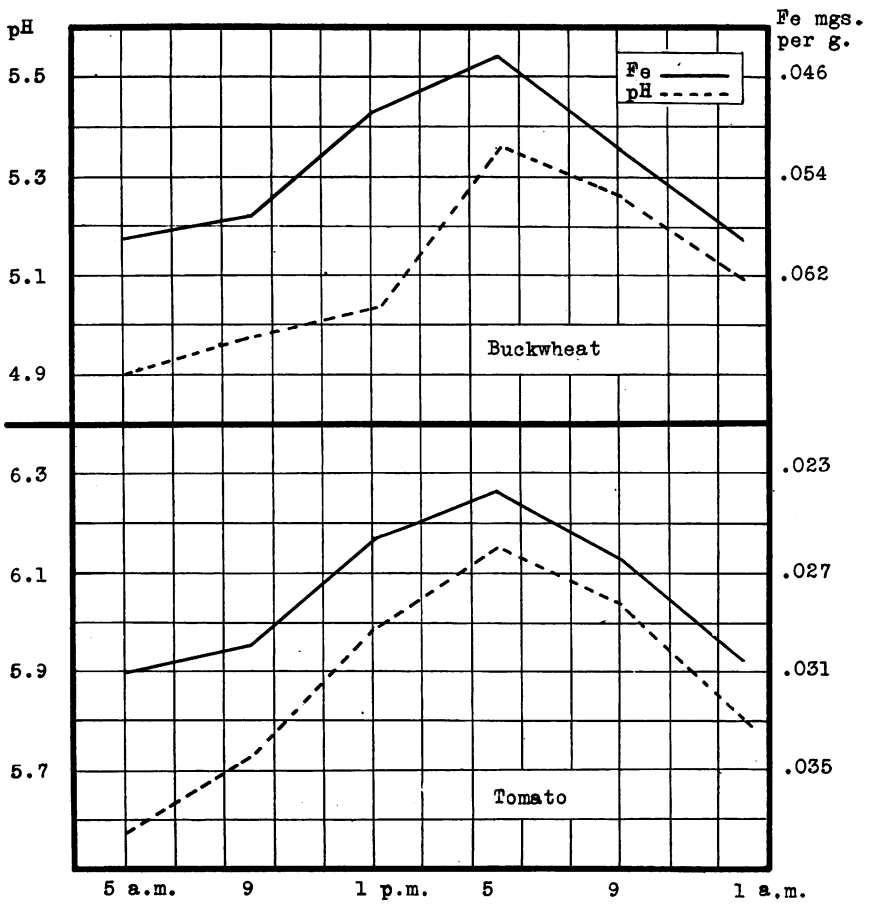
the data for two species of fleshy succulents (*Bryophyllum* and *Sedum*) and for two species of thin-leaved plants showing a relatively low degree of succulency (tomato and buckwheat) have been plotted to form the graphs of figures 7 and 8, and 9 and 10. The pH values and the values for the soluble iron content for each of these species are plotted together to form a pair of graphs with common abscissas, the ordinates on the left indicating

pH values, those on the right expressing soluble iron content (mg. per gram of dry plant tissue). To avoid intersecting of the graphs, the ordinates on the right are written in the inverted order.

The lower graph for each species (figs. 7 and 8) shows the usual course of pH change during a 24-hour period and this, in every case, is almost duplicated by the inverted graph representing the course of fluctuation in the soluble iron content during the same period, thus indicating an intimate relation between pH values of tissue fluids and that portion of iron in the plant which may be regarded as the active fraction, on the reasonable assumption that filterable iron here considered is mobile, readily available, and capable of functioning in the plant processes.

Another significant and important relation is here indicated. A comparison of the data for *Bryophyllum* with those for *Sedum* (fleshy succulents, figs. 7 and 8) brings out the fact that the juices of the former show relatively low pH values, ranging between 3.48 and 4.90, with a relatively very high content of filterable iron, ranging between 0.0861 and 0.1071 mg. per gram of dry tissue; while the juices of the latter show higher pH values, 4.94 to 5.43, with a correspondingly much lower content of filterable iron, ranging from 0.0399 to 0.0536 mg. per gram of dry tissue during a 24-hour period. A comparison of the data for tomato and buckwheat (thin-leaved plants with relatively low degree of succulency, figures 9 and 10) shows this relation in an equally definite manner. Of the four species graphically considered, the tomato shows the highest pH values, varying between 5.58 and 6.15, and the lowest content of filterable iron, fluctuating between 0.0314 and 0.0237 mg. per gram of dry tissue, during an experimental period of 24 hours. This relation holds for all the species the data for which are presented in table IV. Furthermore, the relation holds also for different organs of the same plant, as between stems and leaves, when these organs show considerable difference in the pH values of their juices. This is clearly shown by the data in table IV for stems and leaves of buckwheat, tomato, asparagus and soybeans. The significance of this relation will be further considered in the following section.

It might be well here to suggest that from the data thus far presented it appears that those plants in which the pH values of the tissue fluids lie close to the precipitation point of iron (about 6.0) show greater fluctuation in the filterable iron content from day to night than do those plants in which the pH values of the tissue fluids lie considerably below the precipitation point of iron. This is probably to be inferred from the fact, as will be brought out later, that in plants of the latter type a high percentage of the total iron is in the soluble form, and under such conditions small fluctuations in this fraction might be expected. However, this is merely put forth as



FIGS. 9 (upper) and 10 (lower). Graphs representing the course of variation in pH values and soluble iron content of buckwheat and tomato leaves during a 24-hour experimental interval.

a suggestion, since not sufficient evidence of a positive character is at hand to warrant a definite conclusion.

Comparison of soluble and total iron content of plants with pH of tissue fluids

It has already been pointed out that in certain types of plants, only a very low percentage of the total iron in the tissues is in the soluble (filterable) state, while in other types of plants nearly all the iron is in the soluble state. A study was made of a number of species showing a range in the average pH values of tissue fluids from 4.04 to 6.10, in order to emphasize the relation which pH values of the tissue fluids bear to total, insoluble, and soluble iron fractions in the various species.

TABLE V

AVERAGE pH VALUES, TOTAL AND SOLUBLE IRON CONTENT OF VARIOUS PLANTS OVER A 24-HOUR PERIOD

PLANT	LEAF			STEM		
	Fe, PER GRAM OF DRY TISSUE			Fe, PER GRAM OF DRY TISSUE		
	ACIDITY	TOTAL	SOLUBLE	ACIDITY	TOTAL	SOLUBLE
	pH	*mg.*	*mg.*	*pH*	*mg.*	*mg.*
Bryophyllum	4.04	0.137	0.0958			
Rumex (whole plant)	4.24	0.219	0.0622			
Buckwheat	5.10	0.269	0.0516	4.58	0.082	0.0696
Sedum (whole plant)	5.16	0.278	0.0477			
Tobacco	5.74	0.325	0.0390			
Tomato	5.87	0.297	0.0277	5.38	0.089	0.0349
Asparagus	5.87	0.373	0.0256	5.54	0.111	0.0214
Soybeans	6.01	0.469	0.0246	5.79	0.142	0.0308
Clover	6.10	0.571	0.0281	5.78	0.214	0.0299

The data for the various species presented in table V are arranged in the ascending order of average pH values of the different species. The data were obtained by collecting samples of each species at regular intervals (two or four hour intervals) throughout twenty-four hour periods on clear days. The various measurements were made on these samples and the corresponding values obtained for each species were then averaged; so that each value in the table represents the average of the values obtained over a twenty-four hour period.

It will be observed that the total iron content increases in the different species as the pH value of the tissue fluids increases: that is, high total iron in any given species corresponds to high pH value of the tissue fluids and low total iron with low pH values. Thus, *Bryophyllum* with the low average pH value of 4.04, shows also a relatively low total iron content of 0.137 mg. per gram of dry tissue; while clover with a pH value of 6.10, shows the abnormally high total iron content of 0.571 mg. per gram of dry tissue. On the other hand, the soluble iron content of the different species varies in the inverse order with variation in pH values of the tissue fluids. That is, low pH values correspond to high soluble iron, and high pH values correspond with low soluble iron. Thus, *Bryophyllum* with a low average pH value of 4.04 shows a low total iron content (0.137) but relatively high soluble iron (0.0958); while clover with a pH of 6.10 shows the abnormally high total iron of 0.571 mg. per gram of dry tissue but a very low content of soluble iron (0.0281). These relations hold, not only for the different species, but

also for different organs of the same plant, as between stems and leaves when these organs show considerable difference in the pH values of their tissue fluids, as has already been pointed out.

From the foregoing considerations it is quite apparent that plants like *Bryophyllum, Rumex* and others with relatively low pH values of the tissue fluids, absorb only very small quantities of iron, and that practically all of the iron absorbed remains in a soluble form and is presumably available and capable of functioning in chlorophyll production and other plant processes. But plants such as clover, soybeans, and others with high pH values of the tissue fluids absorb relatively very large quantities of iron, if this is available in the external medium, but only a small proportion of this remains in a soluble form in the plant. Much of the total iron in plants like these is precipitated, probably along paths of translocation, and is therefore unavailable and undoubtedly does not function in the plant processes. If, for any reason, all of the iron in plants of this type should become soluble at any one time, iron toxicity would probably follow and might result in the death of the plant.

The cause for the accumulation of relatively large quantities of unavailable iron in the tissues of plants in which the pH values of the sap lie close to the precipitation point of iron, or the mechanism by which this is accomplished, is at present not clear. It may be suggested, however, that through the precipitation of iron in the plant tissues this element is removed from the field of osmotic activity and thereby a diffusion gradient for it may be maintained from the outside to the inside of the plant, resulting in the accumulation of relatively large quantities of non-available iron.

From the data here presented, it is to be expected that plants in which the pH values of the tissue fluids lie close to or above the precipitation point of iron may yield a high total iron content, but only a small proportion of this total iron can function in chlorophyll production and other plant activities. This is made clear, not only by chemical analyses of the tissues and tissue fluids, but also by the fact that chlorosis is likely to occur in these plants from lack of available iron under slightly unfavorable conditions, and particularly under conditions of high light intensity during periods of which the plant sap attains its maximum pH value and the plant its minimum value for soluble iron. On the other hand, plants in which the pH values of the tissue fluids lie considerably below the precipitation point of iron show relatively low total iron, but nearly all this iron can be extracted with the tissue fluid and is capable of passing through a quantitative filter-paper of the highest quality. This iron appears to be quite mobile in the plant and is uniformly distributed, as is indicated by quantitative analyses of the tissues of the various plant organs, and it is therefore rea-

sonable to assume that this iron is capable of functioning in the plant processes.

Summary

1. The hydrogen ion concentration of tissue fluids varies with light intensity: low hydrogen ion concentration corresponds to high light intensity, and high hydrogen ion concentration to low light intensity.

2. Fleshy or succulent plants show greater variation in hydrogen ion concentration of tissue fluids with change in light intensity than do thin leaved plants, the range of variation occurring in proportion to the degree of succulency of the plants.

3. All plants studied show differences in hydrogen ion concentration between leaf juices and stem juices; fleshy or succulent plants show much lower differences, however, than do non-succulent plants, the degree of difference being in proportion to the degree of succulency.

4. A comparison of hydrogen ion concentration of tissue fluids of different species has no significance whatever unless determinations are made from material collected at the same time from plants grown under approximately identical conditions.

5. Soluble (filterable) iron content of plants varies directly with the hydrogen ion concentration variation brought about by changes in light intensity from day to night.

6. Plants in which the tissue fluids have low hydrogen ion concentration values show high total and relatively low soluble iron content; and those in which the tissue fluids have high hydrogen ion concentration values show low total iron and relatively high soluble iron content.

7. In all plants studied, the iron content of leaves is higher than the iron content of stems.

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