The Detection, Distribution and Mobility of Certain Elements in the Tissues of Plants Growing Under Different Conditions as Determined by the Spectrographic Method
Michigan State University Agricultural Experiment Station
Technical Bulletin
R.P. Hibbard, Botany and Plant Pathology
Issued February 1941
31 pages

TECHNICAL BULLETIN 176 FEBRUARY 1941

THE DETECTION, DISTRIBUTION AND MOBILITY OF CERTAIN ELEMENTS IN THE TISSUES OF PLANTS GROWING UNDER DIFFERENT CONDITIONS AS DETERMINED BY THE SPECTROGRAPHIC METHOD

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INTRODUCTION

Increased interest in the detection and determination of the so-called minor elements, which occur in plants in only minute quantities, brings to attention the necessity for a better, more rapid, and more accurate method for their determination. This is not an attempt to discount gravimetric methods of the ordinary type because these are recognized as being unsatisfactory for the estimation of the exceedingly small quantities of certain elements found in plants. The modern spectrographic technique for analysis is a possibility for use with plant tissues. If that technique can be applied with sufficient accuracy, the way to a better knowledge of the mobility, the distribution, and the rôle of the elements—especially the minor ones—in the physiology of the plant, may be opened.

The detection and determination of impurities in metals, alloys, and solutions have been accomplished formerly by the use of the spectograph. The advance and improvements in this direction since the time of Lockyear (1874), when the first attempts in spectral analyses were made, are described in a thesis by Day (10).

Spectrographic determination of the elements in plant tissues was first successfully attempted by Lündegardh (27, 28) in 1928, but the oxyacetylene flame method used by this author has been superseded because the non-metals were not revealed and a very poor spectrum was obtained. This leaves the arc and spark methods as the only reliable ones to use.

Way and Arthur (45) enumerated the errors to be expected in studies of this sort. In a recent paper by Ewing, Wilson and Hibbard (11) further improvements have been reported and in the paper noted above by Day, a new procedure of photographic plate control, incorporating internal control without the use of computed ratios of spectral intensities, or photographic blackenings, was offered.

This publication is a report of the determination of some ash constituents found in pigmented and non-pigmented parts of certain plants, and a study of the distribution, mobility and possible rôles of certain elements in various tissues of the garden pea, as revealed by the method of spectrographic determination previously worked out at this institution (11).

MATERIALS AND METHODS

Spectrographic Method

The instrument was a Bausch and Lomb quartz spectrograph having a dispersion varying from 7 Å per mm. at 2500 Å, to 21 Å per mm. at 3500 Å. The spectra of impurities in metals can be obtained by arcing electrodes made of the metals, but material from biological sources must be treated in other ways. In these studies, Acheson graphite electrodes were found to be the best, but for calcium determinations even these electrodes were specially treated to free them of metallic impurities. In the preparation of the electrodes for plant material, holes 7 mm. deep were drilled into the ends of the electrode, and into these the test solution was carefully pipetted. These drilled rods were the anodes and were always the lower electrode. The cathodes were from the same lot of rods. The arc was excited by a 15-ampere, 300-volt motor generator. A revolving sector was used to regulate the fraction of incident light reaching the slit.

One entirely new feature in the general method was the preparation of the standards. Preparation of the test sample also received special attention. The base solution in which the sample was dissolved was sodium-ammonium chloride, consisting of 5 grams of sodium chloride, 45 grams of ammonium chloride, and 4.5 grams of hydrochloric acid in a liter of water. Of a large number of solutions tested, the chlorides of the metals gave sharper spectral lines than other negative radicles. Zinc chloride vaporized too quickly and lead was too insoluble. Sodium chloride caused the arc to flare, thus producing uneven exposures. Although ammonium chloride gave even exposures and a good arc, it did not create sharp and clear spectral lines; consequently various mixtures of ammonium and sodium chloride were tried until sharpness and clearness of lines was obtained. The base solution, sodium-ammonium chloride, served two purposes—first as a "filler" giving a greater amount of solid substance in the electrode, and, second, as a means for holding back most of the carbon spectra until volatilization of the sample was complete.

Because no two elements under identical arcing conditions give the same line blackness for an equal change in concentration, it became necessary to try various concentrations until a series of dilutions was obtained which would give gradations of line intensities suitable for analysis. Standards were, therefore, made from the purest chemicals available. For calcium studies, 2 grams of calcium nitrate were dissolved in a liter of base solution and then diluted again so as to give a series of solutions,—milliliter quantities of which would contain 1.0, 0.5, 0.3, 0.2, 0.1, 0.075, 0.05, 0.03, 0.02, 0.01, 0.005, and 0.0025 mg. of calcium. The series of solutions for magnesium was made up in the same way, using magnesium chloride dissolved in the base solution. The series contained 5.0, 2.5, 1.0, 0.5, 0.3, 0.25, 0.2, 0.1, 0.05, 0.025, 0.01, and 0.005 mg. of magnesium per ml.

Potassium behaves differently in that larger amounts can be used to show a marked gradation of line intensities. The higher concentrations may be successfully analyzed directly from the sample without employing a "filler" or a dilution of the sample. In the base solution enough potassium chloride was dissolved to yield 30 grams of potassium in a liter. By dilutions with the base solution a series of concentrations was made up containing 30, 25, 20, 17.5, 15, 12.5, 10, and 5 mg. of potassium per liter. Phosphorus as potassium acid phosphate was dissolved in the base solution. The concentrations made up were 2.0, 1.0, 0.5, 0.2, 0.1, 0.05, 0.02, and 0.01 mg. of phosphorus per ml. Iron, as ferrous ammonium sulphate, was diluted in the base solution and the concentrations made to contain 1.0, 0.5, 0.25, 0.10, 0.05, 0.025, 0.01, 0.005, and 0.0025 mg. of iron per ml.

For the analysis of plant ash, the procedure was to add a 0.05-gram sample to one ml. of concentrated hydrochloric acid and allow it to stand for one hour. Then enough of the base solution was added to this to make a total volume of 10 ml. The potassium samples were similarly prepared with the exception that the sample of ash was 0.2 gm. per 10 ml. of solution instead of .05 gm. For checks, synthetic ash solutions were prepared by dissolving the salts in a 4.5 per cent hydrochloric acid solution so that the resulting concentrations were 0.7 gram of calcium, 0.4 gram of magnesium, 0.15 gram of phosphorus, 0.05 gram of iron, and 14 grams of potassium per liter.

The spectra were photographed. No condensing lens was used between the arc and the slit of the spectrograph. The arc was set at a distance of approximately 30 inches from the slit. A revolving sector was placed in front of the slit to regulate the percentage of light. No increase in the quantity of sample was needed at this distance, and there were no difficulties from "arc wanderings." Sufficient light was used to keep the heaviest lines employed for analysis well under maximum blackening. Eastman "33" plates were suitable for the conditions of these experiments, except where iron interfered with potassium and then the Wratten-Wainwright panchromatic plates gave the best results. Both types of plates were developed in Eastman developer D-11 for 5 minutes at 18° C. The method of "continuous exposure" extensively employed here was used to determine the behavior of the five elements while volatilization was going on. The current was maintained at 10 amperes.

The intensity or relative blackness of the lines was determined with a Bausch and Lomb density comparator. In quantitative analysis the intensity of the light in a given spectral line rather than the photographed image is considered. The intensity of the light in the source depends on the amount of the element in that source that is giving forth the light. However, the photographed spectrum is the best means available to measure the relative intensities of the light of different wave lengths.

Very few lines or "raies ultimes" are suitable for quantitative analysis by the method of arc excitation because they often show maximum blackening in comparatively low concentrations. A line, to be used effectively, must be persistent even in very low concentrations, and it must show a definite change in line density for small changes in concentration. Such effective lines give very satisfactory standard curves over even a short range of concentration. Many lines for each element were studied therefore before final selections were made.

The potassium lines were 3446.37 Å and 3447.38 Å. These were very effective between concentrations of one to 50 mg. per ml., and gave smooth standard curves. The calcium lines were 3158.87 Å and 3179.33 Å. These were effective between concentrations of 0.001 and 5 mg. per ml. and gave good standard curves. The iron lines 2598.39 Å and 2599.40 Å were good for the range of concentrations of 0.0005 to 2 mg. per ml. The magnesium lines were 2776.71 Å and 2779.85 Å and the best range of concentration was 0.001 to 5 mg. per ml. The phosphorus line was 2536.38 Å, and was easily read since impurities were lacking and the lines of other substances were not close by. A satisfactory standard curve was obtained for concentrations between .01 and 10 mg. per ml.

Evaluations of these line intensities or blackness of spectral lines were made only on clear or unfogged plates. First, a reading was made when no light was allowed to enter the slit. Each line was read independently until an accurate check was obtained. The plate was then moved to a clear background in the same region and the reading recorded as "background." The differences between the blackness of the line and absolute blackness and between the "background" and absolute blackness were calculated. The ratio of these differences was plotted against the logarithms of the concentrations to obtain the standard curve. Each plate then held a set of spectra of standards, from which the standard or working curve was formulated, and the spectra of a number of unknowns, which were evaluated from this curve. Duplicate plates were made to check results.

Plant Material and Preparation of Ash

A variety of tissues from selected species of plants was employed. The first studies were made with colored leaves of Iresine, and the green and white areas in the variegated leaves of Euonymus, Aegopodium, and Tradescantia. Water extractions were made from these leaves or leafy parts to obtain the sap pigments and, on further extraction of the residue with acetone, the plastid pigments were isolated. The several extractions in each group were combined and evaporated to dryness, and then ashed in a furnace. Thus, ashes of sap pigments and of plastid pigments were obtained and these were studied spectrographically to determine the relative abundance and distribution of certain elements.

Two separate experiments were conducted in different years using tissues of the pea plant, *Alaska* variety. At first the selected young seedlings were all grown in a so-called complete solution under usual daylight conditions, and then transferred to different light conditions and nutrient supplies. When harvested later the various tissues, such as the roots, stems and leaves, were separated, dried, ashed and studied spectrographically as previously indicated. Further details in the methods will appear in their proper places.

EXPERIMENTATION

EXPERIMENT I. STUDIES WITH IRESINE LEAVES

Leaves from greenhouse-grown Iresine plants were selected for this study because they contained an abundance of anthocyanins as well as plastid pigments. They were shaken, then brushed to rid them of adhering particles and finally dried in an oven. After they had been ground to pass a forty-mesh screen, a magnet was run through the powder to remove any possible metallic iron. Ten grams of this air-dried material were then weighed out and extracted in the following manner: The sample was added to 250 cc. of warm (50° C.) 85% acetone for about a half-hour with frequent shakings. This was filtered and the filtrate saved. The residue was now extracted with warm water as already described, and filtered. This filtrate was saved and the residue again extracted with warm 85% acetone. These operations were repeated a sufficient number of times to insure a complete extraction of all color, plastid as well as sap pigments. All the filtrates were combined and evaporated down nearly to dryness. Then the material was taken up in a mixture of 50 cc. of c.p. diethyl ether in 50 cc. of water. The ether had previously been stored over metallic sodium and was of the highest purity.

After standing over night in a separatory funnel the ether extract was washed five times with water until the water, after passing through, remained clear. The water extract was next washed with ether about 40 times until the ether came through colorless. Each extract was then evaporated down. The plastid sample, in a mushy condition, was transferred to a platinum crucible and dried down in the oven over night. The material remaining in the evaporating dish was washed with ether and again transferred to the platinum crucible. This operation was repeated many times until all material possible had been transferred from the evaporating dish to the crucible. This was dried to constant weight and finally ashed in a muffle furnace.

The sap sample was more easily removed from the evaporating dish to the platinum crucible than the other sample, but this material was so hygroscopic that considerable difficulty was encountered in obtaining a final constant weight. In this treatment the evaporating dish was also washed with water several times in order to transfer all the material possible to the platinum crucible. After drying to constant weight, the sample was set in the muffle furnace and ashed. Data for the amounts of ash, from the three sources, are given in Table 1.

TABLE 1. *Percentages of Ash in Ether and Water Extracts and Tissue Residue from 10 Grams of Oven-dried Iresine Leaf Powder.*

Extraction	Grams Materials Removed	Per Cent Materials Removed	Grams Ash	Per Cent Ash
Ether	.5102	5.10	.0075	0.38
Water	2.8688	28.68	1.1814	60.16
Residue	6.6220	66.22	.7746	39.44
Total	10.0000	100.00	1.9635	99.98

Table 1, column 3, shows that approximately one-third of the sample was extracted by the combined ether and water treatments, with the amount extracted by water far greater than that by ether. Likewise the ash from the water extraction was greatly in excess over that obtained from the ether extraction. The figure for the ash of the residue shows that nearly two-fifths of the total ash was insoluble in these two solvents.

The results from the spectrographic analysis of the sample of Iresine leaf powder appear in Table 2.

TABLE 2. *Percentage Composition of Certain Elements in the Ash of Sap and Plastid Material and Residue of 10 Grams of Iresine Leaf Powder.*

Element	Ash of Leaf Sap	Ash of Leaf Plastid	Ash of Leaf Residue
Calcium	1.960	9.350	22.600
Magnesium	3.440	6.800	16.200
Potassium	59.200	none	none
Iron	0.015	0.061	0.250
Phosphorus	0.300	0.590	0.960
Manganese	0.001	0.003	0.002

Table 2, column 2, gives the percentages of the several elements in the sap ash of Iresine leaf powder. The most abundant of these elements in the ash of mature leaves are potassium, calcium and magnesium. Iron, phosphorus, and manganese are not nearly so abundant. Although the water extract from the leaf (Table 1, column 4) gave the greatest percentage of ash, this excess was due to the large amount of potassium present. No potassium was detected in the ash of the ether extract (Table 2, column 3) or in the ash of the residue (column 4). Potassium in this plant was, at this phase of growth, in the ionic form and all water-soluble.

Much higher percentages of calcium, magnesium, iron, phosphorus and manganese were found in the residue and the ether extract, than in the water extract. In the plastid pigments, calcium and magnesium were two to four times as abundant in the ether-extracted material and the residue. The presence of magnesium in comparatively large quantities in the plastid pigments was expected, since magnesium occurs in the chlorophyll molecule. Calcium also was more abundant in the plastid pigments than in the sap pigments but because it is not a constituent of chlorophyll, it must exist free when absorbed by the roots and moved to the leaves, or chiefly in some organic combination. It is apparent, however, that the leaf contained ether-soluble organic constituents. A glance at Table 2 also reveals the presence of ether-soluble magnesium, phosphorus and iron derivatives. Manganese was present in minute amounts but more in the plastid and residue material than in the sap. It may also form organo-compounds.

EXPERIMENT II. STUDIES ON THE GREEN AND WHITE AREAS OF VARIEGATED LEAVES

Three species of plants showing variegated leaves were selected for this experiment on the possibility that there would be differences in the elemental content of the different areas. The leaves from the woody Euonymus were taken in the mid-forenoon from a large bush on the campus and from a height convenient to reach, on the east side. Samples were approximately of the same size and, therefore, of similar age. Aegopodium, an herbaceous plant, growing as a margin in a large flower bed was the next choice and the larger, healthy leaves were taken. The third plant was Tradescantia, growing in a greenhouse.

In all three cases, the leaves were first brushed and then the white and the green areas cut out. After drying in the oven, the samples were ground to a powder and treated as in case of the Iresine samples. The extractions were made with acetone and water and then with ether, so as to separate the plastid and sap pigments which were present. The residue was discarded. The operator's chief concern was to find, approximately, the distribution of certain elements in the two different areas when extracted with water, acetone, and ether. From the preceding experiment, it was evident that considerable mineral matter is tied up in organic combinations.

The amounts (expressed in milligrams) of potassium, calcium, and magnesium present in the green and white areas of three variegated leaf species tested may be found in Table 3.

As shown in Table 3, the elements considered were potassium, magnesium and calcium. Here, as in the preceding experiment, potassium was the most abundant and it was also found to be absent from the plastid material both in the green and yellow areas. Again, it is evident that potassium is entirely water-soluble inasmuch as it is absent in the ether extract.

TABLE 3. *Milligrams Per 100 Grams of Sample in Green and White Areas of Leaves of Certain Varieties of Plants.*

Plant	Sample	Elements	Aegopodium	Tradescantia	Euonymus
Green Areas	Sap	Ca Mg K	4.3 3.1 583.0	6.4 7.2 380.0	15.4 9.1 380.0
	Plastid	Ca Mg K	19.2 7.1 none	12.2 8.6 none	23.8 11.2 none
Yellow Areas	Sap	Ca Mg K	0.9 1.2 475.0	1.1 2.6 370.0	1.2 1.7 230.0
	Plastid	Ca Mg K	1.6 2.5 none	3.1 2.9 none	4.8 3.2 none

It is shown in the table that Aegopodium contained a higher percentage of potassium than either Tradescantia or Euonymus. This is not surprising because species differ in their absorptive powers even while growing in the same soil or the same nutrient solution. Some plants absorb certain elements, e.g., potassium, in proportions applied to the nutrient medium (16a), up to a certain limit of course, while others may be fed considerable but absorb only small amounts. Finally the plants used here were from different habitats and could, therefore, reveal variations in the amount of the different elements absorbed. In addition, it is well known that plants may take up elements in excess of their requirements. Finally, potassium was found to be slightly more abundant in the green area than in the yellow except in the case of Tradescantia where the percentage difference is only slight.

The next most abundant element was calcium and this occurred both in the plastid and sap material. It was present, however, in greater percentages in the plastid material from the green area than in that of the yellow. The total amount of calcium was very much less, of course, than that of potassium. It was found in larger percentages in the green areas of the sap material than in the yellow. As in Iresine, the ash of the ether extract contained a greater percentage than the ash of the water-soluble material.

Magnesium was present in lesser concentrations than calcium in these plants with the exception of Tradescantia, where there appears to be hardly any difference. It was more abundant in the green areas than in the yellow and also more plentiful in the plastid material than in the sap. Magnesium occurred most abundantly in Euonymus, next in Tradescantia, and least in Aegopodium.

EXPERIMENT III. FIRST EXPERIMENT WITH THE GARDEN PEA, *ALASKA* VARIETY

In the fall of 1935, the first of two water culture experiments was set up. Approximately 2,000 pea seeds were germinated and as the hypocotyl broke through they were set with the hypocotyl down, on paraffined wire netting, laid over large crocks filled to overflowing with a complete nutrient solution.

This afforded a good supply of seedlings with straight roots and shoots of any length desired. From this lot, 560 seedlings with roots 4 cm. long were transferred to three-gallon earthenware crocks, 40 seedlings to each crock. These seedlings were all grown for two weeks in a complete solution, previously found to be suitable for the growth of the pea plant. The cotyledons were then removed. At the end of a week, most of the seedlings had survived the treatment. The few that did not were replaced by others growing in a jar held in reserve. These had had their cotyledons removed at the same time as the others. The removal of the cotyledons was designed to leave the seedlings no other supply of carbohydrates and minerals than that obtained from their environment.

At this time, with a large number of uniform, vigorously growing seedlings, the differential treatments were started. First a group of 40 seedlings was harvested. The leaves, roots and stems were grouped separately, and fresh and oven-dry weights determined. Each group was ashed separately and reserved for later spectrographic analysis for the elements. The data collected from this group of 40 seedlings, represented the distribution of the various elements at the start of the experiment. At the end of the experiment, similar data from groups of 40 seedlings under various conditions of growth make a comparison possible and from this might be deduced the distribution of the various elements in certain tissues of the plant for that period. The leaves as well as the stems were separated into two groups. Those on the upper half of the plants are designated in the tables as terminal leaves and stems; and those on the lower half of the plants as basal leaves and stems.

Some of these cultures were put under long-day light conditions (regular day length plus electric light until midnight). Others for a light period of 9 to 10 hours and still others for a day length of 6 hours. At the time the experiment was conducted (October 12-November 18), the length of day was between 9-10 hours. The long-day light condition was obtained with the use of three 1000-watt lamps under Benjamin reflectors placed at approximately 3 feet from the top of the plants. These lights were automatically regulated to go on at 6:00 p. m. and off at 12:00 m. The short-day period was an exposure of 6 hours, starting at 7:00 a. m. For the rest of the 24-hour period, the plants were housed in a large, well ventilated, cubical dark box, 4½ feet on edge.

The short-light period did not inhibit the growth and development of the pea plants because exposure to light was in that part of the day when photosynthesis was believed to be most active. The final result, however, was some reduction in dry weight of the plant when compared with the check. The lack of calcium was the chief factor that interfered with the growth and development in all these plants since root and top growth almost stopped when calcium was withheld.

All three sets were placed together in the center of the greenhouse. When the short-day plants were exposed to light in the morning all three groups were under approximately the same environmental conditions. Into each of these light conditions, three groups of cultures of 40 plants each were placed. One group consisted of 40 plants growing in a complete solution and this culture was considered the check on the others. The second group consisted of the same number of plants growing in a solution containing all of the elements found in the complete solution with the exception of calcium. The third group was one otherwise like the complete solution but lacking potassium only. When the first sign of injury appeared on the plants in any of the cultures, they were harvested.

Plants in the calcium-deficient solution showed injury before those in the potassium-deficient, the root showing the injury sooner than the leaf. The latter loses color and begins to dry, and the plants grow very little in length (Fig. 1). The roots soften, do not lengthen and may become bulbous at the tips. These and other symptoms are reported by several workers. (11A, 17, 45.)

Fig. 1. Pea plant, *Alaska* variety growing in water cultures. Plants on the right in calcium-deficient solution, those on the left in complete solution. Deficiency of calcium almost immediately inhibits elongation. The difference in height was obtained in a two-week period.

When harvested, the tendrils were discarded but the roots, terminal leaves, basal leaves and terminal stems and basal stems were placed in separate piles, dried and ashed separately. The ash was examined for the presence of calcium, potassium, and magnesium. During the period covering the last 16 days, the solutions were changed twice. The data are shown in Tables 4, 5, and 6.

Of the three elements under consideration, potassium was the most abundant. A glance at Table 4 will show that in the original seedlings and the seedlings in the complete or check solution, there are not such marked differences in percentages between the various tissues as might have been expected, although it is quite evident that the terminal leaves have more

TABLE 4. *Percentage of Potassium in the Ash of Plant Parts Under Certain Environmental Conditions.*

Long Light	Dry Weight*	Roots	Terminal stems	Basal stems	Terminal leaves	Basal leaves
	% K	% K	% K	% K	% K	% K
1. At start	6.50	56.20	60.00	———	58.50	———
2. Complete	19.17	54.00	57.00	60.00	59.60	54.30
3. Minus K	18.46	38.30	42.60	59.00	56.20	59.00
4. Minus Ca	6.73	46.50	60.00	60.00	57.00	60.00
Intermediate Light						
1. At start	6.50	56.20	60.00	———	58.50	———
2. Complete	18.25	56.00	54.60	60.00	60.00	57.30
3. Minus K	17.91	45.00	60.00	60.00	60.00	47.30
4. Minus Ca	6.75	46.70	60.00	60.00	58.30	59.00
Short Light						
1. At start	6.50	56.20	60.00	———	58.50	———
2. Complete	15.71	57.80	58.00	60.00	58.00	56.30
3. Minus K	15.12	48.30	45.00	60.00	49.50	46.30
4. Minus Ca	6.61	43.40	58.60	59.60	58.30	60.00

* 40 plants.

than the basal in all three light conditions in the "complete," and that the basal stems or older stem tissues contain the higher percentages.

The two weeks' additional growth period in the "complete" had not appreciably affected the potassium content, showing, it seems, that this element was absorbed in large quantities early in the life of the plants. This substantiates the general opinion that potassium is very mobile and is widely distributed throughout the plant, and is accumulated in greater amounts than those needed for growth. It is chiefly absorbed in the transpiration stream, and then, if in abundance, stored as a reserve in older tissues.

In the potassium-deficient solutions, the quantity of potassium is reduced in the roots and younger tissues, but few reductions occur in the older stems and leaves. Light duration appears to have no marked effect in this experiment on the distribution of potassium or the amount present in the tissues. The smallest amount occurs in the roots in all three solutions and at all three light intensities, and is the highest in the stems. In the calcium-deficient solutions the same general results seem to hold.

Magnesium was the second most abundant element of those considered. The three-week-old seedlings, as indicated in Table 5, had accumulated a considerable amount in the roots and not much had reached the stem and leaves. Sixteen days later, much of the element was in the aerial portions of the plant and much less was in the root. Apparently, there was a ready translocation by means of the transpiration stream. It is also apparent that the terminal leaves contained the most magnesium while the terminal stems follow with slightly less. The basal leaves had even more than the basal stems, a ready confirmation of the belief that magnesium accumulates in leaves. Its necessity, early in the life of the plant, is apparent.

A drastic reduction in light duration decreased in general the amount of

TABLE 5. *Percentage of Magnesium in the Ash of Plant Parts Under Certain Conditions.*

Long Light	Dry Weight*	Roots	Terminal stems	Basal stems	Terminal leaves	Basal leaves
	% Mg	% Mg	% Mg	% Mg	% Mg	% Mg
1. At start	6.50	1.2950	.0042	——	.0078	——
2. Complete	19.17	.4920	.0534	.0099	.2740	.0385
3. Minus K	18.46	1.3895	.3665	.0210	.1588	.0552
4 Minus Ca	6.73	.1167	.4085	.0118	.1302	.0669
Intermediate Light						
1. At start	6.50	1.2950	.0042	——	.0078	——
2. Complete	18.25	.5033	.0089	.0091	.0456	.0196
3. Minus K	17.91	1.1770	.0875	.0025	.0300	.0189
4. Minus Ca	6.75	.1588	.8800	.0634	.5120	.0578
Short Light						
1. At start	6.50	1.2950	.0042	——	.0078	——
2. Complete	15.71	.3840	.0037	.0063	.0212	.0072
3. Minus K	15.12	1.3850	.0191	.0046	.0178	.0394
4. Minus Ca	6.61	.4220	.4708	.0024	.2738	.0045

* 40 plants.

magnesium present in the roots of plants growing in the complete solutions. In the aerial portions of the plant much less magnesium was found under similar light conditions. The effect of deficient solutions on the magnesium content of the various tissues was very striking. In the roots, magnesium was especially high in the potassium-deficient solution and low in the calcium-deficient, when comparisons were made with check plants. Since antagonistic relationships exist between magnesium and potassium, plants in potassium-deficient solution would therefore absorb and transfer more magnesium. This topic will be dealt with later.

As compared with the checks, the accumulation of magnesium was heavy in the aerial tissues of plants grown in the deficient solutions, either potassium or calcium, and especially heavy in the younger tissues (terminal stems and terminal leaves). Reduction of light in the case of potassium-deficiency seems not to influence the accumulation of magnesium in the roots but it does have a marked reducing effect on the accumulation of this element in the aerial tissues. In the case of calcium deficiency, a reduction in the duration of light increased the amount of magnesium in the roots as well as in most of the aerial tissues, confirming the belief that light increases the absorption and transfer of calcium to the aerial parts of the plants.

In the plants at this age, calcium was the least abundant of the three elements studied. Data in Table 6 show that in plants three weeks old, calcium occurred more abundantly in the roots than in the stem or leaf. Sixteen days later, as shown in the check plants, its amount was lower in the roots but greater in the stems and leaves than in the case of the plants at the start. As was expected, calcium was more abundant in the leafy tissues than any of the other tissues above ground. The transfer of calcium in the transpiration stream was evidently heavy, and its movement into the leaf of young plants is known to be faster than that of most other elements. Not only is this true of the complete solutions, but also of the deficient solutions.

TABLE 6. *Percentage of Calcium in the Ash of Plant Parts Under Certain Conditions.*

Long Light	Dry Weight*	Roots	Terminal stems	Basal stems	Terminal leaves	Basal leaves
	% Ca	% Ca	% Ca	% Ca	% Ca	% Ca
1. At start	6.50	.0891	.0002	———	.0028	———
2. Complete	18.71	.0224	.0011	.0005	.0089	.0178
3. Minus K	18.46	.2240	.0084	.0037	.0188	.0075
4. Minus Ca	6.73	.0251	.0013	.0021	.0182	.0079
Intermediate Light						
1. At start	6.50	.0891	.0002	———	.0028	———
2. Complete	18.25	.0200	.0006	.0030	.0200	———
3. Minus K	17.91	.0820	.0106	.0056	.0100	.0741
4. Minus Ca	6.75	.0178	.0042	.0063	.0141	.0178
Short Light						
1. At start	6.50	.0891	.0002	———	.0028	———
2. Complete	15.71	.0112	.0018	.0005	.0100	.0067
3. Minus K	15.12	.0562	.0047	.0021	.0188	.0266
4. Minus Ca	6.61	.0260	.0012	.0004	.0050	.0032

* 40 plants.

Shortening of light duration reduced the amount of calcium in the roots but showed no effect on the leafy tissue of the check plants. There are some exceptions to this generalization. The differences are not so consistent as those for magnesium. In the potassium-deficient solutions, a short light exposure reduced the supply of calcium in the plant but did not apparently affect its supply in the calcium-deficient solution.

The story of the effects of the deficient solutions on calcium distribution and similar phenomena is in the main similar to that of magnesium. When comparisons are made with checks, it is observed that in the roots calcium was higher in the potassium-deficient solution and approximately the same in the calcium-deficient solution. The younger tissues, especially the leaves, showed the most calcium. Apparently potassium-deficiency did not bring about any injurious effect for this two-week period. More than enough had been accumulated for the need of the plants. In the case of calcium-deficiency, the need of calcium was quickly observed. Calcium is evidently not stored in available form, and is needed continuously.

EXPERIMENT IV. SECOND EXPERIMENT WITH THE GARDEN PEA, *ALASKA* VARIETY

In the fall of 1936 a second similar experiment was conducted in the greenhouse. In this case the plants were older at the start (5 weeks) and the experiment was terminated two weeks later when injury to roots and leaves appeared in the plants in the calcium-deficient solution.

In certain details, this experiment differed from the former. Two light periods, designated as "long-light" and "short-light" were used. In addition to the elements studied in the first experiment, iron and phosphorus were included. No attempt was made to study nitrogen and sulphur because in

TABLE 7. *Milligrams of Ca, Fe, Mg, P and K in 100 Grams of Tissue of Pea Plants.*

Elements	Terminal leaves	Basal leaves	Terminal stems	Basal stems	Roots	Total of 5 Elements in Tissues
Long-Light, Complete Series						
Ca	55.00	43.00	14.80	51.00	50.00	213.80
Mg	21.20	14.00	20.00	8.00	6.80	70.00
Fe	1.10	0.70	0.70	6.00	11.40	19.90
P	36.80	36.80	36.50	15.00	145.00	270.10
K	335.00	313.00	418.00	900.00	244.00	2210.00
Totals	449.10	407.50	490.00	980.00	457.20	2783.80
Long-Light, Calcium-Deficient Series						
Ca	1.00	108.00	0.90	42.00	28.30	180.20
Mg	102.00	82.00	80.00	13.00	6.90	283.90
Fe	< 0.50	1.30	7.80	< 0.50	8.90	19.00
P	25.00	28.00	45.00	25.00	178.00	301.00
K	472.00	572.00	780.00	675.00	359.00	2858.00
Totals	609.50	791.30	913.70	755.50	581.10	3642.10
Long-Light, Potassium-Deficient Series						
Ca	14.00	25.00	20.00	4.10	30.00	93.10
Mg	102.00	95.00	93.00	8.70	30.00	328.70
Fe	1.00	1.20	2.70	< 0.50	7.80	13.20
P	9.20	55.00	79.00	55.00	80.00	278.20
K	170.00	248.00	320.00	248.00	460.00	1446.00
Totals	296.20	424.20	514.70	316.30	607.80	2159.20

< Less than.
> More than.

this procedure these elements volatilized easily on ashing. During the last two weeks, solutions were changed twice a week and between changes the solutions were aerated for three hours daily.

A luxuriant growth was obtained in this way in the check plants. The growth in long- and short-light and deficient solutions varied according to the effects of the conditions. The milligrams of calcium, magnesium, potassium, iron and phosphorus in the roots, terminal leaves, basal leaves, terminal stems and basal stems are shown in Tables 7 and 8, the former showing data for "long-light" conditions and the latter that for "short-light". In view of the fact that the former experiment with peas was more of an exploratory and preliminary type, this one will be treated in greater detail.

The plants in this experiment were two weeks older than those of the preceding experiment but were still actively absorbing the elements of the solution in which they were growing. These plants at harvest time showed the same corresponding difference in appearance as those of the first experiment. Decreases in height of plants according to different light conditions were apparent, and these differences were also registered in differences in dry weight. Plants in the potassium-deficient solutions differed very slightly in height from the

TABLE 8. *Milligrams of Ca, Fe, Mg, P, and K in 100 mg. of Tissue of Pea Plants.*

Elements	Terminal leaves	Basal leaves	Terminal stems	Basal stems	Roots	Total of 5 Elements in Tissues
			Short-Light, Complete Series			
Ca	78.80	88.00	1.10	1.50	7.50	176.90
Mg	19.00	27.30	27.00	4.50	7.50	85.30
Fe	4.30	2.20	.60	.30	7.20	14.60
P	60.50	35.80	37.80	21.50	113.00	268.60
K	370.00	291.00	403.00	451.00	673.00	2188.00
Totals	532.60	444.30	469.50	478.80	808.20	2733.40
			Short-Light, Calcium-Deficient Series			
Ca	.80	.50	25.00	143.00	.44	169.74
Mg	180.00	80.00	68.00	17.00	36.00	381.00
Fe	< .50	1.00	14.00	< .50	6.00	22.00
P	18.00	22.00	45.00	25.00	80.00	190.00
K	375.00	333.00	460.00	700.00	383.00	2251.00
Totals	574.30	436.50	612.00	885.50	505.44	3012.74
			Short-Light, Potassium-Deficient Series			
Ca	25.00	lost	9.70	1.10	28.00	
Mg	54.00	lost	132.00	12.00	54.00	
Fe	8.60	lost	2.00	.40	12.00	
P	26.00	lost	32.00	38.80	53.00	
K	320.00	lost	563.00	527.00	470.00	
Totals	433.60		738.70	579.30	617.00	

< Less than.
> More than.

checks, but a slight reduction in dry weight was apparent later. Those in calcium-deficient solutions were much shorter. The dry weights of the latter plants were much less. The terminal or younger leaves were pale green color.

It is apparent that under long-light conditions the total content of the five elements was highest in the plants growing in the calcium-deficient solutions, next highest in those in the complete solutions and least in the potassium-deficient solutions. Under long-light conditions and in all solutions, potassium occurred in larger quantities in all the plant tissues than any of the other elements investigated. In the check plants in long-light, potassium was 79 per cent of the total ash of five elements; in the calcium-deficient plants, 78 per cent; and in the potassium-deficient plants, 65 per cent. Because in the latter case potassium was not in the solution for a period of two weeks, this would account for the slightly lower percentage (65 per cent) in these plants. Other than in the case of potassium-deficiency it is plain that approximately three-fourths of the ash in all these plants is potassium. Potassium occurs in larger percentages in the calcium-deficient solutions than in either the check or potassium-deficient solution. In its distribution this element is more abundant in the stems, then in the leaves, and, finally, least in the roots.

Phosphorus, the second element to be considered, occurred in much lower quantity than potassium. It was found in all the tissues but in greater quantities in the younger tissues and in the roots. In the various cultures under the long-light conditions, it varied in percentage from approximately 3 to 14. In all cases it was most abundant in the roots. The percentage of phosphorus in the entire plant under long-light conditions, was highest in the plants growing in the calcium-deficient solution and lowest in those growing in the check solution, while those growing in the potassium-deficient solutions contained an intermediate quantity. Though the differences were slight between the last two, there is a significant difference between the checks and calcium-deficient solutions. This will be discussed later.

The third element, magnesium, averaged much higher in plants in the deficient solutions than in the others. It appears that a deficiency of either calcium or potassium increases the absorption of magnesium. A reciprocal relationship is indicated as in the cases of potassium and phosphorus. Magnesium is more abundant in the terminal leaves and stems, therefore in the younger tissues. It is fairly abundant in the basal leaves. Roots contain the least.

Calcium was present in percentages usually greater than those of the other elements, potassium excepted, and was more abundant in the leaves and roots than in the stems. In plants in both deficient solutions, there was significantly less calcium than in the check plants. This would be expected in the calcium-deficient solutions at any rate, but calcium should be high in the potassium-deficient solutions. This will be discussed later.

Iron was the least plentiful of the elements studied. In the various tissues, it totaled slightly more than one-half of one per cent. The total iron in the aerial tissues was not so much affected by a deficiency of calcium as by a deficiency of potassium. The percentage in the roots was considerably curtailed by a deficiency of calcium as well as potassium, while a greater percentage was observed in the aerial tissues.

Reviewing briefly the preceding statements relative to the total amounts and distribution of certain elements in the pea plant growing in a complete solution under long-light condition (the check), one may conclude that potassium was very abundant. It was found in all tissues but occurred in greatest amount in the aerial parts of plants and usually in the older tissues. The remaining elements occurred in far less quantity. Both calcium and magnesium were found in the younger tissue and chiefly in the leaves. Phosphorus was also present in the younger tissues but predominated in the roots. Iron was more abundant in the roots than in the aerial parts of the plant and in the latter it was more plentiful in the green leafy tissue. These results check with those of the first experiment with pea plants.

DISCUSSION OF RESULTS

THE INDIVIDUAL ELEMENTS

Six only of the metallic elements found in plants were determined spectrographically. With the exception of sulphur, which is volatile and difficult to determine by the method used, and the inclusion of manganese, they were the usual ones listed in the old and well known group of "10 essential elements."

A. Potassium. This element was found in greater abundance in the tissues examined than any of the others. It was widely distributed and accumulated in large amounts, bearing out the general conclusions that it is easily absorbed and in quantities much beyond immediate requirements (12, 12A) and exceedingly mobile. It was easily extracted by water and was absent in the ether extract as well as in the residue in Iresine leaf powder. Potassium therefore is present in plants in the ionic form as was shown by Kostytschew and Eliasberg (19) two decades ago. Although this view is now generally accepted, it has only recently been questioned by Mason and Phillis (30) who state that at least 25 per cent of the total potassium is insoluble, held in an adsorbed form, and not released except by hot water extraction.

Potassium, as a rule, was most abundant in the stems, least abundant in the roots and intermediate in quantity in the leaves under long-light conditions and in the complete solution. Under short-light conditions most was found in the roots, next in the stems, and least in the leaves. From this it may be concluded that light is in some way connected with potassium absorption. This agrees with the conclusion of Hoagland (18A). In a calcium-deficient solution the amount of potassium was larger in all tissues with the exception of the basal stems, when compared with those of the checks. Therefore, in the absence of calcium more potassium is absorbed. A reciprocal relationship an adsorbed form, and not released except by hot water extraction.

The short-day period affects the total content of potassium in this plant, as revealed by a marked reduction of approximately 20 per cent in the total amount of the element, and also causes an accumulation of potassium in the root and basal stem and losses in the leaves and terminal stems. This same effect appeared in check plants exposed to short-day conditions.

In potassium-deficient solutions this element is less abundant for obvious reasons. The greater reductions in amounts were in the basal stems and terminal leaves, although there were sizable reductions in all other tissues except in the root where an increase occurred. Transfer was clearly impeded and this is one effect of lack of balance in this type of solution. A reduced absorption of potassium was apparent which was attributed to the presence of calcium in this solution. The element reduces permeability and is negatively correlated with potassium. The effect of a short-light period on potassium content and distribution in the tissues of these plants cannot be definitely determined because of the loss of one sample, but it appears to be slight.

Potassium was slightly more abundant in the green areas of leaves of Aegopodium, Tradescantia and Euonymus than in the yellow, and evidently is essential directly or indirectly to the proper functioning of the leaf. The difference in percentages of potassium in the green and white areas was so small that it was doubtful if this element functions in photosynthesis. Its supposed rôle in this process has been questioned (31). Other than forming salts with organic acids, potassium does not enter into organic complexes, and therefore it is questionable to apply so many important rôles as have been applied to this element. Evidence has been given that it maintains the proper tone and activity of the protoplasm (17) and prevents the breakdown of cytoplasm (39).

B. Calcium, in contradistinction to potassium, was in the "fixed" or organo-complex form. It occurred in much smaller amounts than potassium and was only to a slight extent in the ionic form and then it was not clear that any of this had previously been in the fixed form. In all the experiments, evidence was clear that calcium existed in large part in the organic form. It is apparent that the element could be present in the ionic form in the transpiration stream. Chibnal (6, 7) not long ago detected in cabbage leaves a calcium salt of phosphatidic acid. Steele (42) has stated that this "is the first ether-soluble calcium derivative found in nature." Chibnal also found magnesium and calcium phosphatidates.

Such compounds soluble in ether and at the same time insoluble in water are present in the fat phase of the living cell. On the other hand, sodium and potassium salts are soluble in water but insoluble in ether and therefore exist in the aqueous phase (7). They are believed to be concerned in cell permeability and regulate the different phase relationships in the protoplasmic colloids. These studies also indicate that the tissues examined contained ether-soluble organic magnesium, phosphorus and iron derivatives.

In young pea plants calcium was more abundant in the roots and young leaves, but soon decreased in the root and increased considerably in the leaves where the total amount exceeded that in the stems. This element, then, has its chief function in the leaf. When calcium was removed from the nutrient solution the young leaves became chlorotic, and so calcium either directly or indirectly was essential in the formation of chlorophyll, although it is not a constituent of that compound. This chlorosis of the young leaf also indicated that no transfer of calcium in the ionic form from an organo-calcium compound existed. Thus in a similar way the hypothesis that calcium is immobile has arisen. Under short-day conditions the total accumulation of calcium was reduced 17 per cent, but in its distribution the element differed from that in the check plants by increasing in the leaves and decreasing in the roots. It appeared that the root had donated heavily to the leaves but had not in turn recuperated its losses from the external solution. Absorption had decreased under short-day conditions. Light and calcium absorption as well as potassium absorption are in some way connected.

In Iresine leaves most of the calcium was in the residue, but the ether extraction contained twice as much as that taken out by water. Significant quantities are found therefore in the plastid materials. In the green areas of the variegated leaves of Tradescantia, Euonymus and Aegopodium it was more abundant than in the yellow areas, showing direct or indirect connection with chlorophyll. The greater percentage of calcium in the pea plants than in the others mentioned bears out the information already known that legumes are rich in calcium.

The distribution and total content of calcium in the tissues of plants growing in the deficient solutions showed that losses had occurred when the plants were compared with checks and that these losses were considerable in some tissues. The terminal leaves and stems had lost the most in plants in calcium-deficient solutions. The greatest gains were in the basal leaves and stems. In the roots the loss amounted to about one-half. The loss in the terminal leaves and stems is naturally attributed to the lack of calcium in the external solution and the inability of the young tissue to draw calcium from the older tissue.

The tissues of the plants growing in potassium-deficient solutions contained a reduced amount of calcium when compared with those of the checks. Most of the losses were in the basal stems, roots and the terminal leaves. Terminal stems and basal leaves did not vary much.

Calcium-deficiency symptoms were more quickly indicated than potassium-deficiency symptoms. The injurious effects of calcium exhibited themselves in an almost immediate stoppage of stem elongation, a delayed root development and a loss of green color in the new leaves.

C. Iron. With the exception of manganese this element occurred in the smallest amount of all. Judging from the work on Iresine leaves, and leaves of the variegated type, iron was more plentiful in the plastid than in the sap material. As in the case of the other elements, iron was found in higher percentages in the residue. Therefore ether-soluble organic iron derivatives are present in certain plant tissues. The fact that iron was also found in the water extraction is evidence of its presence in the ionic form.

In the pea plant, iron was most plentiful in the roots and basal stems, next in the leaves, and least in the terminal stems when the plants were grown under long-day conditions and in the complete solution. Under short-day conditions the reverse was the case. The roots and stems held the least and the leaves the most. Light seems to be necessary for the absorption of iron, but its transfer to the aerial parts of the plant appears to be more rapid under short-day conditions. Geriche (14) wrote that the greater the light quantity the greater the need for iron. This should explain why the quantity of iron in the root under short-day conditions was 26 per cent less than in the checks. Light is essential for calcium, potassium and iron absorption.

Pea plants growing in the deficient solutions under the long-day conditions have the same total amount of iron in one case and a little less in the other as the checks, but the distribution differs. The tissues of the plants in the calcium-deficient solutions have less iron in the roots, terminal leaves and basal stems, and more in basal leaves and terminal stems. Several factors are operative here, it is believed, but a change in permeability, and an imbalanced condition in the nutrient solution have both at times been suggested as reasonable explanations. If there is any truth in the hypothesis of reciprocal relationship, as many workers believe, these studies show that iron is negatively correlated with calcium. Under short-day conditions the total amount exhibited an increase of 16 per cent. Other than a large reduction in the root and a good increase in the terminal stems, the iron in the other tissues remained about the same. Absorption was impeded here as in the case of the check plants under short-day conditions.

In the tissue of plants growing in the potassium-deficient solution, the iron is reduced in the basal stems and roots, but increased in the terminal stems and both types of leaves. The evidence is clear that some reduction has occurred in the total iron content also. This also accords with the author's findings in the cases of calcium and potassium accumulation in the plants in deficient solutions. The effect of short-day conditions on the total iron content and its distribution cannot be predicted because one sample was lost.

D. Magnesium. This element was more abundant in the sap and less so in the plastid material than was calcium. The inference is that less magnesium occurs in an organic complex form than calcium; nevertheless, ether-soluble magnesium compounds were present in the leaves of Iresine, Tradescantia, Aegopodium and Euonymus. Only 2.7 per cent of magnesium is found in the chlorophyll molecule and the percentage of this element in the leaf is much above the chlorophyll requirement. The literature (34) concerned with the chemical composition of plant tissues reveals that magnesium is present in pectic substances, in such forms as phosphatides, phospholipin, and proteins. Pollacci (38) has recently shown that *Chlorella vulgaris* grows well and remains green in a culture solution devoid of iron but containing the magnesium salt of pyrrole carbonic acid. If this can be confirmed, then considerable magnesium can be used in building up the pyrrole compounds.

Magnesium was more abundant in the green areas of the variegated leaves than in the yellow ones and more in the plastid material than the sap where the percentage was only half so much. In Iresine leaves the residue contained the largest amount, the plastid material next largest, and the sap the least. Although widely distributed, there is much less magnesium than potassium and in most cases a less percentage of magnesium than that of calcium in the tissues studied.

The roots of young pea seedlings contained an abundant supply of magnesium, but a few weeks later there was much less of the element in the roots and considerable more in the stems and leaves. This implies rather rapid transfer, presumably in the transpiration stream. Under short-day conditions there was little or no effect, possibly there was a slowing up of transfers. It appears in these studies that light is not connected with magnesium absorption. This element seems to be indifferent to light.

Under long-day conditions and in the complete solution the aerial parts of the pea plant contained more magnesium than the roots. Magnesium was more abundant in the terminal stems and leaves of the younger tissue than in the older. The basal leaves contained only a little more than did the root. Green leaves and young tissues require more magnesium, which functions chiefly in the leaf; no attempt is made to show how. By changing the light condition to short-day, one does not induce much change in the total amount of magnesium or its distribution.

Large accumulations of magnesium in the tissues of plants growing in deficient solutions was observed. This accumulation was greater in the tissues of the potassium-deficient plants than in the tissues of the calcium-deficient ones, but in both the percentages far exceed those in the check. This is attributed to the fact that here, as with other elements, there is a reciprocal relationship or modified permeability, or an unbalanced nutrient solution. That more magnesium was found in the plants growing in potassium-deficient solutions than in those in the calcium-deficient solution is thought to be due to the more thrifty condition of the plants in potassium-deficient solutions.

E. Phosphorus. This element occurs in small amounts in the Iresine leaf, but is twice as abundant in the ether extraction as in that by water. Of course, this element is found in the residue in a large amount. It is clear that ether-soluble organic phosphorus compounds are present, which can be confirmed by facts in the literature. This element was more abundant in the pea plant than in Iresine, largely because legumes are high in proteins and these contain phosphorus.

In the tissues of the check plants under long-day conditions, phosphorus was high in the roots, low in the basal stems and intermediate in the leaves. Phosphorus is known to be essential for young tissue. A change to short-day conditions led to a decrease of that element in the root and an increase in the basal stem as well as in the terminal leaves. Those differences were not striking, and presence or absence of light, as in the case of magnesium, did not appear to exert much influence.

The tissues of plants growing in deficient solutions under long-day conditions contained more total phosphorus than the tissues of the check plants under the same condition. In the case of calcium-deficient plants, the percentage was greater than in potassium-deficient plants. There is evidence here of reciprocal relationships but probably not so striking as in the case of the other elements.

F. Manganese. This element was sought for only in the Iresine leaf. It occurred in the least amount of any studied. Although it is doubtful whether on such little evidence any conclusion should be drawn; nevertheless, it is worthwhile to know that manganese was more abundant in the plastid material and therefore ether-soluble. There are manganese-organo compounds in the Iresine leaf. Manganese is now believed to be an essential element and is directly or indirectly connected with chlorophyll formation but not enough work is here reported to confirm this belief.

MOBILITY OF ELEMENTS

The pea experiment affords some information as to the mobility of the various elements. It has been generally believed that of the elements studied calcium and iron are immobile and potassium, phosphorus, magnesium and manganese are mobile, though a study of the literature does not reveal much unanimity of opinion, with the possible exception of potassium. When absorbed and taken up in the transpiration stream, the elements must be mobile. Potassium in these experiments was unquestionably mobile, for it was available at all times. Calcium, however, could not be drawn from older tissues to supply the young chlorotic pea leaves. The latter remained chlorotic until calcium was added to the external solution. The older leaves had accumulated slightly more calcium but that may only be because they were nearer the source of supply. Amar (3) believed in this general view of mobility inasmuch as he demonstrated that no diminution in quantity of calcium oxalate occurred in the older leaves when certain plants were transferred to a calcium-free solution. His inference was that calcium was not in available form to migrate to the younger leaves. Mason and Maskell (29) found a considerable concentration of free calcium in the living cell of the bark of the cotton stem and concluded that in the living cell permeability was inhibited and the calcium could not get out. This accords with Loeb's (25) idea that calcium "tans" the membrane and makes it less permeable or, according to the idea of Osterhout (36), increases the resistance to the passage of an ion or decreases permeability. Sodium, on the other hand, would decrease resistance and make the membrane more permeable. Potassium, which is similar in many respects, would act in this case like sodium.

On the other hand, it is known that cabbage leaves at storage time (37) contained 60 per cent of their calcium in water-soluble form, while six weeks later only 40 per cent was water-soluble. Ames and Boltz (2) found that 40 per cent of the calcium in alfalfa was water-soluble. In the Iresine leaves reported here, about 6 per cent of the calcium was water-soluble; Aegopodium contained 2 per cent, and Tradescantia and Euonymus 30 per cent of the total in the water-soluble form.

Not only do reports differ for calcium but the same is true for the other elements. Sixty to eighty per cent of the phosphorus in certain plants is water-soluble (2, 37, 16), and its reutilization is said to be as rapid as that of potassium or nitrogen. Again a recent report (40) on the oak indicates that phosphorus is mobile. Phosphorus then might be classified in the mobile group (13), yet it must be conceded that there are many phospho-organic compounds such as phytin, nucleic acid, and phosphatides which are certainly immobile.

Immobility of phosphorus can be confused with the phenomenon of interference of transfer. When strawberry plants are grown in a phosphorus-deficient solution, a recent report (9) affirms an injury is produced in certain tissues which slows up the movement of phosphorus from the older to the younger tissues. Mobility is here modified by injury.

The movement of elements, according to recent studies (18, 43), can also be facilitated by aerating the roots.

Magnesium is considered by some (1) to be immobile while others think it mobile (26). Iron is another element in the same category (12A, 14, 35, 44) and it is reported to be relatively abundant in soluble form and even the organic compounds are easily separated out. In the experiments reported here iron was found more abundantly in the ether extraction and the residue, but it also was present in the ionic form, as shown by its presence in the water extraction. In the pea experiment the mobility of iron was influenced by day length and elemental deficiencies in the nutrient solution. In the case of magnesium, the water-soluble form reached 13 per cent, although it varied slightly from this in the other experiments.

To recapitulate, it is possible to find the elements in organic combinations as well as in the ionic form in the plant sap, inasmuch as absorption and transfer from the external medium through the process of transpiration are available. Whether this ionic form could have another source, such as in previously formed organic compounds, has been demonstrated in many cases because reutilization is a very common phenomenon. The matter of mobility or immobility seems to be a relative one, and it would appear that it depends on several factors, the sum total of which expresses metabolic activity. Elements may make organic combination under one set of conditions and then disaggregate under others; this property hinges on the activity or tone of the living matter (17, 39).

RECIPROCAL RELATIONSHIP OF ELEMENTS

The study of the effects of potassium and/or calcium-deficiency in the nutrient solutions, on the absorption, transfer, total content and distribution of the elements in the tissues of plants growing in these solutions brings to one's mind the problem of reciprocal relationship.

According to Davis *et al* (9), calcium and potassium are negatively correlated and so also are potassium and magnesium and potassium and phosphorus. On the other hand, the same authors assert that magnesium and phosphorus, and magnesium and calcium are positively correlated. Fifteen years ago Haas and Reed (38A, 15) observed that more magnesium was absorbed in solutions containing an insufficient amount of potassium. This agrees with the result of the studies here reported. However, Brown (5) has recently shown that high concentrations of potassium decreased the absorption of magnesium. Bartholomew, Watts and Janssen (4) claimed in their experiments that the absorption of magnesium was not significantly influenced by the amount of potassium present. It is possible too that a solution in imbalance may interfere in absorption by increasing or decreasing it. Colby (8) has recently reported that a lack of calcium apparently prevented the absorption of a considerable quantity of any ion. These are quite divergent opinions.

Liebig's Law of Minimum (24) asserts that when one salt is below the critical concentration the remaining salts are absorbed in decreased amounts. On the other hand, Legatu and Maume (20, 21, 22, 23) recently came to the conclusion that an increased absorption of the remaining salts occurred when one was deficient. Thomas (48, 49) has now attempted to reconcile these different views and to show that they are not necessarily contradictory. He writes, "The omission of an element, already abundant in the soil, from a fertilizer, results in an increased absorption by the plant of the remaining elements added in the fertilizer relative to the absorption of these from the complete fertilizer plot until the element omitted from the fertilizer becomes a limiting factor, *i.e.,* until its concentration is reduced below the limiting concentration." The whole problem therefore is one of relativity. The laws of Liebig (24) and of Mitscherlich (33) are merely approximations.

The author's experiments do not lend support to one or the other of these two views because when potassium was absent fewer total salts were absorbed by the plants and when calcium was absent more were taken up. That the absence of one salt causes either a decreased or increased absorption of all the others is not borne out by the work of Hartt (16A) or Ginsburg (14A). The former observed a greater total salt content in a potassium-deficient solution, while the latter noticed a greater total salt content in magnesium-deficient solutions but a reduced total salt content in the potassium-deficient solution. The author's results agree with Ginsburg relative to potassium but do not harmonize with his results on calcium. Apparently more has to be done, but despite these divergent results and opinions, the works of Lündegardh (*27*, *28*), of Alway, Maki and Methley (1), Sampson and Samisch (40), McHargue and Roy (32), Thomas (49), Skeen (41) and others, do demonstrate that the principle of reciprocal relationship and antagonism are tenable.

These phenomena formerly were explained by assuming a change in permeability, as indicated by Loeb (25) and Osterhout (36), or by postulating an influence of one ion on the other. It is now quite evident, as suggested by Hoagland (18) and Steward (43), that those statements are inadequate to explain all similar phenomena. These phenomena must result from metabolic reactions controlled either by enzymes or protoplasm, or both. Aerating the solutions as previously mentioned facilitates the absorption by the root. Light has also its effect and any such modification must involve energy exchanges.

Again the work of Owen (36A) suggests that the repeated addition of the deficient element is not always essential, for even the presence of another element is likely to make what remains of the deficient element more available. He wrote, "that when phosphates are omitted from the manure, the potash content of the foliage is depressed, and that when the phosphates are added to a soil from which they had previously been withheld, the potash content was increased." He wrote elsewhere that "when foliage shows potash deficiency it may be necessary to add phosphates to the soil to enable the plant to make full use of the potash." In other words, the availability of potash in a soil is considerably influenced by the phosphorus status of that soil. Furthermore the amount of phosphorus taken up is conditioned by the potash status of the soil. This puts a rather new light on the subject and shows the necessity for further study.

In the author's experiments, phosphorus and potassium were found to be negatively correlated with calcium. Iron was positively correlated with calcium and potassium. Magnesium was positively correlated with potassium but negatively correlated with calcium.

EFFECTS OF DURATION OF LIGHT

A. Short Day. By exposing the check plants to short-day conditions, instead of long-day, certain marked variations in the total content of salt, their individual totals and their distribution, were observed but these were not so striking as the effects shown by plants in the deficient solutions. Short-day periods reduced the total salt content in most cases and therefore on the individual totals. In the distribution of the elements in the tissues marked effects were shown by some elements, but few or none by others. Calcium and iron had decreased, the former 17 and the latter 26, while magnesium had increased 22 per cent. Potassium and phosphorus showed no significant variations. Calcium had accumulated in the leaves, while losses appeared in the roots and stems. The leaves had drawn ionic calcium from the roots and stems, but they in turn had apparently not absorbed calcium from the external medium. The significance of these results is discussed under the heading of individual elements. Iron acted much the same as calcium.

Magnesium, the only element to gain in amount, showed increases in the root basal stems and leaves, mostly in the latter. The roots had absorbed in excess and therefore light did not appear effective in its absorption of magnesium as in the case of calcium, iron and potassium.

Phosphorus and potassium distribution in the tissues varied from that in the checks. Phosphorus showed an increasing gradient from stem to leaf and also from stem to root. It appears that root was absorbing from the external medium. Like magnesium, phosphorus absorption seems unaffected by light.

The effect of short-day conditions on the plants growing in the deficient solutions showed that there was a reduction of total salt content of about 17 per cent in the tissues of the plants growing in calcium-deficient solutions. The totals of some individual salts were increased—for example, magnesium 35 per cent and iron 15. Those reduced were calcium, phosphorus and potassium. The reduction in the former is probably not significant. Phosphorus had decreased however 36 per cent and potassium 21. These results show that the effect of short light is to reduce the total salt content and to change the totals of the individual elements. These changes are not in general so striking as those brought about by the growth of the plants in the deficient solution.

Considering the distribution of the elements in the tissues of these same plants, calcium was heaviest in the stems and lightest in the roots and leaves, while magnesium showed marked increases in the terminal leaves and roots. The other elements did not differ much from the distribution under long-light conditions. Iron decreased in the root, but increased in terminal stem. The explanation of this is indicated in the discussion of individual elements. Phosphorus was distributed less abundantly in the terminal leaves and root, otherwise not very different from the check.

Considering the data for plants growing in potassium-deficient solutions under short-day conditions, much of real value concerning the total salts content and the variation in the individual totals cannot be obtained because one sample was lost. Something can be noted about the distribution of the elements in the tissues. Losses of calcium were shown in both types of stems, but accumulation was shown in the terminal leaves. Calcium content of roots showed no difference from that of the check. Magnesium had increased appreciably in the roots and basal stems and very markedly in the terminal stems, but the leaves did not contain so much as did those of the checks. Iron increased in the roots and terminal leaves but varied little from the check in other tissues. The interpretation of these data is found under the discussion of the individual elements.

B. Long Day. The long-day plants growing in complete solution were characterized by a noticeably large amount of calcium and phosphorus. This was to be expected because legumes are high in calcium and proteins, and phosphorus is essential for the formation of the latter. An abundance of potassium is noted and this is also according to general belief. Magnesium and iron are not generally required in large amounts. Concerning the distribution of these elements in the tissues, it may be said that calcium is more abundant in the leaves than in the stems or root. That leafy tissue contains considerable calcium was expected and that roots contain much is in accordance to theory.

In the case of phosphorus, the greatest distribution was in the root and the next in the leafy tissue. The old stems contained the least. Phosphorus is known to be high in the regions of rapid growth. Magnesium is more abundant in green leafy tissue, less in the stem and least in the roots, and this is in accordance with general belief. Iron is heaviest in the roots and the gradient is decreasing from this point until the terminal or young leaves are reached and there the iron content is high again. Roots usually contain a high pH and absorb considerable amounts of iron. It is usual to find considerable amounts in the leaf.

With those facts in mind, one finds considerable variation when comparison is made with plants in the deficient solutions. First, the total salt content was increased 30 per cent in the tissues of the plants growing in calcium-deficient solutions and decreased 22 per cent in the potassium-deficient solutions over that in the check. In the calcium-deficient plants all elements except iron increased, and this remained virtually the same. A significant reduction of 15 per cent in the calcium content was to be expected, because that element had been removed from the external solution. Magnesium had increased in the tissues to the extent of 30 per cent, phosphorus 11 and potassium 29.

The distribution in the tissues differed from that in the check, as might be expected. Calcium was greatly reduced in the terminal leaves and stems and somewhat in the root and basal stem, but had accumulated in the basal leaves. The reason for this has been discussed under the individual elements. Iron was more abundant in basal leaves and terminal stems but, like calcium, was reduced in the terminal leaves, old stems and roots. Generally the losses were in the young tissues and gains in the older.

Magnesium showed the most outstanding differences. All tissues with the exception of the roots showed large increases, especially in the young stems and leaves. Though phosphorus had increased slightly in all the tissues over those of the checks the greatest increases were found in the roots and stems. The leaves had not maintained their original amount. Potassium had increased 21 per cent and this increase was spread over all the tissues except the older stems. Roots contained the least, terminal stems the most, and the leaves an intermediate amount.

The losses in the potassium-deficient solutions were due to losses in calcium, iron and potassium. An increase was recorded in magnesium and no significant difference appeared in the phosphorus percentages. The increase in magnesium was even higher than that in the calcium-deficient plants. The loss in calcium amounted to 50 per cent, that in iron 33, and that for potassium 34. In the latter the reduction was expected since it had previously been removed from the solution.

The distribution of the elements in the different tissues was different from that of the checks. Calcium and magnesium showed similar relationships but differed in degree. There was a decrease of calcium in both deficient solutions but less in the potassium-deficient solution. There was an increase of magnesium in both solutions but more in that which lacked potassium. In the case of calcium, the leafy tissues experienced the largest losses and in the case of magnesium the greatest gains were in the leaves, although in all tissues this element had increased with the exception of basal stems. Iron had increased in the terminal stems and basal leaves. Phosphorus was low in the young leaves and roots and high in the basal leaves and stems. The greatest losses of potassium were found in the youngest leaves. These facts are noted under the discussion of individual elements.

SUMMARY

The modern spectrographic technique for analysis, with the modifications worked out at this institution proves to be a possibility for use with plant tissues. The detection, distribution, mobility and functions of the elements can now be given fuller study.

A variety of tissues was examined, and the ether, acetone and water-soluble extractions were obtained. The ash of these were studied for six different metallic elements, potassium, calcium, magnesium, phosphorus, iron and manganese. The ether extractions indicated that all but potassium were fixed in some organic complexes not easily extracted with water. The presence of these same elements in the residue added further confirmation of metallo-organic complexes. Potassium was not found in the residue or ether extract and it is improbable that it occurs in more complex organic combinations than that of salts of certain organic acids. It is possible that the role or functions of potassium has been greatly over-estimated.

The general opinions held as to the distribution of the various elements in the different tissues of the plants under so-called normal conditions have been confirmed. The distribution of the elements under certain peculiar conditions such as a reduction in day length and the growth in calcium-deficient and potassium-deficient solutions have been noted and discussed in detail and certain generalizations may be given here.

The mobility of elements is a matter of relativity. Iron and calcium are not to be considered immobile, and immobility is not merely a matter of chemical precipitation or one of inhibition of permeability. Protoplasm, through its own activity or that of enzymes which it produces, controls metabolic processes and ion-organo compounds are built up and later disaggregated. Energy exchanges, respiration and other vital processes are involved. Admitting that the older theories are not adequate, and the true explatation is more deep-seated augers for ultimate success.

A short-day period slows up the total salt absorption by the root. Full light favors the absorption of calcium, iron and potassium while phosphorus and magnesium appear indifferent. Roots and basal stems are low in mineral content, but the gradient increases from there to the top leaves.

Calcium-deficient plants are higher in total salt content than the check plants while potassium-deficient plants are lower. These phenomena formerly were explained by changes in permeability, reciprocal relationship or solution imbalance. It is now quite evident that the plant's activity regulates metabolic reaction and in some unknown way causes greater absorption with one element and less with the other, and changes or modifies the distribution of the elements in the various tissues of the plant according to a definite plan still to be discovered.

The functions of these elements can only be surmised at this time and the data add little in this direction, but through the use of the spectrographic technique, a better approach to the subject is possible and readily appreciated. The place of greatest accumulation would not then offer the key to the rôles that the elements serve.

ACKNOWLEDGMENTS

The writer takes pleasure in acknowledging the help and cooperation of those who made this work possible; to Prof. D. T. Ewing for directing the spectrographic work and for his counsel and criticism; to H. C. Beeskow for the ashing and ether and water extractions; to Dr. Francis W. Lamb and Dr. Mable F. Wilson for spectrographic tests; and to Prof. F. C. Bradford for interchange of opinion and criticism of the manuscript.

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