IRON IN THE LEAVES AND CHLOROPLASTS OF SOME PLANTS IN RELATION TO THEIR CHLOROPHYLL CONTENT

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(WITH ELEVEN FIGURES)

Introduction

That lime-induced chlorosis can be alleviated by the application of iron salts has been recognized for a hundred years. As early as 1845, GRIS (8) treated chlorotic plants with iron and obtained positive results; his experiments have been repeatedly confirmed. Opinions differ widely, however, as to the comparative amount and state of iron in chlorotic leaves. Various investigators have presented evidence indicating that more, the same, or less iron is present in chlorotic than in green leaves. Those who have reported the first two conditions have, in general, explained chlorosis as an immobilization or inactivation of iron which occurs mainly, or at least to a greater extent, in chlorotic leaves than in green ones. Of the more recent workers, WALLACE (20) and VIDAL (19) found no relation between total iron and chlorophyll content in lime-induced chlorosis, but GILE and CARRERO (5) and CHAPMAN (2) did. OLSEN (13) was unable to relate total iron to chlorophyll content in plants grown in iron-deficient culture solution whereas SCHOLZ was able to find such a relationship.

Both MOORE (12) and GRIESSMEYER (7) demonstrated the qualitative presence of iron in chloroplasts by employing staining reactions. LEIBICH (10) found up to 82 per cent. of the leaf iron in the chloroplasts of spinach. HILL and LEHMANN (9) found the isolated chloroplasts of Claytonia to contain four times as much iron as would be expected were the iron equally distributed throughout the leaf.

That the iron in the leaf is not all in the same chemical combination and does not all perform the same function seems indicated by the necessity of iron for non-green plants and the presence of iron containing enzymes in all plants. Hence it is probable that only a fraction of the leaf iron is closely related to chlorophyll formation. Although PARSCHE (15) found only a poor correlation between total iron and chlorophyll, he did find that the water soluble (filterable) iron was, as a rule, higher in green leaves. OSERKOWSKY (14), working with pear, apricot, and peach leaves, developed a method for determining a fraction of iron which was proportional to the chlorophyll content. His method consisted of extracting the dried leaf material with 1.0 N hydrochloric acid. He then plotted this acid-soluble iron against the chlorophyll content and obtained a straight line which, when extrapolated, intersected the iron axis at some point other than the origin. The difference beween the iron axis intercept and the acid-soluble iron for a given sample represented the amount of iron directly proportional to the chlorophyll. OSERKOWSKY called this "active iron" by which he

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meant a specific iron fraction, or fractions, active for the formation of chlorophyll. The difference between the iron axis intercept and the origin he called the "acid-soluble inactive iron."

In the work reported here, this concept of active and inactive iron has been confirmed and extended. The total iron and chlorophyll content have been studied with particular reference to quantitative relationships. Since the chloroplasts are the chlorophyll containing bodies of the leaf, iron determinations were made on isolated morphologically intact chloroplasts.

Materials and methods

FIELD MATERIAL

Leaves were collected from Hardy pear trees about 25 years old. These trees were growing in calcareous soil and showed varying degrees of lime-induced chlorosis. Four sets of green and chlorotic leaf samples were collected at intervals during the growing season. These samples were placed in jars and held at 2.5° C. until used within 48 hours after collection.

For the chlorophyll determination, two samples of ten or fifteen leaves each were selected at random, cut into small pieces, thoroughly mixed, and two grams carefully weighed out. This process was carried out as rapidly as possible to minimize drying.

For dry weight, ash, and iron determinations, leaves were weighed and washed in water to remove loose dirt. Then the surfaces of each leaf were carefully rubbed with the fingers in 0.3 N hydrochloric acid in order to remove encrusted dirt and spray residues. The acid was changed frequently and the washing conducted as rapidly as possible. There were no signs of injury. After the acid washing, the leaves were thoroughly rinsed in distilled water and dried in a ventilated oven at 55° C. for 48 hours. The dried leaves were weighed and then ground in porcelain ball mills with glass marbles.

GREENHOUSE MATERIAL

Two species of plants, Turkish tobacco and Golden Bantam corn, were grown in solution in the greenhouse in 8-liter asphaltum painted stoneware crocks. The solution (half strength HOAGLAND) had the following composition: KNO_3, 0.0025 M; $Ca(NO_3)_2$, 0.0025 M; $MgSO_4$, 0.0010 M; KH_2PO_4, 0.0005 M. In order to make certain that the plants would not suffer from minor element deficiency, B, Mn, Zn, Cu, Mo, V, Cr, Ni, Co, W, and Ti were added in the amounts recommended by ARNON (1). Aeration of solutions was provided by compressed air entering through sintered glass aerators.

The plants were transferred to fresh nutrient solution every three weeks. The pH of the fresh solution was 5.5. At the end of the three-week period, the pH was about 7.5 for the corn cultures and about 6.0 for the tobacco cultures. At first, the plants were given small, but adequate, amounts of iron (1.0 ppm.) occasionally to maintain normal color and growth. Later, the amounts of iron and the frequency of its addition were adjusted so that the

desired degree of chlorosis was produced. By this means, plants ranging from very chlorotic to deep green were obtained.

In each experiment the plants, both corn and tobacco, were harvested when they were about two feet tall and had eight or ten pairs of mature leaves. Only the mature or nearly mature leaves from the upper half of the plant were used. The midribs of all leaves, and the basal and apical thirds of the corn leaves, were removed with a brass knife and discarded. From these prepared samples, numerous discs of leaf tissue were punched out with a brass cork borer for the chlorophyll determinations. Usually from 1.00 to 1.50 gm. were taken for analysis. For the other determinations, the remainder of the leaf sample, about 100 to 500 gm., was weighed, washed, dried, reweighed and then ground in the manner previously described for pear leaves.

All chlorophyll determinations were carried out on fresh material. The samples were thoroughly ground with sand, a pinch of calcium carbonate, and 80 per cent. acetone. The ground material was washed into a filter and washing with 80 per cent. acetone continued until a volume of 100 ml. had been reached. The transmission of this, diluted when necessary, was determined in a photoelectric colorimeter similar to that described by EVELYN (4). The filter used had a transmission band of 6000 to 7000 Å. Solutions of pure carotene and xanthophyll gave the same reading as the solvent blank, indicating that the filter transmitted no light in the absorption range of the carotenoids. The instrument was calibrated with known amounts of pure chlorophylls a and b, obtained through the courtesy of DR. G. MACKINNEY of this University. Because of the relatively small difference (7 per cent.) between the calibration curves of the two chlorophylls, the ratio could vary considerably without introducing a serious error. The chlorophyll content of a group of samples determined with the colorimeter and with a Bausch and Lomb spectrophotometer agreed to about ± 5 per cent.

Total iron was determined by a modification of the method of SAYWELL and CUNNINGHAM (16). From 50 to 200 mgm. of dried leaf tissue were digested with 1 ml. concentrated H_2SO_4 and a few drops of 30 per cent. H_2O_2. The digest was diluted and treated with 1 ml. of 10 per cent. hydroxylamine hydrochloride. The solution was now adjusted to pH 5 with NH_4OH, using a stirring rod and Congo red paper as an external indicator. One ml. of a 0.1 per cent. alcoholic solution of ortho-phenanthroline monohydrate was added and the volume adjusted to 10.0 ml. The color intensity was determined in the previously described colorimeter, using a filter with a transmission band of 4400 to 4800 Å.

To determine the acid soluble iron, 200 to 500 mgm. of dried leaf tissue were extracted with 10.0 ml. of 1.0 N HCl in 15-ml. centrifuge tubes. The extraction period was 6 hours for corn and tobacco, 24 hours for pear. During extraction, the samples were shaken from time to time. At the end of extraction, the tubes were centrifuged, aliquots of the supernatant liquid evaporated to dryness, and iron determined as described.

Ash weights were determined by igniting a 1.00-gm. sample of the dried leaf tissue in a muffle at 650° C.

Results

Dry matter and ash weights

The percentage dry weight of chlorotic leaves is frequently much lower than that of green leaves. Table I shows that this relationship was present in young leaves of field material, but was less evident in older leaves and in the material grown in the green house. In pear leaves there was an accumulation of dry matter as the growing season progressed. With approximately the same chlorophyll content (about 0.8 per cent. in the deep green leaves) the dry matter increased from 29.8 to 40.7 per cent. between April and September. At the same time, the difference in dry matter between the chlorotic and green leaves of a set tended to diminish, so that at the beginning of the season, there was a difference of about 25 per cent. between the most chlorotic and the greenest samples, whereas near the end of the season the difference was about 10 per cent.

In the corn and tobacco leaves there were no significant differences in percentage of dry matter except in the greenest sample of each set. Since these plants were first grown under normal conditions and then transferred to iron-free culture solutions, the chlorotic tissues were perhaps able to draw upon the lower green leaves for mobile nutrients. The larger percentage of dry matter in the deepest green leaves was probably due to higher photosynthesis.

There appeared to be no consistent difference in the ash content of the dried chlorotic and green samples of a set. However, the percentage ash was frequently less when the percentage dry weight was high. This was particularly true with pear leaves collected early in the season and, to some extent, with corn and tobacco leaves. Presumably the increased dry matter diluted the ash. The change in the ash content was probably an indirect result of chlorosis.

Iron in whole leaf

In table I, the data for the acid-soluble and total iron are given for several series of pear, corn, and tobacco leaves. These values are represented graphically in figures 1–8, inclusive. In every case the acid-soluble iron increased as the chlorophyll increased. As pointed out by Oserkowsky (14), it is only the iron which is proportional to the chlorophyll that can be considered as active in chlorophyll formation and not all of the acid-soluble fraction. The active iron fraction plotted against chlorophyll content displays a typical limiting factor type of curve. It is least evident in the pear leaf samples. In field material, one must select samples on the basis of greenness only, the range of iron content being determined by uncontrolled soil conditions. In greenhouse material, the iron content of samples is subject to some degree of control so that a large range of iron content in relation to the chlorophyll content could be obtained.

TABLE I

ANALYSES OF CHLOROTIC AND GREEN LEAVES

DESCRIPTION OF LEAVES	PER-CENTAGE DRY WEIGHT	EXPRESSED ON DRY WEIGHT BASIS			
		CHLORO-PHYLL	ASH	ACID-SOL. FE	TOTAL FE
	%	%	%	ppm.	ppm.
Hardy pear spur leaves, 4/28/41					
Severely chlorotic	22.3	0.170	6.78	12.4	19.0
Moderately chlorotic	23.9	0.248	6.14	15.6	26.9
Slightly chlorotic	25.0	0.341	6.02	17.0	23.6
Pale green	26.7	0.614	5.60	26.7	36.4
Green	28.3	0.735	4.90	30.4	40.3
Deep green	29.8	0.893	4.92	35.7	46.9
6/3/41					
Severely chlorotic	30.3	0.129	6.62	10.7	21.4
Moderately chlorotic	34.5	0.214	5.44	11.5	22.3
Slightly chlorotic	35.7	0.340	6.08	13.3	22.7
Green	36.7	0.654	6.16	19.8	27.8
Deep green	36.6	0.943	6.64	29.4	39.2
7/21/41					
Very sev. chlorotic	30.4	0.0883	8.50	12.0	21.8
Severely chlorotic	33.8	0.173	7.86	12.7	26.4
Moderately chlorotic	37.0	0.263	8.08	15.0	30.1
Pale green	39.6	0.525	8.08	17.6	29.2
Green	39.0	0.693	7.21	26.2	36.6
Deep green	37.3	0.777	7.46	31.7	45.8
9/22/41					
Moderately chlorotic	38.3	0.258	6.68	17.0	31.6
Slightly chlorotic	42.0	0.372	7.28	18.4	32.6
Pale green	42.4	0.460	7.32	20.4	36.1
Green	42.8	0.582	7.44	24.4	38.7
Deep green	40.7	0.816	7.64	32.8	47.2
Corn, 3/20/42					
Severely chlorotic	17.6	0.243	8.05	19.6	38.3
Moderately chlorotic	18.2	0.566	7.25	30.1	49.6
Pale green	17.5	0.798	7.15	36.9	56.5
Green	17.8	1.180	7.10	53.4	77.3
Deep green	19.3	1.540	7.20	131.0	178.0
Corn, 5/2/42					
Severely chlorotic	14.0	0.246	8.20	12.7	24.2
Moderately chlorotic	14.1	0.543	7.18	21.8	30.6
Pale green	14.9	0.810	7.35	28.8	40.8
Green	14.4	1.220	7.50	44.3	56.3
Deep green	16.1	1.710	6.83	79.4	100.0
Tobacco, 2/22/42					
Severely chlorotic	7.66	0.242	17.1	39.6	78.9
Moderately chlorotic	7.22	0.507	18.8	40.0	87.0
Pale green	7.82	0.743	17.3	42.1	70.2
Green	7.38	1.110	16.2	46.5	73.2
Deep green	8.67	1.540	14.9	92.7	115.0
Tobacco, 6/16/42					
Moderately chlorotic	8.89	0.471	14.5	30.1	63.7
Pale green	8.92	0.769	15.3	34.7	68.2
Green	9.41	1.200	13.6	42.3	67.7
Deep green	9.23	1.760	13.7	68.4	105.0
Very deep green	11.60	2.070	10.2	103.0	140.0

In all but one set, the total iron curve paralleled the acid-soluble curve. Since the total iron was found to be related to the chlorophyll content and this finding disagreed with much of the published data, this aspect was inves-
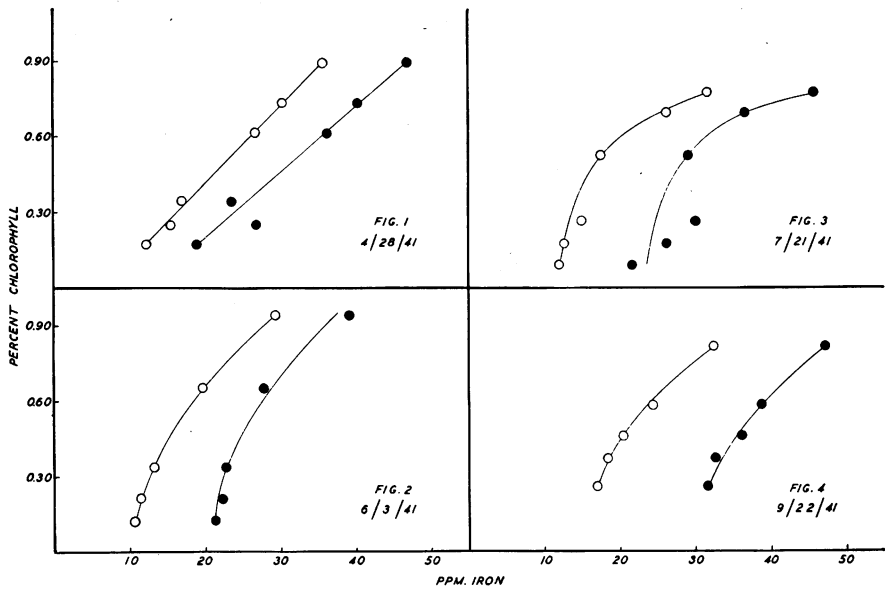
FIGS. 1, 2, 3, 4. Acid-soluble and total iron in pear leaves, expressed on a dry weight basis. ◯, acid-soluble iron; ●, total iron.

tigated further.   It is possible that the large number of leaves collected per sample, and the fact that selection was distributed among several trees or plants, may have tended to cut down the individual variation and thus give
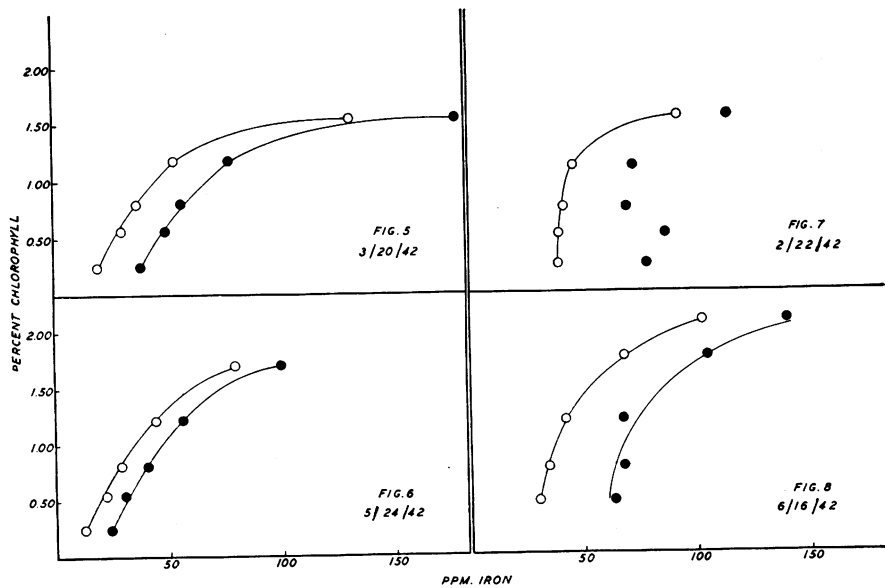
FIGS. 5, 6.   Corn leaves.   Acid-soluble and total iron, expressed on dry weight basis. ◯, acid-soluble; ●, total iron.

FIGS. 7, 8.   Tobacco leaves.   Acid-soluble and total iron, expressed on dry weight. basis. ◯, acid-soluble; ●, total iron.

more consistent results. Also the thorough washing employed could account for more consistent results. Very few investigators mention washing their samples preparatory to analysis.

CZAPEK (3) states that the usual iron oxide content of leaf ash is 1 to 4 per cent., sometimes lower and frequently higher. He then cites values as high as 10.2 per cent. (*Vitis vinifera*), 16.17 per cent. (*Triticum repens*) and even higher in other plants. The deep green sample of corn leaves harvested May 20, 1942 was the richest in iron of any sample analyzed. Yet its iron content, expressed as percentage iron oxide of ash, amounted to only 0.35 per cent. Even allowing for differences in species, the values reported by CZAPEK seem rather high in comparison to the above value. In view of this, it was felt that an examination of the magnitude of surface contamination was warranted.

Samples of chlorotic and green pear leaves were collected fom trees growing in calcareous soil and also, for comparison, a sample was collected

TABLE II
EFFECT OF WASHING ON LEAF IRON

	PPM. FE OF DRY WEIGHT		
TREATMENT OF LEAVES	CHLOROTIC LEAVES FROM CALCAREOUS SOIL	GREEN LEAVES FROM CALCAREOUS SOIL	GREEN LEAVES FROM NON-CALCAREOUS SOIL
	ppm.	*ppm.*	*ppm.*
Unwashed	348.0	250.0	547.0
Washed in water only	82.0	82.4	177.0
Washed in acid once	21.6	36.1	80.4
Washed in acid twice	20.0	36.1	79.5

from an orchard in non-calcareous soil. The leaves, which were quite dusty and covered with spray residue, were first washed carefully in distilled water, then in 0.3 N hydrochloric acid, rinsed thoroughly in distilled water, washed a second time in acid, rinsed again, and dried. The iron was determined in the dried leaves and various wash waters. Since the volumes of the latter were known, as well as the dry weights of the leaves, the respective amounts of iron in unwashed leaves, water washed, and acid washed leaves were calculated. The data are presented in table II.

It is evident that analyzing field samples for iron without preliminary washing may cause a 1700 per cent. error (compare acid-washed and unwashed chlorotic sample). This may be the explanation for the extremely high iron values cited by some authors. It is further to be noted that washing in water alone is not sufficient: the leaves still contain enough surface contamination to cause more than a 100 per cent. error. The first acid washing removed practically all remaining surface iron, since the second removed no more than traces. The method of acid washing as used here probably does not leach out any iron from the interior of the leaf. Such

leaching would require that the acid penetrate into the leaf but since no signs of injury were observed, it is doubtful if this occurred. All of the above considerations would apply to greenhouse-grown material, although to a lesser extent, since these plants were not exposed so long or to so much wind-blown dust as orchard trees. Moreover, the orchard trees were sprayed while the greenhouse plants were not. Much of the surface iron on leaves from the former may have been in the spray materials.

CHLOROPLAST IRON

Chloroplasts were isolated from three sets of tobacco plants by the procedure of GRANICK (6), except that the leaves were ground in a "blendor" instead of with mortar and pestle. Essentially, the method consisted of

TABLE III

ACID-SOLUBLE IRON IN WHOLE LEAF TISSUE AND CHLOROPLASTS OF TOBACCO, CALCULATED ON DRY WEIGHT BASIS

DATE	PERCENTAGE CHLOROPHYLL OF WHOLE LEAF	PPM. ACID-SOLUBLE IRON EXPRESSED ON WHOLE LEAF BASIS	
		WHOLE LEAF	CHLOROPLASTS
	%	ppm.	ppm.
10/1/41	0.584	53.8	35.3
	0.989	66.3	44.8
	1.430	83.5	64.3
	2.110	120.0	100.0
2/22/42	0.242	39.6	22.0
	0.507	40.0	23.0
	0.743	42.1	24.5
	1.110	46.5	29.0
	1.540	92.7	58.1
6/16/42	0.471	30.1	25.3
	0.769	34.7	25.7
	1.200	42.3	32.7
	1.760	68.4	49.4
	2.070	103.0	52.6

liberating the chloroplasts in 0.5 molar glucose solution and isolating them by differential centrifugation at controlled centrifugal forces. Microscopic examination showed the presence of only chloroplasts and starch granules in the preparations. The plastids as well as the original leaf material were analyzed for acid-soluble iron and chlorophyll. From these values, the fraction of acid-soluble leaf iron, localized in the chloroplasts, was calculated. In table III, the chlorophyll content and acid-soluble iron of whole leaf tissue, as well as chloroplast acid-soluble iron, are given. Figures 9, 10, and 11 are graphical representations of this data. In the region where iron is limiting, the acid-soluble iron of the chloroplasts parallels that of the whole leaf; i.e., active iron can be determined from either curve. Since the acid-soluble iron in the non-chloroplast portions of the leaf remains essentially constant when iron is a limiting factor, the active iron (fraction of iron directly proportional to chlorophyll content) must be localized in the chloro-

plasts.   As pointed out by OSERKOWSKY, there is no stoichiometric relation
between this fraction of iron and chlorophyll content.   This is shown graphi-
cally by the different slopes for each set of data.

Because of the structure of corn leaves, a comparison between the isolated
chloroplasts and whole leaf tissue was not possible.   In chlorotic corn leaves,
the interveinal region is much lighter in color than the region immediately
adjacent to the vascular bundle.   Because of the fibrous nature of the latter,
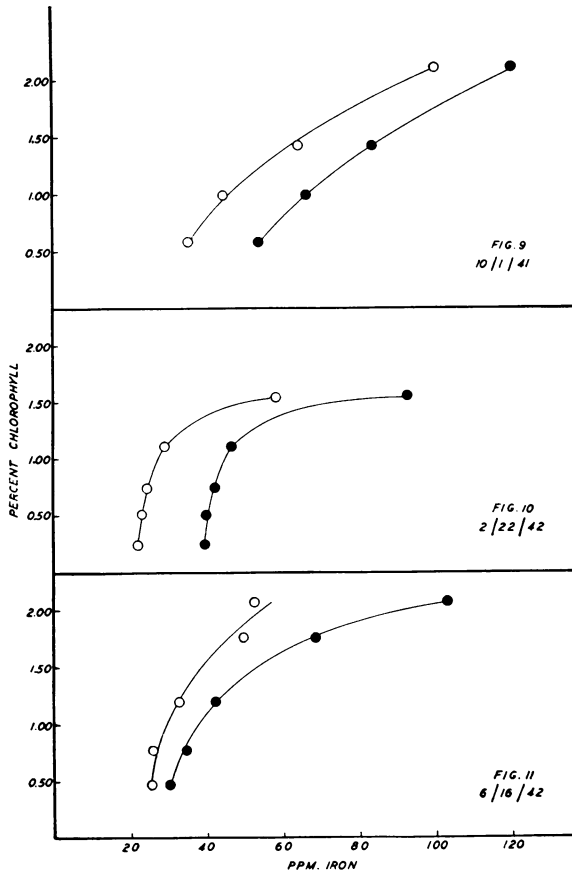
FIGS. 9, 10, 11.   Acid-soluble iron in chloroplasts and whole leaf tissue of tobacco,
expressed on dry weight basis of whole leaf.   ○, chloroplasts;   ●, whole leaf tissue.

these greener cells are not readily ruptured by the grinding process and
therefore one does not obtain representative chloroplast samples.   This was
not the case with tobacco leaves, in which grinding ruptures more or less the
same proportion of cells regardless of position in the leaf.

However, microscopically clean chloroplast preparations (though not
representative) could be obtained from corn leaves and analyses made of
them.   In table IV, acid-soluble and total chloroplast iron are presented in
terms of micrograms per ml. of chloroplast suspension for both corn and

tobacco.   From 57 to 74 per cent. of the total iron in the chloroplast is acid-soluble.   There are no particular trends: in two cases, the chlorotic samples have a greater percentage of acid-soluble iron than the green samples; in the others, the converse is true.

In the tobacco samples, for which the active iron values were determined, there is a decided difference between the chlorotic and green plastids with respect to this fraction.   The chlorotic chloroplasts contain a much smaller percentage of iron in the active form than do the green plastids.   On the other hand, the change in slope of the chloroplast curves in figures 9, 10,

TABLE IV

IRON FRACTIONS IN CHLOROPLASTS

DATE	DESCRIPTION	MICROGRAMS PER ML. OF CHLOROPLAST SUSPENSION		
		ACTIVE FE	ACID-SOL. FE	TOTAL FE
		γ	γ	γ
3/20/42	Corn Chlorotic	....	1.210 (65.1%)*	1.860
	Green	....	3.310 (57.9%)	5.720
5/2/42	Chlorotic	....	0.519 (67.9%)	0.764
	Green	....	3.260 (65.2%)	5.000
2/22/42	Tobacco Chlorotic	0.0567 (3.73%)	0.931 (61.3%)	1.520
	Green	1.120 (29.3%)	2.830 (74.1%)	3.820
6/16/42	Chlorotic	0.138 (9.08%)	0.865 (56.9%)	1.520
	Green	0.884 (36.5%)	1.570 (64.9%)	2.420

* Indicates percentage of total iron.

and 11 indicate that at low chlorophyll values, whatever active iron is present is utilized more efficiently than at high chlorophyll values.

In recent years, the idea that chlorophyll exists in the leaf in combination with a protein has received considerable attention.   Since MOMMAERTS (11) reported the presence of iron in a purified chlorophyll-protein complex, such a complex was isolated from both corn and tobacco leaves and investigated. The complex was prepared by the ammonium sulphate method of SMITH (18).   Only carefully tested iron-free reagents were used.   Iron was found to be present in significant amounts in all preparations.

Since slightly alkaline solutions had been used, the possibility existed that iron had been precipitated by the alkali, so the procedure was carried out using ammonium sulphate adjusted to pH 5.0.   Although the protein complex appeared to have become denatured as a result of the acidity, iron was still present.   This suggests that the iron was closely associated with a

protein.   Experiments with papain, to be described in a later paper, tend to confirm this supposition.

Discussion

If the acid-soluble iron be plotted against the varying chlorophyll content for a series of samples grown under similar conditions, a curve is obtained which does not pass through the origin, but intersects the iron axis. Two assumptions are made: (1) that the iron given by this intercept is constant throughout the set; and (2) that it is inactive in chlorophyll formation.   These two assumptions provide the simplest interpretation of, and are consistent with, the observed data.   Even if the inactive iron were not constant but varied uniformly, either decreasing or increasing with chlorophyll content, the general concept would not be changed.   The quantitative relationships, however, would be altered.

In all but one set of samples, the curve for total iron parallels that for acid-soluble iron.   That is, the active iron values are the same whether derived from either the acid-soluble or total iron curve.   This would indicate that the chlorophyll-forming mechanism involving iron is the first to suffer when iron becomes deficient.   The intercept of the total iron curve with the iron axis denotes the minimum amount of iron necessary for the maintenance of the leaf aside from chlorophyll formation.   When iron is a limiting factor, there is assumed to be no partition of it among the various fractions when it enters the cell; but rather it is suggested that certain other requirements (iron containing enzymes, etc.) must be satisfied before active iron and chlorophyll can be formed.

The slope of the active iron curve is not constant; it is greatest in the chlorotic region.   Although chlorotic leaves are low in active iron, whatever active iron is present is used more efficiently than in green leaves.   The variation of the active iron efficiency (slope) between different sets shows that other factors, internal or external, determine the quantitative relation between active iron and chlorophyll.

Because chlorosis may be cured by treatment with iron salts and yet analyses apparently show chlorotic leaves to contain as much or more iron than green ones, it has frequently been assumed that abnormal inactivation or precipitation of iron occurs in chlorotic leaves.   However, washing experiments indicated that this entire problem required examination.   It was found that with few exceptions properly acid-washed chlorotic leaves contained less total iron than green leaves.

The probable source of the surface contamination is spray materials and dust particles which contain iron as the oxide or silicate.   Both of these are essentially insoluble in dilute acid and hence, even if present, would not appear in the acid-soluble fraction.   Confirmation of this was obtained by the fact that the iron in the acid wash water could be removed by centrifugation and only reacted with iron reagents after boiling with strong acid for several minutes.   The efficacy of the acid washing does not depend on the dissolv-

ing off of the iron contamination, but rather on dissolving the spray materials and permitting the dust particles to be more readily dispersed in the wash solution.

An analysis of isolated chloroplasts clearly indicates that the active iron fraction is localized in the chloroplasts, although the latter also contain acid-soluble inactive iron. The nonchloroplast, acid-soluble iron of the cell remains approximately constant as long as iron is a limiting factor. When iron is no longer limiting in chlorophyll formation, it accumulates more rapidly in the nonchloroplast fraction of the cell than in the chloroplasts. The presence of acid-soluble iron throughout the cell is in agreement with the work of MOORE (12) who found a labile (reacting with hematoxylin) fraction of iron in the chloroplasts, cytoplasm, nucleus, and occasionally in the cell wall.

Summary

1. The active iron concept of OSERKOWSKY is valid for corn and tobacco leaves as well as for pear leaves.

2. A proportionality exists between total iron and chlorophyll content in the leaves of the plants studied.

3. Before chlorophyll formation can occur, the total iron content of the leaf must exceed a certain minimum level, which is determined by the species and the growth conditions.

4. Thorough washing of the leaves with dilute acid is a prerequisite for valid quantitative analyses of iron. Neglect of this point may lead to highly erroneous results.

5. In tobacco, the active iron is localized solely in the chloroplasts; but other fractions, both acid-soluble and acid-insoluble, are also present.

6. Iron is present in the chlorophyll-protein complex prepared by ammonium sulphate precipitation from corn and tobacco leaves.

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