132. STUDIES ON IRON IN PLANTS WITH SPECIAL OBSERVATIONS ON THE CHLOROPHYLL : IRON RATIO

By R. HILL and H. LEHMANN[1]

From the Biochemical Laboratory, Cambridge

(*Received 24 October 1941*)

THE starting point for the investigations about to be recorded was that one of us [Hill, 1937; 1938] found that chloroplasts, which he was able to separate from the cells of green leaves, produced oxygen if iron salts were added to the medium in which they were suspended.

The connexion between chlorosis and iron deficiency in green plants is well known [cf. Czapek, 1920]. Investigations by Oserkowsky [1933] show that not all of the iron is available during the formation of chlorophyll. He found that one of the factors deciding the development of chlorosis in leaves is lack of iron in the earlier part of the season. Oserkowsky differentiates between 'active' and total iron; active—we would call it available—iron is described by him as the part of the iron in dried leaves obtainable by extraction with *N* HCl. The chlorophyll content of chlorotic leaves is proportional to the active iron and not to the total amount of that mineral present.

In the present work measurements of iron and chlorophyll simultaneously in normal green leaves of different plants have been made. In several cases the chlorophyll : iron ratios throughout a season were followed. We have been concerned with measurements of the total iron in the plant tissue, as the nature of the iron compounds present is still obscure. It will be seen that the iron compounds fall into three main classes based on solubility.

METHODS

The chlorophyll was estimated by measuring the degree of extinction of a band in the red part of the spectrum of acetone extracts of fresh leaves. We measured the extinction at a wave-length of 6600 Å. using the spectrophotometer. The actual point where the greatest absorption could be found was always chosen for measurement. This was between 6550 and 6650 Å.

Control experiments showed that it made no difference if the acetone extracts were prepared in diffuse daylight or in the dark room illuminated with a green-coloured lamp.

The molecular absorption coefficient $\log_{10}(I_0 \times I)$ for chlorophyll in acetone was taken as $0{\cdot}46 \times 10^{-3}$ [see also Hill & Scarisbrick, 1940].

The measurement of the iron followed the principles laid down by Hill [1930]. After ashing of dried leaves at low temperature the inorganic residue was boiled in *N* HCl following McCance & Shipp [1933]. The additional manipulations for complete extraction suggested by McCance *et al.* [1936] could be omitted provided that the temperature of ashing was kept sufficiently low. $\alpha'\alpha'$-Dipyridyl in excess was added followed by 4 mol. of sodium acetate for each mol. HCl, and a

[1] Beit Memorial Fellow.

reducing agent added in solid grains, usually sodium hydrosulphite. The developing colour was compared with that of standard solutions of different amounts of reduced iron and $\alpha'\alpha'$-dipyridyl. These solutions were standardized by titanium titration.

The reagents used were always tested for the presence of iron and were found to be practically free. The sodium acetate solutions were boiled and filtered, the HCl stock was specially chosen; the sodium hydrosulphite contained on the average 0·02 μg. iron/mg.

Both wet and dry methods of ashing were used. The dry ashing method was found reliable for dried leaves but not for leaf extracts unless a platinum crucible was used. In the case of fluids containing biological material a large surface of dried residue was in contact with the porcelain crucible, and, as it was invariably alkaline, it attacked the crucible and set free iron which was soluble in acid afterwards. When phosphorus was measured, the method of Fiske & Subbarow [1925] was used.

Several estimations of chlorophyll and iron respectively on different samples of the same material were made. Values were only taken into account when the different estimations were in agreement within the limits of experimental errors.

If the midrib and veins of the leaves or leaflets were big enough to have appreciable influence on the results—as in the case of horseradish or chestnut—these parts of the plant tissue were removed before the chlorophyll and iron were examined.

The dry weight of the leaves was determined and usually the ash. Both will be expressed as percentage of the fresh weight. Chlorophyll and iron will be given in terms of molarity $\times 10^{-3}$, for this makes easier a comparison between the concentrations of the two. The molarity is expressed per g. fresh leaf as we do not know yet how much of the iron and the chlorophyll are actually in solution or are incorporated with the solid parts of the plant tissues. Since the dry weight and ash are measured at the same time and recorded in the text, the results can be calculated on any other basis.

The time of the year and the number of leaves per g. will give an idea of the seasonal stage of the plants in which the different measurements were taken.

I. Seasonal observations

The iron and chlorophyll contents of green leaves and leaflets throughout a season have been determined in:

(*a*) Elder (*Sambucus nigra*).
(*b*) Chestnut (*Hippocastanum*).
(*c*) Dead nettle (*Lamium album*).
(*d*) *Claytonia perfoliata.*

(*a*) *Elder*

It will be observed in Table 1 that in May, during the time of the most active growth, the chlorophyll : iron ratio rises, but it returns in June to the values measured in April, and even the appearance of the iron-containing fruits does not influence the ratio to any great extent.

Some special observations on flowers and fruits were made. Though both these organs of the elder contained iron, their provision did not affect the iron content of the leaflets (Tables 1*a* and 1*b*).

Table 1. *Chlorophyll and iron in elder throughout a season*

Date	No. of leaflets per g.	Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron	Special remarks
4. iii. 37	70	22	—	2·1	1·5	1·4	—
16. iii. 37	45	22	—	2·0	1·1	1·8	—
2. iv. 37	28	23	—	1·6	1·3	1·2	—
16. iv. 37	12	21	—	3·2	0·8	4·0	—
7. v. 37	3–20	21	1·7	3·0	0·4	7·5	—
12. v. 37	2½	19	1·6	3·0	0·4	7·5	—
19. vi. 37	2	22	2·5	3·6	0·7	5·1	In flower
7. viii. 37	1	24	3·7	4·0	1·0	4·0	Fruits appear
26. viii. 37	1–1½	21·5	3·2	3·4	0·5	6·8	—
8. ix. 37	Green 1–1½	23	—	4·0	0·2	20·0	—
	Yellow 1–2	14	2·5	0·2	0·2	1·0	—

Table 1*a*. *Iron in leaflets and corollae of elder*

19. vi. 37	Green leaflets	Corollae
Dry weight %	22·4	21·0
Ash %	2·5	1·9
Iron, $M \times 10^{-3}$	0·67	0·42
Chlorophyll, $M \times 10^{-3}$	3·64	—

Table 1*b*. *Iron in leaflets and fruits of elder*

	7. viii. 37		8. ix. 37	
	Green leaflets	Fruits	Green leaflets	Fruits
Dry weight %	24	17·5	23	8·4
Ash %	3·6	1·0	—	4·3
Iron, $M \times 10^{-3}$	1·0	0·3	0·2	0·1
Chlorophyll, $M \times 10^{-3}$	4·0	0·4	4·0	—

The absolute iron content per individual fruit remained constant (Table 1*c*).

Table 1*c*. *Iron in individual berries of elder*

	7. viii. 37	8. x. 37
Average weight of berry, mg.	100	350
Dry weight %	17·5	8·4
Average weight of iron per berry, μg.	11·9	11·9

(*b*) *Chestnut*

In chestnut (Table 2) as in elder, it will be seen that the iron precedes the chlorophyll both in appearance and in disappearance.

Table 2. *Chlorophyll and iron in chestnut throughout a season*

	Date	No. of leaflets per g.	Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron	Special remarks
A.	24. iv. 37	4	19	1·0	2·5	0·25	10·0	—
	14. v. 37	¾	19	0·9	2·8	0·25	11·2	—
B.	17. v. 37	1	—	—	—	0·5	—	In flower
	9. viii. 37	½	30	2·5	6·8	1·2	5·7	Developing fruits
	22. ix. 37	¾	35	3·4	6·8	0·4	17·0	Fruits fully developed and shedding
	27. x. 37	1	32	3·2	1·0	0·5	2·0	Yellow leaflets

Two different trees were investigated: A and B. A was much later in seasonal development.

The blossoms again contain quite an appreciable amount of iron, and this has no effect on the iron content of the leaflets (Tables 2*a* and 2*b*).

Table 2*a*. *Iron in leaflets and corollae of chestnut (tree B)*

B. 14. v. 37	Green leaflets	Corollae	Stamens, pollen removed
Dry weight %	—	10·4	12·7
Ash %	—	1·0	0·7
Iron, $M \times 10^{-3}$	0·45	0·2	0·1
Iron, % of ash	—	4·7	1·0

Table 2*b*. *Iron in leaflets and young fruits of chestnut (tree B)*

B. 9. viii. 37	Green leaflets	Young fruits
Dry weight %	30	40
Ash %	2·5	1·5
Iron, $M \times 10^{-3}$	1·2	0·4
Iron, % of ash	2·7	1·4

In contrast to what was found in the elder, the appearance of the fruit is accompanied by a considerable fall in the iron (Tables 2*a* and 2*b*).

The distribution throughout the fruit was investigated (Table 2*c*).

The brown 'shells' are comparatively rich in iron; next to the plumules contain more iron than the rest of the seed per g.

Table 2*c*. *Iron in fruit of chestnut*

22. ix. 37	Whole fruit	Husk one pericarp	Two chestnuts	Shells	Two plumules	Rest of embryo
Average weight of fruit, g.	67	25	42	4·8	0·3	37
Dry weight %	57	45–63	59	73	36	57
Ash %	2·9	2·9–4·1	2·5	4·4	2·2	2·6
Iron, μg./g. wet weight	8·4	6·25	9·5	44	15	5
Iron, μg./fruit	560	160	400	210	5	190

(*c*) *Dead nettle*

In the case of dead nettle, plants growing in different localities had the same iron content and chlorophyll : iron ratio.

Again the iron precedes the chlorophyll in both accumulation and removal (Table 3). The provision of the flowers with iron (Table 3*a*) does not affect the iron content of the leaflets any more than it did in elder and chestnut.

Table 3. *Chlorophyll and iron in dead nettle throughout a season*

Date	No. of leaves per g.	Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron	Special remarks
8. iv. 37	6	18	—	3·4	0·8	4·25	—
19. iv. 37	5	17	—	3·8	0·5	7·6	—
19. v. 37	4	17	2·8	3·6	1·4	2·6	In flower
10. viii. 37	Green 7	22	3·5	3·8	1·3	2·9	—
	Yellow 7	22	4·3	1·4	0·7	2·0	—

Table 3*a*. *Iron in leaflets and corollae of dead nettle*

19. v. 37	Green leaflets	Corollae flower	Stamens
Dry weight %	17	10	9
Ash %	2·8	1·1	1·7
Iron, mol. $\times 10^{-3}$	1·4	0·4	0·2

(*d*) Claytonia perfoliata

The inflorescent leaves, which differ anatomically from the radical leaves and are only half the size, have the same iron content and chlorophyll : iron ratio. The iron precedes the chlorophyll in the leaves in both accumulation and removal (Table 4).

Much of the iron which the inflorescent leaves lose in the later part of the season can be found again in the seed.

The seed of *Claytonia* was collected in June. Each inflorescence produced about 45 seeds. If the iron found in 45 seeds is added to the amount found in the leaf, the iron content rises from $0{\cdot}2 \times 10^{-3}$ to $0{\cdot}4 \times 10^{-3}$ *M*, nearly as high as in April, when the iron was $0{\cdot}5 \times 10^{-3}$ *M*.

Table 4. *Chlorophyll and iron in* Claytonia perfoliata *throughout a season*

Date	No. of leaves per g.	Dry weight %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	$\frac{\text{Chlorophyll}}{\text{Iron}}$	Special remarks
27. ii. 37	70	7	1·2	1·0	1·2	—
14. iv. 37	15	7	2·4	0·5	4·8	In flower
1. vi. 37	2–3	6	1·8	0·2	9·0	Seed

The seed had a dry weight of 76·6 and 1·9 % ash. In 230 mg. seed (66 seed $\approx$ 100 mg.) were found 27 μg. iron.

Four inflorescent leaves containing 20 μg. iron and weighing 1·513 g. (iron $0{\cdot}2 \times 10^{-3}$ *M*) have 180 seeds $\approx$ 119 mg. 119 mg. seed contain 14 μg. iron. Adding the iron content of the seed to that of the leaves four inflorescent leaves contain 34 μg. iron or 22·5 μg. iron/g. fresh weight. This would correspond to a molarity of $0{\cdot}4 \times 10^{-3}$ iron.

Iron content of chloroplasts

A comparison has been made in the case of *Claytonia* between the iron content of the whole leaf and that of the isolated chloroplasts. Suppose the quantity of iron in the whole leaf is 300; if this iron were evenly distributed through the leaf, the amount present in the chloroplasts would be represented by 8; the amount actually found was 31. This is the first time that the high iron content of chloroplasts as compared with other parts of the cell has been demonstrated by chemical measurement. It was hitherto only assumed on the basis of histological staining methods.

II. Leaves of evergreens

Measurements were made of the chlorophyll : iron ratio in evergreen plants, younger leaves being compared with older ones of the same individual plant. It

was generally found that the chlorophyll : iron ratio was higher in older than in younger leaves. The plants investigated were:

(*a*) *Euonymus japonicus.*
(*b*) Japanese laurel (*Aucuba japonica*).
(*c*) Box (*Buxus sempervirens*).

(*a*) Euonymus

Leaves of *Euonymus* of different kinds were investigated in May 1937. Dark green leaves surviving from 1936 and dark green ones from 1937 were chosen, and also the very yellow leaves on the end of the twigs and the light green ones below them which were just changing from the yellow state into the normal dark green appearance.

The difference in dry weight between green and yellow leaves may be due in part to differences in starch content.

After a sunny day (31. v. 37) four yellow and four green leaves of equal length (4–5 cm.) were picked, weighed and extracted with alcohol. The yellow leaves weighed 0·647 g., the green ones 0·813 g. Whereas the residue contained plenty of starch in the case of the latter, no trace of starch could be found in the residue of the yellow samples.

Whereas the chlorophyll : iron ratio in the 1936 leaves was about ten times as great as in the youngest of those of 1937, it will be noted that the phosphorus : iron ratio is the same in all leaves, i.e. about 70 (Table 5). It might be suggested that phosphorus was connected with the carbohydrate metabolism in the absence of chlorophyll. The state of the yellow leaves may recall the findings in haemosiderosis in man, in which an enormous accumulation of iron in the liver goes hand in hand with a lack of respiratory pigment.

Table 5. *Chlorophyll, iron and phosphorus in* Euonymus

Date	Kind of leaves	No. of leaves per g.	Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron	Phosphorus, $M \times 10^{-3}$	Phosphorus / Iron
24. v. 37	Dark green of 1936	2	29	1·9	3·0	0·2	15·0	13·9	69
24. v. 37	Dark green of 1937	3	25	2·5	2·2	0·4	5·5	29	72
21. v. 37	Light green of 1937	7	17	2·2	0·4	0·5	0·8	—	—
21. v. 37	Yellow of 1937	7	19	1·5	0·2	0·7	0·3	47·5	68

Keilin [1925] has shown *Euonymus* to be rich in haematin. We measured the actual amount in our leaves but found only a concentration of $0{\cdot}6 \times 10^{-4} M$ of haematin iron. Though the enormous acoumulation of iron cannot be accounted for as haematin, it is significant to find so much just before chlorophyll is formed.

(*b*) *Japanese laurel*

In Japanese laurel the distinction between the light green leaves of the current year and the dark green ones of the previous season was obvious in the month of April and similar, though less marked, differences in the chlorophyll and iron distribution were found as in *Euonymus* (Table 6).

Table 6. *Chlorophyll and iron in Japanese laurel, 26. iv. 37*

Kind of leaves	No. of leaves per g.	Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron
Young	15	29	1	0·4	0·2	2·0
Old	¼	31	2	2·6	0·3	8·7

(*c*) *Box*

In this plant (Table 7) it will be noted again that iron accumulates before the chlorophyll. Whereas in May the old leaves contained more iron than the new ones, in August the relation is reversed; most of the iron is now to be found in the leaves of 1937 and least in those of 1933.

Table 7. *Chlorophyll and iron in box*

Date	Kind of leaves	No. of leaves per g.	Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron
20. v. 37	Leaves of 1933–36	25	44	0·7	3·4	0·5	6·8
	Leaves of 1937	25	27	1·2	1·4	0·3	4·7
11–14. viii. 37	Leaves of 1933	22	43	2·8	1·5	0·25	6·0
	Leaves of 1934–36	25	44	2·8	4·0	0·3	13·3
	Leaves of 1937	31	44	2·3	3·0	0·5	6·0

III. Single measurements of chlorophyll : iron ratio in several plants

(*a*) Tulip (*Tulipa*).
(*b*) Horseradish (*Cochlearia armoracia*).
(*c*) Spinach (*Spinacia oleracea*).
(*d*) *Tropaeolum*.
(*e*) Garlic mustard (*Alliaria officinalis*).
(*f*) Golden elder (*Sambucus nigra*, golden variety).

(*a*), (*b*) Very little iron was to be found in tulips, though it must be kept in mind that the high water content of this plant may be responsible (Table 8). The blossoms and pistils were also investigated. It can be seen that the iron is fairly equally distributed throughout the different organs, if the ash as a whole is taken as a comparative basis.

Table 8. *Chlorophyll and iron in tulip, 16. v. 37*

Organ	Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron	Iron, % of ash
Leaf (1/20 per g.)	11·7	0·89	1·0	0·29	3·4	1·8
Perianth leaves	8·1	0·34	—	0·11	—	1·7
Pistil, pollen removed	9·5	0·58	—	0·15	—	1·4

Table 9. *Chlorophyll and iron in horseradish, 12. vi. 37*

Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron
19	2·1	3·2	0·45	7·1

(*c*) In spinach, as in tulip, a low iron content goes hand in hand with a low dry weight (Table 10).

Table 10. *Chlorophyll and iron in spinach, 21. x. 37*

Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron
10–15	2	1·9	0·2	9·5

The chlorophyll : iron ratio is particularly high. It is difficult to understand why it has been recommended as an iron-containing food with an iron content of only $0{\cdot}2 \times 10^{-3} M$. But it may be noted that large amounts of spinach leaves have to be used in preparing a dish of the cooked vegetable.

Table 11. *Chlorophyll and iron in* Tropaeolum, *30. x. 37*

Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron
14·5	1·4	2·8	0·3	9·3

(*d*) As we had always observed that the iron content of the leaves falls before the chlorophyll disappears, we used the leaves of *Tropaeolum* to find out if the removal of iron is a necessary condition for the yellowing of leaves. We left the leaves of *Tropaeolum* in water and observed their yellowing. We then measured the iron content of the leaf as a whole and of the green centre of the leaf which we separated from the yellow border. We also determined the concentration of iron in the stalks. Whereas the chlorophyll content decreased from $2{\cdot}8 \times 10^{-3} M$ in 9 days to $0{\cdot}26 \times 10^{-3} M$, the iron content remained constant at $0{\cdot}3 \times 10^{-3} M$. The iron in the stalks remained throughout at a molarity of $0{\cdot}1 \times 10^{-3}$. The green centres of the yellowing leaves contained rather less iron than the whole organ, namely $0{\cdot}25 \times 10^{-3} M$.

(*e*, *f*) *Garlic mustard and golden elder*

Differences in chlorophyll content were found in leaves of the same plant. This may be due to differences in illumination, leaves growing in the shade being richer in chlorophyll, as if they needed more pigment to make the greatest possible use of what light they can obtain. The difference may be due, on the other hand, to an inherited factor (variety). As an example of the influence of the former factor, garlic mustard was chosen; as an example of the latter, common and golden elder were used.

The specimens of garlic mustard were of two kinds, though they were grown at the same place and picked at the same time. We could differentiate between dark leaves which had grown in the shade and light sun leaves, yet the chloro-

Table 12. *Chlorophyll and iron in garlic mustard (shade and sun leaves) and in golden and common elder*

		No. of leaves per g.	Dry weight %	Ash %	Chlorophyll, $M \times 10^{-3}$	Iron, $M \times 10^{-3}$	Chlorophyll / Iron
Garlic mustard 4. v. 37	Shade leaves ...	1	11	—	3·6	0·45	8·0
	Sun leaves ...	3	15	—	2·6	0·3	8·7
Elder 7. v. 37		3–20*	21	1·7	3·0	0·4	7·5
Golden elder 5. v. 37		8–16*	18·5	—	1·4	0·45	3·1

* The number of leaves per g. had no great influence on the iron and chlorophyll content.

phyll : iron ratio was the same in all leaves. At the same time an example is given in which leaves of two different varieties of the same species with differing chlorophyll content were compared. Golden elder and common elder differ in pigment but not in iron content (Table 12).

IV. Nature of the plant iron

The observations of Keilin [1925] and Mann [1938] have established the fact that part of the iron is present as haematin, though this proportion is only small. Some of the iron found in plants is soluble in acetone, some in water, some is insoluble in both (Table 13).

Table 13. *Different solubilities of iron found in elder leaflets*

13. v. 37. 10 g. fresh leaflets contain 240 μg. Fe
10 g. fresh leaflets (dry weight 18%) extracted with acetone. 1·2 g. powder are obtained

	Amount of Fe per 10 g. leaflets, μg.
Acetone extract concentrated and ashed	19
42% of powder extracted with water; extract ashed	50
42% of powder ashed after water extraction	168
	237

Table 14. *Iron in water extract of acetone powder of dead nettle leaves*

	μg. Fe
Total iron/g. fresh leaf (17% dry weight)	24
Total iron/g. acetone powder	150
Available iron in 10 : 1 water extract of 1 g. powder	5
Available after 7 min. boiling of extract in HCl	6
Available after 180 min. boiling of extract in HCl	6

We observed several kinds of water-soluble iron in plant extracts and extracts of acetone powders of leaves.

(*a*) A proportion reacts at once with the dipyridyl reagent.

(*b*) A certain proportion reacts after boiling with acid. This can be either an iron protein complex or iron phosphate (Table 14).

(*c*) A proportion varying in amount reacts with dipyridyl only after ashing.

This last fraction seemed to correspond with that which Chibnall & Channon [1929] found in the ether-soluble part of cabbage leaves. We repeated Chibnall & Channon's experiments and confirmed their findings. This fraction may of course result from mechanical adsorption, but even so the adsorption of iron by lecithin should be of biological importance for the transport of this element in the plant.

Summary

The iron and chlorophyll contents of green leaves and leaflets throughout a season have been determined in elder (*Sambucus nigra*), chestnut (*Hippocastanum*), dead nettle (*Lamium album*), and *Claytonia perfoliata*. Iron was always found to precede the chlorophyll both in appearance and disappearance. Blossoms and fruits contain an appreciable amount of iron, yet this fact is without any influence on the iron content of elder and chestnut leaves. 2/5 of the iron of the whole chestnut fruit were found in the woody shells of the embryo. In

Claytonia much of the iron which the green leaves lose in the later part of the season can be found again in the seed. The chloroplasts in *Claytonia* leaves contain four times as much iron as they would be expected to have were the iron equally distributed throughout the leaves. This is the first time that the iron content of the chloroplasts has been chemically measured.

During the development of the leaves of evergreen plants iron storage precedes that of chlorophyll. Green leaves of *Euonymus japonicus* left over from the previous year have a chlorophyll : iron ratio which is ten times as great as that of leaves of the current season. The phosphorus : iron ratio is the same in all leaves. It is suggested that the high phosphorus content in yellow leaves may be connected with carbohydrate metabolism in absence of chlorophyll. Japanese laurel (*Aucuba japonica*) and box (*Buxus sempervirens*) behave in a similar way to *Euonymus*. The chlorophyll : iron ratio was also determined in tulip (*Tulipa*), horseradish (*Cochlearia armoracia*), spinach (*Spinacia oleracea*), *Tropaeolum*, garlic mustard (*Alliaria officinalis*) and golden elder (*Sambucus nigra*, golden variety). The chlorophyll : iron ratio is the same in the dark (shade) leaves and the light (sun) leaves of garlic mustard. Golden elder and common elder differ in pigment but not in iron content.

The iron content in most plants investigated was about a fourth to a tenth of the chlorophyll compared per mol. Some iron in plants is soluble in acetone, some in water, some insoluble in both. Three kinds of water-soluble iron could be observed:

(*a*) A large proportion reacts at once with $\alpha'\alpha'$-dipyridyl.

(*b*) A certain proportion reacts after boiling in acid; this can be either an iron protein complex or iron phosphate.

(*c*) A proportion varying in amounts reacts with $\alpha'\alpha'$-dipyridyl only after ashing.

One of us (R. Hill) was a Beit Memorial Fellow during part of this research. Some of the expenses of the work were defrayed from a research grant from the Ella Sachs Plotz Foundation to H. Lehmann.

REFERENCES

Czapek (1920). Biochemie der Pflanzen. Jena: G. Fischer.
Chibnall & Channon (1929). *Biochem. J.* **23**, 176.
Fiske & Subbarow (1925). *J. biol. Chem.* **66**, 375.
Hill (1930). *Proc. roy. Soc.* B, **107**, 205.
—— (1937). *Nature, Lond.*, **139**, 881.
—— (1938). *Proc. roy. Soc.* B, **127**, 192.
—— & Scarisbrick (1940). *Proc. roy. Soc.* B, **129**, 238.
Keilin (1925). Private communication.
McCance & Shipp (1933). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 187, 640.
—— Widdowson & Shackelton (1936). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 213, 1936.
Mann (1938). *Arch. Nauk. biol.* **9**, 10.
Oserkowsky (1933). *Plant Physiol.* **8**, 449