

Proc. Fla. State Hort. Soc. 83:372-375. 1970.

## THE IRON-CHLOROPHYLL RELATEONSHIP IN YOUNG HASS AVOCADO LEAVES

E. F. Wallihan and R. G. Sharpless

*University of California, Riverside*

### ABSTRACT

Young summer leaves of uniform size on a single tree were tagged and then picked at intervals as they matured. Prior to the time of maximum leaf expansion, the relation between Fe and chlorophyll concentrations changed rapidly in the direction of increased chlorophyll per unit of Fe. Samples from three orchards taken in September revealed a correlation coefficient of .70 between Fe and chlorophyll concentrations in spring cycle leaves whereas summer cycle leaves gave a more random distribution. It is concluded that the use of leaf analysis for diagnosing Fe status of Hass avocados provides more useful information when spring cycle leaves are collected in September than when summer cycle leaves are collected in September.

### INTRODUCTION

Avocado leaves bearing clear symptoms of Fe deficiency usually contain less than 30 ppm of Fe in the dry matter. Occasionally we find iron chlorotic leaves containing 50 to 60 ppm Fe, which is the common range found in dark green leaves. Two possible explanations for this discrepancy have been suggested in connection with studies on other kinds of plants. First, the suggestion that part of the leaf Fe may be precipitated in the tissues and thus become physiologically inactive, seemed to be supported by Oserkowsky's (4) investigation of solubility of Fe from pear leaves in dilute HCl. Subsequent work by Jacobson (2) showed that the internal precipitation hypothesis was not necessary if surface contamination was removed from the leaf prior to analysis. Second, Jacobson and Oertli (3) subsequently showed, with sunflower plants, that chlorophyll formation could be regulated by Fe supply up to the time the leaf matured. After that, the leaves became incapable of forming chlorophyll, even after taking up added Fe. As a result, the Fe-chlorophyll relationship deviated from that established when the leaves developed under a constant rate of supply.

This study was undertaken to determine if these or other explanations applied to the Fe-chlorophyll relation in avocado leaves.

### MATERIALS AND METHODS

Leaf samples were obtained from three orchards, one in Riverside County and two in Ventura County. In one phase of the study, 30 young leaves of uniform age and degree of chlorosis were selected and tagged on a single tree. These were sampled at

intervals. All other samples were selected on the basis of visual inspection.

Each sample was put immediately into a plastic bag in an ice chest, then transferred to a refrigerator at 5°C in the laboratory. Each leaf to be analyzed was washed with soap, using a 1" nylon paintbrush, then placed in a vial so that the end of the petiole was immersed in water. After weighing, a band of tissue was removed across the widest part of the leaf and weighed for chlorophyll determination by the official AOAC method (1). The remainder of the leaf was dried at 60°C for Fe analysis by the o-phenanthroline method (5). Suitable precautions were taken against contamination from glassware, filter paper, and dust. Estimates of Mn and Zn concentrations were made in some samples by methods previously described (6).

## RESULTS AND DISCUSSION

Table 1 gives the average concentrations and weights per leaf of Fe and chlorophyll at each of the four sampling dates for the tagged leaves. The reduction of Fe concentration between July 9 and 28, during which time the weight of Fe per leaf nearly doubled, illustrates the diluting effect of leaf growth. During the same period, chlorophyll content increased four fold and the concentration increased by half. Thus, rapid growth was accompanied by rapid translocation of Fe into the leaves and rapid production of chlorophyll. The chlorophyll content per unit of Fe present in the leaf changed from the lowest to the highest measured value. After July 28, this ratio decreased somewhat. Continued movement of Fe into the leaves resulted in progressive rise in Fe concentration.

Figure 1 shows the distribution of Fe and chlorophyll values for the individual leaves. The inset graph shows that they reached maximum area about August 1. Samples taken at that time and later show a fairly clear relation between Fe and chlorophyll concentrations, whereas the July 9 samples are distinctly separate. Thus, the immature leaves did not provide a clear relation between Fe and chlorophyll concentrations.

To test this observation farther, analyses of all samples from the 3 orchards are plotted in Fig. 2, in which a distinction is made between leaves from the current or recent growth cycle and those from the next older cycle. Field observation indicates that this is a distinction between summer and spring cycles, both represented on the same shoot.

The entire body of data does not show any consistent trend but data from the spring cycle gives a distribution having a linear coefficient of correlation of .70, represented by the line in Fig. 2. This correlation is significant beyond the .01 level.

Table 1. Average concentrations of iron and chlorophyll, weights per leaf of iron, chlorophyll, and dry matter, and chlorophyll to iron ratios in Hass avocado leaves tagged on July 9. 5 leaves were taken on each date.

Sampling date	Concentrations in leaves <sup>†</sup>		Weights per leaf <sup>†</sup>			Chlorophyll <sup>†</sup>
	Fe	Chlorophyll	Fe	Chlorophyll	Dry matter	Fe
	µg/g <sup>*</sup>	mg/g <sup>**</sup>	mg	mg	g	
July 9	37.2 <sub>a</sub>	.592 <sub>a</sub>	5.5 <sub>a</sub>	.41 <sub>a</sub>	.15 <sub>a</sub>	.074 <sub>a</sub>
July 28	18.2 <sub>c</sub>	.826 <sub>b</sub>	10.4 <sub>b</sub>	1.71 <sub>b</sub>	.58 <sub>ab</sub>	.165 <sub>c</sub>
Aug. 20	22.6 <sub>bc</sub>	.932 <sub>b</sub>	13.9 <sub>b</sub>	1.80 <sub>b</sub>	.62 <sub>b</sub>	.133 <sub>b</sub>
Sept.23	25.6 <sub>b</sub>	1.022 <sub>b</sub>	18.5 <sub>c</sub>	2.42 <sub>b</sub>	.72 <sub>b</sub>	.132 <sub>b</sub>

<sup>†</sup> Within a column, figures not having a subscript letter in common differ at the .05 level.

<sup>\*</sup> Dry weight basis.

<sup>\*\*</sup> Fresh weight basis.

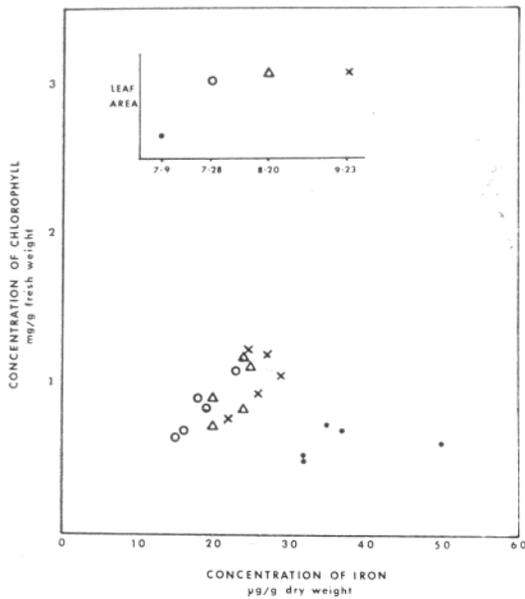


Figure 1.—Changes in leaf area and concentrations of Fe and chlorophyll in summer leaves of Hass avocado.

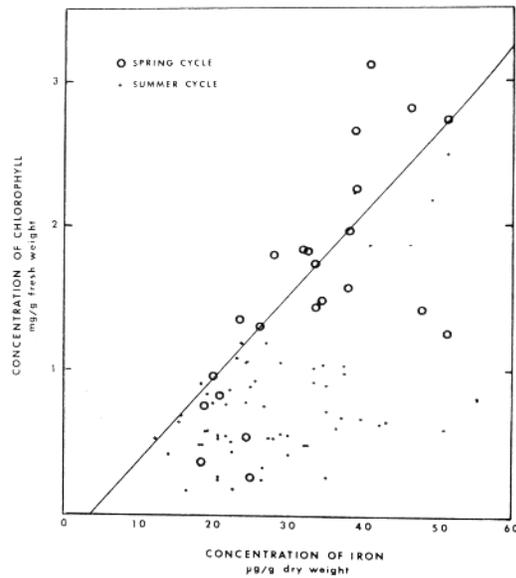


Figure 2.—Relation between Fe and chlorophyll concentrations in spring cycle and summer cycle leaves of Hass avocado.

Many of the points representing summer cycle leaves fall close to the correlation line but there is no obvious basis for identifying such leaves by inspection. Also, of the 23 old cycle leaves plotted, 3 or 4 may be far enough from the statistical average to suggest that additional factors influenced the Fe-chlorophyll relation. It is possible that chlorophyll production was partially limited in some leaves by other nutrient deficiencies, in view of leaf concentrations as low as 21 ppm Zn and 20 ppm Mn found in some of the sampled trees. Also, these samples do not provide a clear test of the effect of varying Fe supply, such as that found in sunflower (3).

The most probable explanation for low chlorophyll in the young leaves seems to be that, during periods of rapid leaf expansion, chlorophyll production lagged Fe uptake by the leaf. This is supported by a previous observation that visible response to Fe spray treatments required from 1 to 6 months, depending on temperatures. Considering this variable, and the effect of soil factors on rate of Fe uptake by trees, it does not seem likely that a closer correlation between Fe and chlorophyll than that observed in this study will be achieved in field samples taken under a variety of conditions. It is self-evident that the best correlation will be obtained when Fe is the only factor limiting chlorophyll production. Thus, studies of the Fe-chlorophyll relation in leaves require recognition of possible deficiencies of Zn, Mn, and N, and toxic levels of Cl, B, and other substances, as well as chlorosis-inducing effects of insects, viruses, and other pathogens.

## **SUMMARY AND CONCLUSION**

The following generalizations may be useful as a guide in connection with leaf analysis for Fe as a diagnostic procedure:

1. Eight of 9 samples of spring cycle Hass avocado leaves, sampled in September and containing less than 30 ppm Fe (dry weight basis), contained less than 1.5 mg/g of chlorophyll (fresh weight basis). Summer cycle leaves did not conform to this pattern.
2. All of the 14 spring cycle leaves having more than 30 ppm Fe contained more than 1.25 mg/g of chlorophyll. This was not true of summer cycle leaves.

These observations suggest that useful information regarding the level of Fe nutrition of the tree can be obtained from analysis of spring cycle leaves harvested in September.

## **LITERATURE CITED**

1. Association of Official Agricultural Chemists. 1955. Official Methods of Analysis. 8th Ed.
2. Jacobson, L. 1945. Iron in the leaves of chloroplasts of some plants in relation to their chlorophyll content. *Plant Physiol.* 20: 233-45.
3. Jacobson, L., and J. J. Oertli. 1956. The relation between iron and chlorophyll contents in chlorotic sunflower leaves. *Plant Physiol.* 31: 199-204.
4. Oserkowsky, J. 1933. Quantitative relation between chlorophyll and iron in green and chlorotic pear leaves. *Plant Physiol.* 8: 449-468.
5. Sandell, E. B. 1950. Colorimetric determination of traces of metals. Second Ed.

Interscience Publishers, Inc., N. Y.

6. Wallihan, E. F., M. J. Garber, J. E. Hammond, Wilma L. Printy, D. S. Rayner, and R. G. Sharpless. 1967. Iron requirement studies of navel orange trees in solution cultures. *Hilgardia* 38: 247-264.