
Chlorophyll Concentrations in Navel Orange Leaves in Relation to Iron Status, Leaf Age, and Season

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Abstract. Young shoots on field-grown trees [Citrus sinensis, (L.) Osbeck] were the source of repeated chlorophyll measurements on individual leaves by light absorbance and occasional leaf samples for laboratory measurement of chlorophyll and Fe. These concentrations increased or decreased during the period of leaf expansion, depending on comparative rates of Fe uptake, chlorophyll formation, and leaf growth. Chlorophyll/Fe ratio in spring cycle leaves increased until leaves were fully expanded (May-June) and maintained the same level in December. Fall cycle leaves reached the same ratio in December. Linear regression of chlorophyll concentration on Fe concentrations was \( r = 0.907 \) at \( n = 46 \) for mature leaves having < 50 ppm Fe. Mature leaves having > 50 ppm Fe had essentially equal chlorophyll concentrations, indicating a maximum value of about 3 mg/g F.Wt. A graph of loci for immature leaves showed the majority with low chlorophyll in relation to Fe concentration. It is concluded that the maximum concentration of chlorophyll per unit of Fe is not achieved until leaf maturity.

General agreement has not been reached on the relation between leaf Fe concn and degree of chlorosis or measured chlorophyll concn (2, 5, 8, 9, 10). Leaves classified according to severity of chlorosis exhibit overlapping Fe concn between groups when precautions are taken to remove surface contamination from fresh leaves prior to analysis (8, 10). Similarly, plots of Fe against measured chlorophyll sometimes include erratic points even though the general trend is clear (2, 10). Such observations lend doubt to the usefulness of Fe concn in the leaf as an index of Fe sufficiency for plant function.

The usual procedure for testing the diagnostic value of tissue analyses is to compare them directly with the desired feature of plant performance, such as growth rate, yield, fruit color, etc. We have found a reasonably good relationship between leaf Fe concn and fruit production in navel orange trees (12). Most studies of Fe-chlorophyll relations appear to be based on the assumption that chlorophyll production responds instantly to Fe in the leaf. Contrary evidence (3) led us to examine seasonal changes in the Fe-chlorophyll relationship in leaves of navel orange.

Materials and Methods

The study area comprised about 25 ha of commercial orchards from 10 to 50 years old by Byn Mawr, about 13 km NE of Riverside. The soil was San Emigdio fine sandy loam with 0-2% slope and weak crumb structure in the Ap horizon (0-20 cm). The C horizon below 20 cm was massive and hard. The entire profile was calcareous.

Variable severity of Fe deficiency among trees, limbs, individual shoots, and seasons was judged to be typical of the Fe problem in citrus. Individual terminal shoots were selected and tagged for subsequent sampling. Selections were made at the time terminal leaf buds opened, either in early spring (spring cycle) or late summer (fall cycle). Each twig was classified as green (uniform green color) or chlorotic (interveinal tissues distinctly less green than the veins) based on conditions of existing mature leaves. Twigs with intermediate status or bearing flower buds were omitted.

Leaf samples were analyzed for total Fe by the o-phenanthroline procedure of Sandell (7) after thorough washing (11), and for chlorophyll by solvent extraction (1). Repetitive measurements of chlorophyll in individual leaves in the field were done by light absorbance (11). A random sample of 12 to 20 leaves in the same age bracket and representing the full available range of chlorophyll concn (CC) was picked on each reading date and analyzed for CC by extraction to serve as a calibration of the absorbance meter for that date, calculated by best fit to the formula \( \log y = A + B \log x \), where \( x = \) absorbance and \( y = \) CC.

Care was given to keep sampled leaves fresh and turgid by using polyethylene bag and a portable ice chest inasmuch as CC values were to be expressed on a fresh weight basis. Weights were taken after the individual leaves had been rolled and inserted into test tubes so that the petioles were immersed in water for an hour or longer.

Results and Discussion

Thirty-five twigs on one tree were tagged and classified in early April 1966. Each new leaf in the spring flush was measured with the absorbance meter starting on April 11 and at intervals depending on rate of change. Fig. 1 shows the changes in CC over a period of 10 months for 2 leaves on each of 2 shoots selected to illustrate the range of values observed among individual leaves on a single shoot and between green and chlorotic shoots.

The relatively small difference in CC between the initial and terminal leaves on a given shoot indicates that any leaf on an expanding shoot could be used to characterize the shoot for subsequent studies. The highest CC occurred in shoots that arose from green twigs, indicating that the general level of chlorophyll production was characteristic of individual shoots and presumably reflected differences in Fe supply from various parts of the root system. The fact that all leaves on the chlorotic shoot abscised prior to August 3 suggests that a CC of 0.25 mg/g was insufficient for survival.

A parallel series of 140 tagged shoots, distributed among several trees, were sampled at intervals through the same 10 months' period. Each sample consisted of 12 to 15 leaves. Fig. 2 shows the average CC and total Fe concn for the respective dates and shoot classes and corresponding changes in average weights of dry matter, Fe, and chlorophyll per leaf.

Seasonal trends of CC and differences between green and chlorotic leaves were similar to those observed by repeated measurements of individual leaves (Fig. 1) except that the chlorotic leaves that achieved CC levels averaging 0.6 mg/g on July 5 survived into the following winter.

CC in green leaves increased by about 50% during the period

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Fig. 1. Trends of chlorophyll concn in initial and terminal leaves of 2 spring cycle shoots, one on a green branch (dots) and the other on a chlorotic branch (circles), of 1 tree. Data from field measurements by light absorbance. Chlorotic leaves abscissed between June 30 and August 3.

of leaf growth, which ended about June 3, while Fe concn remained rather steady; total weights of chlorophyll and Fe per leaf increased threefold. Concns increased abruptly during the ensuing month. Thus, concn of both chlorophyll and Fe were strongly affected by dilution during leaf expansion.

Chlorotic leaves lost a significant amount of chlorophyll without any change in Fe during the period from Dec. to Feb. The mechanism for chlorophyll loss is not clear but the fact that it occurred provides one possible explanation for the limited success in correlating Fe and chlorophyll concn among random leaf samples.

A similar series of shoots was tagged in early April 1967 and CC measured by absorbance during the period from 10 April to 11 May. The shoots were not classified visually but, for the purpose of comparing trends with those in the spring of 1966, an arbitrary group of shoots was selected for statistical analysis on the basis of having CC on 10 April between 0.5 and 0.9 mg/g fresh wt. This procedure limited the sample variability. The data are summarized in Fig. 3.

Table 1. Chlorophyll/iron ratios on successive sampling dates.

<table>
<thead>
<tr>
<th>Date</th>
<th>Spring cycle 1966</th>
<th>Fall cycle 1966</th>
<th>Spring cycle 1967</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 10</td>
<td>—</td>
<td>—</td>
<td>18.3</td>
</tr>
<tr>
<td>April 11</td>
<td>22.8d2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>April 19</td>
<td>23.0d</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>April 27</td>
<td>23.2d</td>
<td>—</td>
<td>18.3</td>
</tr>
<tr>
<td>May 5</td>
<td>31.1c</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>May 11</td>
<td>—</td>
<td>17.4</td>
<td>—</td>
</tr>
<tr>
<td>May 20</td>
<td>41.7ab</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>June 3</td>
<td>42.4ab</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>June 5</td>
<td>40.0ab</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sept. 15</td>
<td>—</td>
<td>26.4b</td>
<td>—</td>
</tr>
<tr>
<td>Sept. 30</td>
<td>—</td>
<td>32.0ab</td>
<td>—</td>
</tr>
<tr>
<td>Nov. 4</td>
<td>—</td>
<td>33.5ab</td>
<td>—</td>
</tr>
<tr>
<td>Dec. 8</td>
<td>—</td>
<td>44.4a</td>
<td>—</td>
</tr>
<tr>
<td>Dec. 14</td>
<td>45.5a</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Feb. 8, 1967</td>
<td>35.7bc</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

2Mean separation in columns by Duncan's multiple range test, 5% level.
The comparison between young and mature orange leaves resembles that previously found in leaves of 'Haas' avocado (13) and supports the qualitative statement by Hill and Lehmann (3) that "iron was always found to precede the chlorophyll . . . ," based on analyses of field samples from 13 species. The x-intercept indicates that about 8 ppm Fe were required before chlorophyll production occurred, which is similar to findings by Jacobson (4) on pear, corn, and tobacco leaves. Our adoption of linear regression in the Fe-deficiency range is based on the slightly higher correlation coefficient than was obtained when log Fe (r = 0.883) or log chlorophyll (r = 0.851) was used. Inspection of Jacobson's curves suggests that they might be interpreted the same way, given the assumption that the upper 1 or 2 loci lie in the maximum chlorophyll region in some cases.

**Conclusions**

The quantitative Fe-chlorophyll relationship in orange leaves is subject to variation due to 1) time required for chlorophyll formation, and 2) loss of chlorophyll during winter. Mature navel orange leaves sampled during the growing season and containing 50 ppm Fe (dry wt basis) or less should exhibit a close linear correlation between Fe and chlorophyll concn. Inclusion of samples containing Fe in the luxury consumption range can be expected to weaken the linearity and lower the correlation coefficient.

The use of correlation between Fe and chlorophyll concn as a basis for evaluating the diagnostic value of leaf Fe concn will be valid for the plants studied only in mature leaves sampled during the growing season.

**Literature Cited**