# HILGARDIA 

A Journal of Agricultural Science PUBLISHED BY THE California Agricultural Experiment Station

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Sap Concentration and Inorganic Constituents of Mature Citrus Leaves

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## HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE published by<br>CALIFORNIA AGRICULTURAL EXPERIMENT STATION

# SAP CONCENTRATION AND INORGANIC CONSTITUENTS OF MATURE <br> CITRUS LEAVES ${ }^{1}$ 

A. R. C. HAAS ${ }^{2}$ and F. F. HALMA ${ }^{3}$

## INTRODUCTION

Previous investigations, Haas and Halma, ${ }^{(2)}$ on the sap concentration and inorganic constituents of citrus leaves suggested the need for further data as regards seasonal fluctuations. In general the sap of immature citrus leaves is very dilute and becomes more concentrated as the leaves become mature. It appears that the sap of mature leaves increases in concentration as the season advances and that the highest concentration is reached in the winter. The supposition prevails that the sap concentration remains at this level until the leaves absciss. It will be shown, however, that the high sap concentration during the winter months is only transitory and that it is probably related to ineffective translocation of elaborated food rather than to increased age of the mature leaves. As the tree becomes active in the spring, the leaf sap concentration decreases to approximately the same level as that reached during the previous spring when the leaves were mature, but a year younger ; in other words, fluctuations in sap concentration of mature leaves is seasonal. On the other hand, the changes that occur in inorganic constituents of similar leaves appear to be related to the age of the leaves rather than to seasonal changes.

[^0]This report is based on an investigation carried on for two years. During 1927-1928 the freezing-point depression of the sap was determined on leaves taken from several orchards. During 1928-1929 studies were made on leaves taken from one orchard throughout the year and in addition to determinations of sap concentration, inorganic analyses were made of the sap and dried leaves.

For the sake of clarity it may be mentioned that the growth of citrus takes place in cycles. Depending upon the age of the tree and the variety, two or three well-defined growth cycles are made during the season. Not all twigs produce new terminal growth at the same season; hence, it was considered necessary to ascertain whether the leaves from the two kinds of twigs (those with and without new growth) are comparable in their physical and chemical characteristics.

## MATERIAL AND METHODS

The studies were made on Eureka lemon (a variety of Citrus limonia Osbeck), and on Valencia and Navel orange (both varieties of Citrus sinensis Osbeck). The leaf samples were taken from trees growing in the orchards of the Citrus Experiment Station, Riverside, California. Our previous studies (Halma and Haas ${ }^{(4)}$ ) have emphasized the importance of careful sampling. For example, it was shown that although the inorganic constituents of the leaves are not affected, yet a great difference in sap concentration exists between the leaves exposed to direct sunlight and those not so exposed. For this reason the samples were always collected between 8 and $8: 30$ a.m. on the shady side of the trees, at intervals of four to six weeks throughout the year.

The samples of $1927-1928$ represented mature leaves of approximately the same age. During 1928-1929, however, mature leaves were taken from a certain growth cycle chosen at the beginning and adhered to throughout the year. In this case, the samples represented leaves of increasing age. The leaves were divided into two lots: one lot was prepared for inorganic analysis and the other for the determination of the freezing-point depression, after which the sap was used for inorganic analysis. A few inorganic analyses were made of the leaf meal after the sap was expressed.

For the determination of the freezing-point depression, the leaves were frozen for approximately 20 hours and then ground in a meat chopper. The sap was expressed with a hand press. Realizing that the amount of pressure applied in obtaining the sap is an important
factor, care was taken to apply as uniform a pressure as possible to all samples. The uniformity of the data obtained justifies the belief that the errors which might have resulted from this source are negligible. The freezing point of the sap was determined with the Beckmann apparatus and corrected for undercooling by the formula given by Harris and Gortner. ${ }^{(5)}$

## SEASONAL CHANGES IN SAP CONCENTRATION

The results obtained for the freezing-point depression of the leaf sap during the two years are represented by figures 1 and 2. It is obvious that all four curves show a striking similarity. All show a comparatively low sap concentration during the growing season and a high concentration during the winter months.

The fact that three different citrus varieties (Eureka lemon, Navel and Valencia orange) are represented, and that the method of sampling the leaves was different in the two years, points to the conclusion that an explanation for the seasonal fluctuations in sap concentration cannot be due to inherent characteristics of the species but must be sought elsewhere.

The growth habit of Eureka lemon on the one hand and that of Navel or Valencia orange on the other are dissimilar in some respects. The lemon is more easily awakened from its semi-dormant state during the cold season than is the orange. Lemon trees produce flowers and fruit practically throughout the year, whereas orange trees normally produce only one set of blossoms and one crop of fruit. If differences in growth habits of lemon and orange trees were related to the fluctuations in sap concentration, the curves should show a dissimilarity, which is not the case. The marked similarity even in the minor fluctuations of lemon and orange, excludes soil moisture as a factor because the two species being in different orchards, were not irrigated at the same time. This does not exclude the possibility, however, that lack of soil moisture may affect sap concentration, but in this investigation the trees had an adequate supply of water.

Examination of meteorological data recorded at the Citrus Experiment Station showed that sunshine or humidity bore no relation to the fluctuations in sap concentration.

It was found, however, that the air and soil temperature curves (figs. 1, 2) bear a striking inverse relationship to that of the sap concentration. The air and soil temperatures chosen were the mini-
mum which occurred during the night previous to sampling. It may be mentioned that the maximum air temperature also shows a similar inverse relationship to the sap concentration. It will be seen that as the minimum air or soil temperatures decrease, the sap becomes more


Fig. 1. Freezing-point depression $(\Delta)$ of sap of Eureka lemon and Valencia orange leaves and its relation to soil temperature and minimum air temperatures; 1927-1928.
concentrated. It appears that the sap concentration increases as the soil temperature drops to about $60^{\circ} \mathrm{F}$ or below. Similarly, it increases as the minimum air temperature during the previous night falls to about $40^{\circ} \mathrm{F}$ or below. It is of interest to note from the graphs that
the sap concentration is affected to a greater extent by the minimum air temperature than by the soil temperature.

Citrus being an evergreen, undoubtedly carries on photosynthesis during the winter months, especially under semi-arid conditions, although the root system is relatively inactive, due to low soil temperatures. For example, citrus roots that were pruned at regular intervals throughout the year, regenerated new roots within a short time


Fig. 2. Freezing-point depression ( $\Delta$ ) of sap of Eureka lemon and Navel orange leaves, and its relation to soil temperature and minimum air temperature.
when pruned during the growing season, but failed to respond when pruned during the season when the soil temperature was low (Halma ${ }^{(3)}$ ). It is reasonable to assume, therefore, that under unfavorable soil temperatures translocation of carbohydrates must be either very slow or completely arrested. A somewhat similar condition may be brought about artificially during the growing season by girdling the trunk or twigs in which case the sap concentration also increases. From the nature of the curves it appears that the temporary chilling of the twigs and perhaps also of the leaves by low air
temperatures during the night previous to sampling may arrest translocation. But this cannot be the primary reason because during the day as the air temperature rises and translocation should become normal, the sap should become more dilute, which is not the case. It appears that the high sap concentration during this period is due to a condition under which the roots are unable to make use of the photosynthetic products.

It has already been pointed out that the leaves taken during the latter part of 1928-1929 were older than those taken throughout 1927-1928, yet in both cases the sap concentration at the end of the year was practically the same as that at the beginning of the year. This excludes, therefore, the possibility that the sap concentration is related to the age of the leaves, provided they are mature.

Incidentally, it may be mentioned that in the case of the lemon the one-year-old leaves showed symptoms of old age and began to absciss. Orange leaves of similar age, however, were in good condition. This indicates that under the conditions of this investigation the lemon leaves are shorter lived than orange leaves.

The consistently lower values (figs. 1, 2) for the sap of lemon leaves as compared with that of Valencia and of Navel orange have previously been pointed out by Haas and Halma. ${ }^{(2)}$

## CHANGES IN INORGANIC CONSTITUENTS OF DRIED <br> LEAVES

We have seen that the changes in sap concentration are seasonal. It will be shown in this section of the paper that changes in the inorganic constituents are not seasonal but appear to be due largely to the age of the leaves.

As already mentioned, during 1928-1929 analyses were made of the sap after the freezing point was determined and also of dried leaves. These analyses include ash, calcium, magnesium and potassium and are given in tables 1 and 2 . In order to clarify the data, they are graphically represented by figures and an attempt will be made to point out the differences that exist.

The total ash as a percentage of dry matter (fig. 3) is similar for both varieties up to about April, when the lemon rises sharply until it reaches 25 per cent or more as compared with 17 per cent at the beginning. No such marked rise occurred in the orange.

The curves for the soluble ash as a percentage of dry matter (fig. 3 ) are parallel, with the lemon showing lower values than the orange.

TABLE 1
Inorganic Constituents of Eureka Lemon Leaves; Expressed as Percentages of Dry Matter

| Date | Total ash | Soluble ash | Soluble calcium | Total calcium | Soluble magnesium | Total magnesium | Soluble potassium | Total potassium |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1928 |  |  |  |  |  |  |  |  |
| August 21........... | 16.95 | 6.40 | 1.60 | 5.71 | 0.42 | 0.52 | 1.31 | 1.34 |
| September 13..... | 15.30 | 5.83 | 1.08 | 5.32 | . 38 | . 78 | 1.47 | 1.78 |
| October 1............ | 15.97 | 5.96 | 1.22 | 5.00 | . 35 | . 48 | 1.28 | 1.33 |
| October 18......... | 16.53 | 5.75 | 1.47 | 5.61 | . 38 | . 48 | 0.77 | 0.82 |
| November 6....... | 15.12 | 4.95 | 1.16 | 5.83 | . 37 | . 80 | 0.89 | 1.22 |
| November 19...... | 15.64 | 5.37 | 1.36 | 5.25 | . 39 | . 51 | 0.74 | 0.77 |
| December 17..... | 15.93 | 5.55 | 1.62 | 5.48 | . 38 | . 48 | 0.84 | 1.06 |
| 1929 |  |  |  |  |  |  |  |  |
| January 8........... | 15.89 | 5.64 | 1.41 | 5.23 | . 33 | . 45 | 0.81 | 0.86 |
| February 11. ...... | ......... | 5.32 | 1.74 | ..... | . 32 | ....... | 0.47 | ....... |
| February 26........ | 13.52 | 4.62 | 1.16 | 5.34 | 29 | . 66 | 0.73 | 1.03 |
| March 25............ | 15.62 | 6.29 | 1.84 | 5.33 | . 39 | . 48 | 0.61 | 0.84 |
| A pril 29.............. | 16.39 | 6.75 | 2.05 | 6.51 | . 35 | . 66 | 0.78 | 1.06 |
| May 29................ | 20.98 | 7.76 | 2.28 | 7.44 | . 44 | . 54 | 0.76 | 0.98 |
| July 8................. | 23.58 | 7.01 | 2.08 | 8.61 | . 37 | . 47 | 0.66 | 0.72 |
| July 23................ | 24.18 | 8.43 | 2.58 | 8.78 | . 47 | . 56 | 0.56 | 0.63 |
| August 12........... | 24.77 | 8.26 | 2.64 | 9.28 | . 50 | . 58 | 0.54 | 0.58 |
| August 27........... | 25.14 | 8.18 | 2.45 | 9.09 | . 49 | . 58 | 0.55 | 0.60 |
| November 5........ | 25.56 | 7.65 | 2.26 | 9.35 | 0.55 | 0.65 | 0.57 | 0.60 |

TABLE 2
Inorganic Constituents of Navel Orange Leaves; Expressed as Percentages of Dry Matter

| Date | Total ash | Soluble ash | Soluble calcium | Total calcium | Soluble magnesium | Total magnesium | Soluble potassium | Total potassium |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1928 |  |  |  |  |  |  |  |  |
| August 21........... | 19.23 | 10.57 | 2.65 | 5.89 | 0.73 | 0.84 | 0.91 | 1.11 |
| September 13..... | 13.03 | 7.05 | 2.12 | 4.57 | . 50 | . 64 | 1.12 | 1.36 |
| October 1. | 17.55 | 9.85 | 2.82 | 5.77 | . 45 | . 54 | 1.08 | 1.12 |
| October 18....... | 15.68 | 9.10 | 2.58 | 4.86 | . 49 | . 58 | 0.92 | 0.95 |
| November 6... | 15.99 | 9.68 | 2.95 | 5.42 | . 59 | . 70 | 1.07 | 1.33 |
| November 19...... | 17.35 | 9.40 | 2.70 | 5.78 | . 46 | . 54 | 0.96 | 1.02 |
| December 17...... | 16.28 | 9.24 | 2.71 | 5.29 | . 46 | . 57 | 0.86 | 0.91 |
| 1929 |  |  |  |  |  |  |  |  |
| January 8........... | 15.91 | 8.93 | 2.60 | 5.36 | . 43 | . 51 | 0.95 | 0.98 |
| January 11......... | 13.80 | 7.79 | 2.22 | 5.57 | . 36 | . 44 | 0.96 | 1.19 |
| February 26........ | 15.02 | 8.40 | 2.56 | 5.87 | . 36 | . 68 | 0.81 | 1.16 |
| March 25............. | 13.84 | 8.23 | 2.62 | 4.95 | . 36 | . 43 | 0.57 | 0.61 |
| April 29.............. | 13.67 | 8.02 | 2.57 | 4.88 | . 33 | . 39 | 0.92 | 1.16 |
| May 29................ | 16.43 | 9.60 | 3.18 | 5.72 | . 31 | . 37 | 0.76 | 0.96 |
| July 8................. | 16.33 | 9.81 | 3.13 | 5.65 | . 39 | . 46 | 0.75 | 0.78 |
| July 23................ | 17.81 | 10.07 | 3.29 | 6.26 | . 40 | . 48 | 0.65 | 0.70 |
| August 12........... | 18.01 | 10.25 | 3.32 | 6.10 | . 39 | . 47 | 0.74 | 0.75 |
| August 27........... | 18.32 | 10.82 | 3.59 | 6.33 | . 41 | . 50 | 0.76 | 0.78 |
| November 5........ | 19.29 | 11.44 | 3.62 | 6.69 | 0.47 | 0.55 | 0.97 | 1.01 |

This difference in solubility between lemon and orange has already been pointed out by Haas. ${ }^{(1)}$ Otherwise the general trend of the curves is similar to that of total ash as a percentage of dry matter.

The calcium and potassium may be considered together since they show a constant relationship. The soluble and total calcium as percentages of the dry matter (fig. 4) follow the same course as soluble and total ash as percentages of the dry matter (fig. 3). This indicates a close relation between calcium and ash. The orange contains


Fig. 3. Total ash and soluble ash as a percentage of dry matter in citrus leaves.
a greater percentage of soluble calcium than the lemon. The total calcium, however, shows no constant difference, but beginning about April the percentage in the lemon rises considerably above that in the orange, the differences becoming increasingly greater.

In contrast to the calcium, the soluble and total potassium as a percentage of dry matter (figs. 5, 6) show a downward trend instead of an upward trend; that is, as the season advances the values decrease. The soluble potassium does not show consistently higher values for the orange as is the case with soluble calcium.

The soluble magnesium as a percentage of dry matter (fig. 7) shows an initial downward trend followed by a weak recovery in the
case of the orange and a steady upward trend in the lemon. The first part of the curve shows lower values for the lemon, whereas the reverse occurs at the end. The total magnesium as a percentage of the dry matter (fig. 8) varies in a direction similar to that of the soluble mag-


Fig. 4. Total and soluble calcium as a percentage of dry matter in citrus leaves.
nesium but the fluctuations are much greater. For example, there are four distinct peaks in the lemon (fig. 8) which the soluble magnesium does not show.

The results obtained for the inorganic constituents of the ash of leaf sap are given in table 3. It will be seen that the ash in the sap


Fig. 5. Soluble potassium as a percentage of dry matter in citrus leaves.


Fig. 6. Total potassium as a percentage of dry matter in citrus leaves.


Fig. 7. Soluble magnesium as a percentage of dry matter in citrus leaves.


Fig. 8. Total magnesium as a percentage of dry matter in citrus leaves.

TABLE 3
Inorganic Constituents of Eureka Lemon and Navel Orange Leaf Sap

| Date | Ash, grams in 30 cc of sap |  | Calcium, per cent of ash |  | Magnesium, per cent of ash |  | Potassium, per cent of ash |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Lemon | Orange | Lemon | Orange | Lemon | Orange | Lemon | Orange |
| 1928 |  |  |  |  |  |  |  |  |
| August 21.. | 0.9810 | 1.9347 | 24.07 | 35.22 | 5.36 | 3.55 | 17.33 | 5.96 |
| September 13.... | 0.9133 | 1.1773 | 23.38 | 27.72 | 5.40 | 4.73 | 18.33 | 12.44 |
| October 1........... | 0.9526 | 1.5556 | 24.06 | 29.85 | 5.49 | 4.02 | 17.92 | 9.57 |
| October 18... | 1.0388 | 1.7376 | 25.74 | 31.09 | 6.54 | 4.14 | 12.31 | 8.49 |
| November 6..... | 1.0520 | 1.6536 | 26.79 | 32.18 | 6.63 | 4.21 | 11.25 | 8.54 |
| November 19...... | 1.0672 | 1.6661 | 27.72 | 35.35 | 6.84 | 3.99 | 10.12 | 8.14 |
| December 17...... | 1.1952 | 1.7754 | 31.11 | 32.51 | 5.25 | 3.97 | 10.39 | 8.42 |
| 1929 |  |  |  |  |  |  |  |  |
| January 8......... | 1.0710 | 1.7064 | 28.26 | 32.33 | 5.09 | 3.82 | 11.76 | 8.58 |
| February 11....... | 1.2503 | 1.6616 | 30.91 | 29.21 | 5.33 | 3.95 | 7.13 | 8.31 |
| February 26....... | 1.0890 | 1.8189 | 28.08 | 34.76 | 5.23 | 3.19 | 10.61 | 6.02 |
| March 25.......... | 1.4652 | 1.9923 | 29.11 | 27.74 | 5.35 | 3.63 | 7.03 | 6.28 |
| April 29............ | 1.5746 | 2.0716 | 28.74 | 29.18 | 3.80 | 3.28 | 8.95 | 5.74 |
| May 29.............. | 1.8644 | 1.8124 | 30.28 | 32.38 | 4.29 | 3.23 | 6.57 | 5.83 |
| July 8............... | 1.5398 | 1.7458 | 28.96 | 31.44 | 4.14 | 3.21 | 8.06 | 7.35 |
| July 23 ............... | 1.7410 | 2.0595 | 32.42 | 34.32 | 6.20 | 3.88 | 6.77 | 6.23 |
| August 12........... | 1.7629 | 1.9484 | 33.17 | 34.62 | 6.60 | 3.86 | 5.80 | 5.70 |
| August 27........... | 1.7257 | 1.9505 | 30.09 | 33.15 | 5.78 | 3.45 | 6.38 | 5.92 |



Fig. 9. Comparison of soluble calcium as a percentage of soluble ash in dried leaves, with calcium as a percentage of the ash of sap.
of both species increases as the leaves become older, the orange being consistently higher than the lemon. Toward the end of the year the ash of the lemon rises more rapidly with a resultant smaller gap between the two species.

Figures 9, 10, and 11 compare the analytical results obtained with dried leaves and sap. It is here assumed that the soluble inorganic


Fig. 10. Comparison of soluble potassium as a percentage of soluble ash in dried leaves, with potassium as a percentage of the ash of sap.
constituents as a percentage of soluble ash are comparable to the percentage of these same constituents in the ash of the leaf sap. In general, with the possible exception of magnesium, the curves are comparable; hence, there seems to be very little choice of methods. Whether the minor differences between the dried leaves and sap are significant can be ascertained only by more extensive investigations.

Attention may be called to the fact that so-called mature citrus leaves vary considerably in their inorganic composition; hence a
mature citrus leaf cannot be defined as having a rather definite inorganic composition. Perhaps a better basis of maturity is the ability of a stem, to which the leaves are attached, to initiate new growth when conditions are favorable.


Fig. 11. Comparison of soluble magnesium as a percentage of soluble ash in dried leaves, with magnesium as a percentage of the ash of sap.

## EFFECT OF YOUNG GROWTH CYCLE UPON PREVIOUS CYCLE

It will be recalled that during 1928-1929 leaves from two types of twigs of the same age were studied; one without and one with terminal growth. Tables 4 and 5 give the results obtained for the freezing-point depression and the inorganic constituents of leaves of these two types of twigs. It will be seen (table 4) that the lemon




* Sample A in each case, having no new terminal growth; sample B having new terminal growth.
leaves on twigs having terminal growth show slight, but consistently lower values for the freezing-point depression, ash, and calcium, but higher values for potassium. On the other hand, in orange leaves (table 5) only the ash shows these differences, the freezing-point depression, calcium and potassium values being irregular. The magnesium values were omitted from tables 4 and 5 since they fluctuated widely. If new growth develops partly at the expense of the cycle immediately back of it, then it may well be that the loss may occur either in the stems, or leaves, or both. Data given in table 6 suggest

TABLE 6
Effect of a New Growth Cycle on the Inorganic Constituents of Mature Navel Orange Stems; Expressed as Percentages

| $\begin{aligned} & \text { Date } \\ & (1929) \end{aligned}$ | Sample | Dry matter |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Ash | Calcium | Potassium | Magnesium |
| Jan. 5 | Stems without new growth.................. | 9.79 | 3.29 | 0.37 | 0.30 |
|  | Stems with new growth....................... | 7.35 | 2.44 | 0.48 | . 0.15 |
| Jan. 15 | Stems without new growth.................. | 11.40 | 3.86 | 0.41 | 0.38 |
|  | Stems with new growth.................. | 8.05 | 2.74 | 0.43 | 0.14 |

that in the case of the Navel orange the stem may be of more importance than the leaves as far as supporting new growth is concerned. While orange leaves (table 5) showed a reduction only in the ash, the stems also show a decrease in calcium and magnesium. The slight increase in potassium again suggests the inverse relation between calcium and potassium.

It is believed that the data represent a true cross section of the changes that occur throughout the year. In general they suggest a relationship between the inorganic constituents and the age of the leaves. It will be recalled that the lemon and orange trees from which the samples were taken, grew in the same locality but not in the same orchard. It is of special interest, therefore, that in figures 6 and 8 the fluctuations in the curves for both species occur on the same date. This condition cannot be explained on the basis of meteorological data such as temperature, wind velocity, sunshine, and humidity. Although the growth habits of the two species differ in many respects, certain phases of their nutrition may be similar, at any rate the coinciding fluctuations cannot be ascribed to mere chance.

## SUMMARY

Sap concentration of mature leaves of Eureka lemon, Navel and Valencia orange is related to seasonal changes in soil and air temperatures.

The sap of lemon leaves is consistently more dilute than that of orange but the fluctuations coincide.

Inorganic constituents of dried citrus leaves and of sap seem to be related to the age of the leaves rather than to environmental changes.

There are indications that a new growth cycle draws on the inorganic supply of the subtending growth cycle.

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The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:
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5. Citrus Blast and Black Pit, by E. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
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