

PREPARATION OF AVOCADO LEAVES FOR CHEMICAL ANALYSIS OF NUTRIENT ELEMENTS

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Leaf analysis is practiced nowadays as a means of studying the nutritional status of many plant species. For avocados, leaf sampling procedures have been developed (2). After the sampled leaves are picked from the trees, they must be transferred as soon as possible to the laboratory, where they are cleaned and washed thoroughly to free them of dust or any other surface contamination.

Various cleaning methods are being used for leaves of different plant species (1). The method usually depends on the characteristics of the leaf surface; smooth, rough, or covered with warts or hair. Rough-surfaced leaves tend to absorb dust or any foreign particles much more than smooth-surfaced leaves. Sometimes the leaves are contaminated with residues of various spray materials such as insecticides, pesticides or certain foliar-applied nutrients. Although such leaves are usually not sampled for chemical analysis, a short time after spraying, some materials may adhere to the leaves for quite a long time, and in such cases it is necessary to remove them by a thorough washing of the leaves, sometimes with the aid of a diluted acid or various detergents.

The elements to be analyzed play, of course, a decisive part in the choice of a method by which to clean the leaves. If these are major elements such as nitrogen, calcium, potassium, etc., a small surface-absorbed quantity of these elements may not change the results to a great extent. If, however, micronutrients such as zinc, copper, etc., which are usually present in the leaves in very minute quantities, are being analyzed, even the slightest contamination may affect the results considerably. In such cases, the leaves require a thorough cleansing. It must be kept in mind, though, that too long immersion of the leaves in water or in a washing solution may cause various elements to leach out from the leaves and thus render the results of analysis unreliable (3). The question is, what, under normal conditions, would be the best method of washing the leaves so that all surface-absorbed materials would be removed without causing the leaching of nutrient elements from inside the leaves. In order to answer this question, two experiments were carried out with various methods of cleaning and washing of avocado leaves. These methods and their results are described in the present article.

Materials and methods

FIRST EXPERIMENT

In October 1963, leaf samples were taken from Fuerte trees growing in a 10-year-old avocado orchard in which most trees suffered from salinity. From each tree were taken 15 healthy complete leaves (blade and petiole) of the spring flush from the northern side, at a height of 5-6 ft. Altogether, 360 leaves were sampled in this experiment.

The leaves were brought to the laboratory and divided into three treatment groups of 120 leaves each. To each group of leaves, one of three methods of washing was applied, as follows:

- a) Rinsing under running tap water, then washing twice in de-ionized water;
- b) Rinsing under running tap water, then washing in 0.25% "Ama" detergent solution, and washing twice in deionized water;
- c) Rinsing under running tap water, then washing in 0.25% "Ama" detergent plus 3% HCl; solution and washing twice in deionized water.

After washing, the leaves of each group were divided into six subgroups (replications) of 20 leaves each, for the chemical analysis. All leaves were then oven dried for 24 hours at a temperature of 65°C, ground to powder by means of a Wiley mill through a 40-mesh screen. The powder of each subgroup was kept in a sealed plastic vial for the chemical analysis of 12 elements.

SECOND EXPERIMENT

On June 5, 1968, leaf samples were taken from 20 Fuerte trees, 11 years of age, growing in an experimental avocado orchard of The Volcani Institute of Agricultural Research. From each tree we sampled 25 healthy, complete leaves (including blade and petiole), about 6 months of age, from the northern side of the tree at a height of about 5-6 ft. Five hundred leaves were sampled in this experiment.

After reaching the laboratory, all the leaves were thoroughly mixed together and then divided into five treatment groups of 100 leaves each. The leaves of each group were then cleaned or washed by one of the following methods:

- a) Wiping the leaves with cotton moistened in deionized water.
- b) Slight rinsing under running tap water, then dipping once in tap water, and twice in deionized water.
- c) Slight rinsing under running tap water, then washing once in 0.25% "Ama" detergent solution and twice in deionized water.
- d) Slight rinsing under running tap water, then washing once in 0.25% "7X" detergent solution and twice in deionized water.
- e) Slight rinsing under running tap water, then washing in 0.25% "7X" plus 3% HCl solution.

After washing, the leaves of each group were divided into ten subgroups of ten leaves each, which comprised ten replications for the chemical analysis. All leaves were then oven dried for 24 hours at a temperature of 65°C, and ground to powder by means of a

Wiley mill through a 40-mesh screen. The powder of each subgroup was kept in a sealed plastic vial for the chemical analysis.

The following is a brief description of the methods used for the chemical analysis of 12 elements:

Nitrogen (total): Wet burning in microkjeldahl, colorimetric reading of the complex ammonia-phenol-hypochloride (in a "Technicon" autoanalyzer).

Chlorine: Extraction in nitric acid and potentiometric microtitration by means of an Ag-AgCl electrode (with a Metrom potentiometer).

Dry ashing at 550°C for the following elements:

Potassium and sodium: Reading by means of a flame photometer (E.E.L.)

Calcium and magnesium: Microtitration with versenate with calcein indicator for Ca, and Eriochrome black for the sum of Ca + Mg.

Phosphorus: Colorimetric reading with Molybdate-Vanadate (by means of a "Bausch and Lomb" spectrophotometer).

Iron: Colorimetric reading with phenantrolin (B.L.)

Manganese, zinc and copper: Reading by atomic absorption ("Unicam").

Boron: Ashing separately with CaO and colorimetric reading by the Carmín method.

Results and conclusions

Table 1: Mean values for each of 12 analyzed elements (each number represents a mean value of six replications).

Table 2: Mean values for each of 12 analyzed elements (each number represents a mean value of ten replications).

From the results presented in the two tables it can be seen that for most elements not much difference exists between the various treatments applied. In Table 1, Ca shows a significant reduction due to the use of detergent (tr. b) or detergent with acid (tr. c) as compared with water (tr. a). The Ca contamination of the leaves was probably due to the dust from the calcareous soil on which the orchard was planted. In Table 2, phosphorus showed significantly higher values in the two treatments where 7X detergent was used (trs. d and e) than in the other three treatments (a, b, c). This is explained by the fact that 7X detergent contains phosphorus. It is also possible that it contains traces of copper, which may explain the higher amount of Cu in the leaves cleansed with 7X.

Most striking is the highly significant reduction of the iron content in both tables in those treatments where detergents with or without acid were used (treatments b and c in Table 1 and c, d and e in Table 2). The explanation for this could be that dust particles from soils containing iron oxides adhere to the leaf surface and are removed much more thoroughly with detergent or acid solutions than with water.

TABLE 1

<i>Treatment</i>	<i>a</i> <i>Water wash</i>	<i>b</i> <i>Ama-detergent wash</i>	<i>c</i> <i>Ama + HCl wash</i>	<i>Significance</i>
<i>In dry matter %</i>				
N	1.41	1.37	1.44	N.S.
P	0.10	0.10	0.10	N.S.
K	0.81	0.88	0.90	N.S.
Ca	2.19	2.04	1.93	**
Mg	0.77	0.75	0.73	N.S.
Na	0.050	0.054	0.049	N.S.
Cl	0.70	0.68	0.60	N.S.
<i>ppm</i>				
B	51.0	54.5	52.3	N.S.
Fe	78.5	53.0	53.0	**
Zn	34.0	41.0	32.5	**
Cu	33.0	23.0	39.0	N.S.
Mn	116.5	116.5	115.0	N.S.

** Significant at the 1% level.
N.S.: Not Significant at the 5% level.

TABLE 2

<i>Treatment</i>	<i>a</i> <i>Wet cotton wipe</i>	<i>b</i> <i>Water wash</i>	<i>c</i> <i>Ama-detergent wash</i>	<i>d</i> <i>7X-detergent wash</i>	<i>e</i> <i>7X + HCl wash</i>	<i>Significance</i>
<i>In dry matter %</i>						
N	1.92	1.97	1.93	1.98	1.93	N.S.
P	0.131	0.124	0.131	0.146	0.140	*
K	1.11	1.18	1.12	1.21	1.13	N.S.
Ca	1.40	1.38	1.43	1.30	1.37	N.S.
Mg	0.63	0.64	0.63	0.60	0.59	N.S.
Na	0.006	0.008	0.005	0.005	0.006	N.S.
Cl	0.461	0.458	0.465	0.447	0.447	N.S.
<i>ppm</i>						
B	49	48	45	43	43	N.S.
Fe	114	78	62	60	58	**
Zn	21.6	23.5	20.0	22.1	21.7	N.S.
Cu	13.5	12.0	10.0	14.7	17.0	**
Mn	75.6	64.6	73.0	74.0	75.0	N.S.

* Significant at the 5% level.
** Significant at the 1% level.
N.S.: Not significant at the 5% level.

In conclusion, it can be said that under normal conditions, if the leaves are not too contaminated, a good rinse with tap water and washing with deionized water may be sufficient to enable one to obtain reliable results from leaf analysis of most elements. This is actually the method practiced in our laboratory for analyzing avocado leaves, except for specific cases where highly accurate results are needed for the study of microelements such as iron.

LITERATURE

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