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# Leaf Sap Concentration and Cold Resistance in the Avocado

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Harris and Popenoe (2) reported in 1916 that the leaf sap of avocado varieties belonging to the West Indian race was less concentrated than that of varieties belonging to the Guatemalan and Mexican races. These results, they pointed out, agree with horticultural experience in that the West Indian avocado is less resistant to cold than the other two races. They found, however, no consistent differences in the freezing point depression between the leaf sap of the Guatemalan and Mexican races, although varieties of the latter are much more resistant to cold than varieties of the former. Moreover, there are varietal differences in cold resistance within all three races.

Harris and Popenoe were aware of the complexity of the problem, but thought it highly probable that a thorough knowledge of the freezing point lowering of leaf sap would be of some service in predicting ability to withstand cold. This report deals with an attempt to explore this probability.

All commercial varieties in California belong to the Guatemalan or Mexican races; a third group whose members have characters intermediate between the two are referred to as hybrids. The most important variety in California, the Fuerte, belongs to the latter. Varieties of the West Indian race are unsuited climatically to California conditions.

As to the botanical classification there is disagreement. Some botanists place the West Indian and Guatemalan under one species, *Persea americana*, *Mill.*, and the Mexican under another species, *P. drymifolia*, *Cham.* & *Schlecht*, while others refer to all three races as *P. americana*. The latter view is shared by Popenoe (4) who believes that there are insufficient grounds for separating the three races botanically.

# MATERIALS AND METHODS

In general the procedure was the same as that previously followed for citrus by Halma (1) which in turn was based on Walter's recommendation (5). At 2 weeks intervals between May, 1938 and April, 1940, the osmotic value (Ov) and the per cent of total soluble solids of the leaf sap were determined for the following varieties: Mexican race — Puebla, Mexicola, Duke, and Topa Topa; Guatemalan race — Nabal, Dickinson, and Benik; Hybrid — Fuerte. These varieties are growing in the orchard at the University of California, Los Angeles. All were of bearing age but their bearing behavior differs, some fruiting yearly and others irregularly. All are on Mexican seedling rootstock.

Care and experience are essential in the collection of the leaves. The time of the day, location on the tree, condition and age of leaf are factors which affect the sap concentration. For this reason leaves of comparable age were gathered between 7:45 and 8:15 a.m. from the west side of the tree which, at that time of day, had not received direct sunshine. A sample consisted of from two to four leaves, depending upon their

size. They were wrapped immediately in damp cloth to prevent water loss. The green weight of the samples ranged from 8 to 14 grams, and the amount of sap obtained from the killed leaves varied from 3 to 5 cubic centimeters. After removing surface moisture and dust each sample was placed in a vial 108 millimeters in height, 27 millimeters in diameter, capacity of 40 milliliters, and provided with a black, molded screw cap. A rubber washer (cut from a discarded automobile inner tube) was placed inside the cap to provide an air-tight fit.

The leaves were killed with heat by placing the vials in a covered vessel which contained sufficient water to cover the vials to the lower edge of the screw cap. The water was heated before immersing the vials and then brought to a boil rapidly. The leaves were kept in this atmosphere of boiling water for 30 minutes, then taken out and allowed to cool for at least 3 hours before making the determination. As previously pointed out by Walter (5), Mallory (3), and Halma (1), the main advantage of killing leaves by heat is that they can be stored without significantly affecting the Ov of the sap. Leaves killed by cold are unstable, thus if avocado leaves are killed by cold (solid carbon dioxide), their Ov may increase from 2 atmospheres after a 3-hour storage at room temperature (between 70 and 80 degrees F) to 5 atmospheres after 72 hours. This change is less rapid when the leaves are kept at 40 degrees F. If killed by heat, the Ov of the sap of comparable leaves may show a plus or minus, variation of 1 atmosphere.

Each sample, wrapped in a piece of strong muslin cloth 10 square centimeters, was placed between two steel plates held by a 6-inch vise. By gradual increase in pressure a good extraction was obtained. No tests as to the effect of pressure on the Ov were made, but on the basis of previous investigation with citrus (1), it was assumed that the avocado behaves similarly; namely, that with small samples there is no consistent difference between the Ov of the sap obtained with a 9000 pounds per inch pressure and the Ov of sap obtained with a 6-inch vise.

The freezing point was determined with a Drucker-Burian micro-thermometer. This thermometer has a fixed scale ranging from 0.5 to -5.5 degrees C in divisions of 0.02 degrees. The entire length of the thermometer is only 28 centimeters. The mercury column cannot be set for various ranges in temperature depressions as it can be in the Beckmann thermometer. With an ordinary hand lens the temperature can be read to 0.01 degree C, which is sufficiently accurate for plant material. The thermometer scale is too short to determine the total amount of undercooling; hence it is necessary to arrest the undercooling by quickly introducing and withdrawing a glass capillary or a piece of platinum wire containing particles of frozen distilled water and then stirring the sap rapidly.

The brine temperature was kept between -8 and -10 degrees C. 2 cubic centimeters of sap were used which allowed complete immersion of the thermometer bulb without touching the bottom of the tube. The undercooling was arrested at approximately 1 degree below the actual freezing point of the sap. The values obtained were translated into atmospheres.

An additional index of the physico-chemical characteristics was obtained by determining the per cent of the total soluble solids by means of a refractometer.

#### DISCUSSION OF THE DATA

Before discussing the data it is necessary to refer briefly to the relative cold hardiness of the eight varieties included in this investigation. On the basis of observations made after the severe freeze of January, 1937, the range in cold hardiness is about 10 degrees F with Duke, and probably Mexicola, the hardiest, and Benik the least hardy. The range in hardiness among members of the Mexican race is greater than that among Guatemalan varieties. Thus the difference between Duke and Puebla is about 7 degrees F. Topa Topa, one of the Mexican varieties, needs special mention. Information concerning it and Mexicola are meagre due to the fact that they are used only for rootstock purposes. While there is little doubt concerning Mexicola, the status of Topa Topa is not certain. It was considered very hardy until one observer reported after the 1937 freeze that a tree of this variety suffered as much injury as an adjacent Puebla.

Puebla and Fuerte, the latter considered a Mexican-Guatemalan hybrid, are about equal in hardiness. The range between these two varieties on the one hand, and the three Guatemalan on the other, probably does not exceed 4 degrees F. Nabal seems to be slightly hardier than Dickinson, but Benik is definitely the most tender.

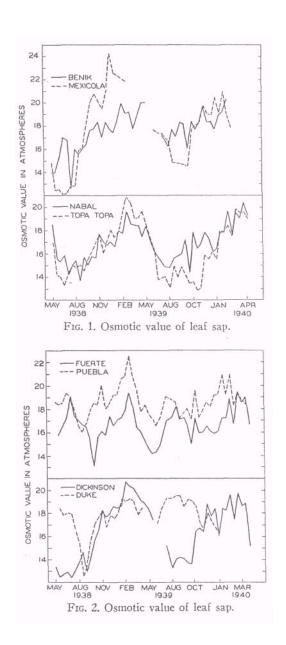
Assuming the existence of a relationship between cold hardiness and leaf sap concentration, as implied by Harris and Popenoe, one should find a higher Ov for a hardy variety like Duke than for a relatively tender one like Benik. However, the data do not support this assumption. In each graph (Figs. 1 and 2) a Mexican variety is compared with a Guatemalan, except in one case where Fuerte is compared with Puebla. The gaps in the graphs for Duke, Mexicola, Dickinson, and Benik are due to lack of suitable leaves; it seems that leaves of these varieties are shorter lived than those of the others used in this investigation.

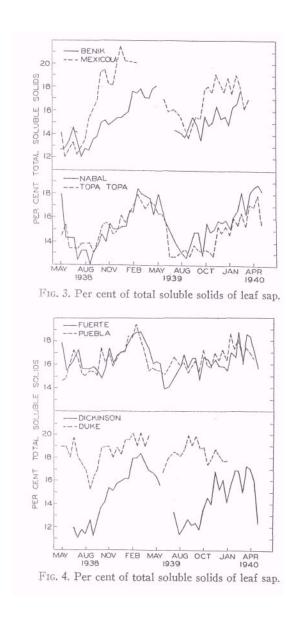
Attention should be focused on the period between November and March when low temperatures are most likely to occur. The graphs indicate that during this period there is no consistent relationship between cold hardiness and Ov. Thus, during the 1938-39 winter the Ov for Mexicola is higher than that for Benik, but the values during the following season are practically the same. Dickinson, which is relatively tender, shows values similar to those of the hardy Duke, and there is practically no difference between Nabal and Topa Topa. Finally it should be noted that Puebla values are higher than those for Fuerte during both seasons, a condition which does not agree with the observed similarity in hardiness of the two varieties.

The percentages of total soluble solids (Figs. 3 and 4) are suggestive. With only one exception, Topa Topa, the values are higher for the hardy varieties than those for the relatively tender ones. The consistently higher values for Duke and Mexicola as compared to Dickinson and Benik, together with the similarity in values between Puebla and Fuerte, are in accordance with field observations on cold hardiness.

## CONCLUSION

Leaf sap concentration studies of eight Avocado varieties covering a period of 2 years, revealed no relationship between the osmotic value of expressed leaf sap and cold hardiness. Percentage of total soluble solids, however, were, in most cases, consistently higher for the hardy varieties than for the relatively tender ones.





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