pH DETERMINATION IN PLANT TISSUE

A. R. C. HAAS

In a recent investigation[1] a procedure was found that permitted fairly accurate determinations of the pH of soils of low moisture percentages. One of the essentials of the method was the use of a horn spoon in spreading the moisture or soil films uniformly throughout the rather dry soil mass.

When plant tissue is finely cut in a grinder, relatively few of the cells are actually opened. The important fact is that the contents of the cut cells is smeared over the uncut groups of cells. On account of the somewhat analogous nature of the state of the soil and of the finely divided plant tissue, it appeared highly probable that the same methods that were applied in the determination of the pH of soils could be applied equally as well in the pH determination of plant tissue.

Materials and methods

The pulp of date fruits, the peel of lemon fruits, and the leaves of avocado seedlings furnished the material for the tests. A Beckman pH meter (model g) with a shielded glass electrode, and ten-foot extension cables attached to both electrodes, served as the means of measuring the pH.

The date fruits were wiped clean without being previously washed. The lemons and the avocado leaves were washed in running distilled water and then wiped dry. The date fruits were cut with a knife in order to discard the seed. The lemon fruits were peeled and the pulp was discarded, care being taken in the peeling process to avoid piercing the bounding membranes of the acid pulp. The samples were finely cut by means of a Universal food chopper, no. 3, making use of the finest of the four cutters. The chopper was used in place of the Wiley mill because of the relative ease in the washing and drying of the chopper, although most any cutting machine may be used. With very woody material the mill is most useful, although pencil sharpeners or whittling with a knife may be usefully employed.

The pH was determined immediately after each lot of material was cut in the chopper. The same sample of finely cut plant tissue that was used in the determination of pH was at once placed in a wide-mouthed glass jar that was tightly closed by means of a glass cover with suitable rubber gasket and metal clamp. As each sample was finished it was temporarily stored in a refrigerator until the group of samples was completed. Refrigeration was then obtained by storing the jars overnight in an underground freezing room of a cold storage company. The following day the jars were taken to the

[1] HAAS, A. R. C. The pH of soils of low moisture content. Soil Science (In press).

laboratory where, after thawing the frozen plant tissue, the juices were expressed and the pH values determined in the extracted juices. Twenty thousand pounds pressure was used for the sap extraction from the date pulp and avocado leaves, while fifteen thousand pounds was used for the sap extraction from the lemon peel.

After the plant tissue was finely cut with the chopper it was thoroughly mixed in a shallow, wide glass dish. The juice from the cut cells was uniformly spread throughout the mass by means of a horn spoon which was used in the same manner as a spatula is used in mixing ointments. Heating of the material was overcome by working rapidly and by spreading out the material immediately after it emerged from the chopper. With some materials brief refrigeration may be of assistance in this regard. The cut tissue is placed in a beaker which is tapped on a folded towel on the table top. With the hand or any suitable tool, the tissue is well compacted within the beaker and the electrodes and thermometer are quickly inserted.

Both electrodes were held in an electrode holder provided with a spring clamp on a metal support. Upon squeezing the clamp the electrodes could be raised or lowered. The electrodes were pressed into the cut tissue and the surface tissue was firmly compacted about the electrodes in order to cover the unshielded portion of the glass electrode. A sufficient depth of well compacted tissue should occur between the bottom of the beaker and the sensitive part of the glass electrode. This material acts as a bumper or buffer in preventing the glass electrode from being broken against the bottom of the beaker. These electrodes are supposed to withstand pressures of thirty-five pounds and probably will withstand much higher pressures. In the inserting of the glass electrode into soils,[1] whenever care was taken to avoid contacting the bottom of the beaker, these electrodes withstood any slow steady pressures that could be applied with the hands.

After the temperature adjustment of the pH meter was made, the pH readings of the tissue were made. The electrodes then were more firmly pressed into the tissue and the surface tissue again was tightly compacted about the electrodes. Thus, pH readings were made a second time. This repetition or renewal of the electrode pressures and the surface compacting about the electrodes with the accompanying pH readings was continued until three successive repetitions of the process showed no appreciable change in the pH readings. This insures that the contact between the juice films on the tissue and the glass electrode is the most intimate that is obtainable.[1]

The sample of tissue was then removed as described and after being frozen, the sap was extracted under pressure. The pH of the juice was then determined and compared with that of the finely divided and freshly cut, but unfrozen, plant tissue.

Results

pH OF CUT FRESH FRUIT TISSUE AS COMPARED WITH THAT OF THE JUICE OF THE FROZEN TISSUE

A total of 100 unripe green dates were collected at Indio on July 24, 1939; the pulp weighed 158 grams and the seed 19 grams. The peel of seven ripe healthy lemons was used in the test with lemons. Table I shows the

TABLE I

pH OF FRESHLY CUT PLANT TISSUE AND OF THE SAP OBTAINED FROM THE FROZEN TISSUE

SAMPLE NO.	FINELY CUT FRESH PLANT TISSUE	JUICE EXTRACTED FROM FROZEN PLANT TISSUE
	DEGLET NOOR DATE PULP	
	pH	*pH*
1	5.55	5.45
2	5.65	5.47
3	5.55	5.41
4	5.60	5.50
5	5.62	5.50
Distilled water	5.27	
	PEEL OF RIPE HEALTHY LEMONS	
1	5.30	5.31

agreement between the pH values of finely cut fresh fruit tissue and those of the sap extracted from these tissues after being frozen. With date pulp which is very high in sugars there should be considerable opportunity for chemical changes to take place after the pulp is cut and yet the results were most promising, even in these preliminary tests.

pH OF FINELY CUT FRESH AVOCADO LEAVES AND OF THE JUICE EXTRACTED FROM THE TISSUE AFTER FREEZING

This experiment should not only serve to reveal whether the pH values of cut fresh tissue differ from those of the sap of the same sample of tissue after freezing, but should also indicate the effect, if any, of the pH of the soil upon that of the sap in plant leaves.

Hanford soil (pasture soil), obtained near the Citrus Experiment Station, was used in these cultures. An avocado seed was planted in each container of soil. The containers were six inches in diameter by seven inches high and contained 4000 grams of air-dry soil. Distilled water was used at all times. The cultures each received a total of 1.0947 grams of nitrogen applied in the form of calcium nitrate solution. This nutrient was divided into three equal applications to the soil during the growth of the cultures from May 1, 1939, to June 5, 1940. Various amounts of sulphur (table II) were applied to the surface of the soil after the tops were several inches high.

TABLE II

RESULT OF COMPARATIVE METHODS OF DETERMINING THE pH OF AVOCADO LEAF SAP OF PLANTS GROWN IN SOILS OF DIFFERENT pH

SULPHUR ADDED TO THE SURFACE OF SOIL CULTURES	SOIL SAMPLES AT END OF THE EXPERIMENT		MOISTURE PERCENTAGE OF THE SOIL AT END OF THE EXPERIMENT	FRESH WEIGHT OF LEAVES	FRESH WEIGHT OF TRUNK	FRESHLY CUT AVOCADO LEAVES	JUICE EXTRACTED FROM FROZEN AVOCADO LEAVES
	pH AT THE CULTURE SOIL MOISTURE PERCENTAGE	pH OF SUSPENSION OF OVEN-DRIED SOIL 1-5 SOIL-WATER RATIO					
gm.	*pH*	*pH*	%	*gm.*	*gm.*	*pH*	*pH*
0	6.42	6.86	10.1	55	64	5.45	5.47
0.05	6.53	6.72	10.3	85	101	5.47	5.50
0.10	6.49	6.84	11.8	69	90	5.41	5.44
0.50	5.90	6.37	11.0	86	132	5.40	5.40
1.00	5.26	5.95	8.2	88	106	5.43	5.42
1.50	4.82	5.42	6.9	98	104	5.55	5.57
2.00	4.72	5.04	9.1	91	104	5.40	5.37
2.50	4.39	4.83	6.8	89	99	5.63	5.70
3.00	4.21	4.55	6.6	98	102	5.80	5.98
3.50	4.31	4.46	6.8	65	77	5.63	5.61
4.00	4.02	4.24	10.6	16	36	5.65	5.76
4.50	3.80	4.10	10.6	Leaves dead	30		
5.00	3.75	4.11	11.7	Leaves dead	31		
	Soil samples divided into upper and lower halves						
0	6.10	6.70	8.0				
	7.27	7.38	8.1				
0.05	5.85	6.60	5.9				
	6.70	7.27	5.8				
0.10	6.05	6.66	7.8				
	6.82	7.24	8.3				
2.00	5.06	5.31	4.5				
	5.14	5.20	5.4				

Soil samples were obtained when the experiment was terminated. Six cores of soil, taken the full depth of the container, were used for the pH determination at the culture soil-moisture content. When oven dried, these soil samples were used to determine the culture soil-moisture percentage at the time of sampling and were also used for the pH determination of soil suspensions at the 1 to 5 soil-water ratio. A few days after the soil sampling, additional samples in a few containers were taken at the upper and the lower three-inch depths.

Table II shows the pH values of the soil when the experiment was concluded. In every case the pH values obtained in suspensions at the 1 to 5 soil-water ratio exceed those determined at the culture soil-moisture percent-

age occurring at the termination of the experiment. The pH at this latter moisture content more nearly represents the pH that affects the growth of these plants than does the pH at the 1 to 5 soil-water ratio.

In the soil samples (table II) taken from the upper portion of the soil mass, the pH at the culture soil-moisture percentage was less than that of the lower or deeper portion of the soil mass. Any acidity produced by oxidation of the sulphur or by the use of the distilled water (pH 5.30) brings acidity to the upper portion of soil before that to the lower or deeper portion.

Where no sulphur was applied the growth was poor. Even the smallest application of sulphur greatly improved the growth. With a sulphur application above 3 grams, growth was retarded, while at the 4.5- to 5-gram application, the leaves were dead and remained attached to the trunk which was alive.

Table II shows the range of soil acidity at the termination of the experiment. At or below pH 4.00 the growth was seriously retarded. The pH values for the leaves by the two methods of preparing the tissue agree very well. It is of interest that at the pH values of the soil (determined at the culture soil-moisture percentage) above pH 4.50, the pH values of the leaf tissue or juice are all approximately the same. Below this soil pH value, the pH readings for the tissue, or its juice, show a very slight tendency to be higher. Why the reaction of the tissues or their juices should tend to be less acid while the reaction of the soils are more acid, will require further study. At any rate, at the pH values which are comparable to those already found in orchard soils, the pH of the leaf tissue is notably constant.

In tables I and II it will be seen that the pH of the leaf or fruit tissue experimented with, was close to 5.5. Other pH determinations, for example those of the juice of the pulp of citrus fruits, are very much lower than this. With tissues other than these, as for example in the tissues of roots, very low pH values were also encountered. These pH determinations were made by whittling the root into many shavings which were treated in the manner described for cut fresh tissue. This technique should prove most useful for tissues from which it is difficult to obtain much juice, such as woody twigs, and for those tissues in which high air-suction or pressures are being avoided.

Summary

The method used for the pH determination in soils of low-moisture content was found to be equally applicable to plant tissue. The agreement between results obtained in this manner and those obtained with juice extracted from frozen tissue was most satisfactory. Over a wide range of pH in the soil of avocado cultures, no significant change in the pH of the leaf juice was noted.

CITRUS EXPERIMENT STATION
UNIVERSITY OF CALIFORNIA