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COMPARISON OF THE CALCIUM AND SULPHUR CONTENT OF PLANT FLUIDS EXPRESSED FROM TISSUES KILLED BY AUTOCLAVING AND BY FREEZING WITH SOLID CARBON DIOXIDE

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Sap analysis in combination with analysis of the complete tissue is particularly useful in gaining information concerning the distribution of elements between fluids and solids of a given tissue and the relative availability of elements for conditioning metabolic processes of the plant.

Two methods, killing by heat and killing by freezing with solid carbon dioxide, are readily adapted to the pretreatment of plant tissues in the field for subsequent studies on the expressed sap. Both procedures have been used but few comparisons of results of mineral analyses of sap obtained by the two methods have been published. Results reported in this paper are intended to call to the attention of investigators of plant tissues the possibility of obtaining expressed fluids of different composition depending upon the methods of pretreating the materials. Particular emphasis is placed on the calcium and total sulphur of such saps because these two constituents showed the greatest change as a result of the type of pretreatment for the plants and elements studied. With other plants, other constituents may differ.

BROYER (1) recently reviewed methods for preparing tissues for analysis in physiological studies with plants, but most of the investigators he cited have been primarily interested in the osmotic concentration, the carbohydrate content, or the nitrogen fractions of the expressed saps. Freezing of plant tissues at low temperatures prior to expression of the sap has been studied and used by BROYER and HOAGLAND (2), MASON and PHILLIS (6), GREATHOUSE (4), MEYER (7), and others.

DONEEN (3) found no difference in the freezing point depression, total solids, total sugars, or nitrate-nitrogen in the sap of small wheat plants as a result of freezing or heating. A decrease in the calcium content of sap as a result of heating has been noted by ILJIN (5) for several different plants. While studying the "partition indices" of potassium, magnesium, and calcium in cotton leaves, PHILLIS and MASON (9) noted a difference in the concentrations of potassium and calcium between sap expressed from frozen and from boiled leaves and attributed this to the occurrence of base exchange during boiling.

Experimental technique

Tissues from cotton and tomato plants grown in nutrient solutions containing added chloride and sulphate salts were chopped into pieces approximately one centimeter or less in length, mixed thoroughly, and divided into equal parts. Each portion was sealed in a pint glass fruit jar. One series was placed in contact with "dry ice" for 16 to 20 hours in a small refrigeration box and then stored in a cold storage locker at 6” to 8” F. until time of analysis. The other series was autoclaved immediately at 15 pounds pressure for 15 minutes. After thawing, the frozen samples were subjected to a pressure of 2,000 pounds per square inch in a Carver Press. Identical pressing technique was used on all samples. The expressed juices were centrifuged at 1500 r.p.m. for 5 minutes.

TABLE I

CALCIUM AND TOTAL SULPHUR ANALYSES OF PRETREATED PLANT TISSUES

SAMPLE DESCRIPTION	MILLIEQUIVALENTS PER LITER OF SAP			
	CALCIUM		TOTAL SULPHUR AS SULPHATE	
	FROZEN	AUTOCLAVED	FROZEN	AUTOCLAVED
	m.e./l.	m.e./l.	m.e./l.	m.e./l.
Cotton leaves				
1	263	208		
		200		
2	251	230		
3	241	192		
4	381	211	183	135
	388	212	194	132
5	397	223	164	106
		214		108
		211		103
6	421	227	167	103
7	427	249	154	94
	457	249	155	97
8	352	273	236	207
	330	248	233	197
Tomato leaves				
A-1	64	57	148	129
A-2	56	56	155	132
A-3	45	43	179	129
A-4	25	27	183	165
B-2	49	51	116	103
B-3	49	46	176	119
B-4	30	35	182	163

Results

Table I presents the calcium and total sulphur analyses of expressed saps from cotton and tomato tissues which were pretreated by freezing and by autoclaving. Duplicates shown represent portions of the original material carried through the entire procedure including the pretreatment. Standard analytical procedures were used.

Autoelaving before pressing cotton leaves yielded a sap which had less calcium and less total sulphur than the sap from leaves killed by freezing. The total sulphur content of sap expressed from tomato leaves and young stalks was likewise lower from the autoclaved than from similar frozen tissues. The calcium content, in this latter case, was not affected by the method of pretreatment and was lower than that from the cotton.

Other constituents (Mg, K, Na, and Cl) were also determined but are not shown since differences due to treatment were generally small and not conclusive. Total chlorine content of the saps obtained by the two methods agreed closely. This indicates that there were no concentration changes which could be accounted for by a differential loss of water as a result of the type of pretreatment.

Table II shows the partial composition of sap expressed from cotton leaves subjected to two additional treatments. One set of samples was

TABLE II

EFFECT OF TREATMENT OF COMPOSITION OF SAP OF COTTON LEAVES

Treatment	Milliequivalents per liter of sap		
	Calcium	Total Sulphur	Total Chlorine
Frozen	352 350	236 233	20 19
Autoclaved	261 244	195 185	19 20
Frozen and autoclaved	273 248	207 197	19 19
Frozen, expressed and autoclaved	229 217	168 168	20 20

frozen as previously described ; a second set was autoclaved ; a third set was frozen and then autoclaved; and the fourth set was frozen and thawed, the sap expressed and centrifuged, and then autoclaved. All saps were centrifuged and analysis started immediately after expression. When the frozen sap was autoclaved, a coagulated mass settled to the bottom of the container. This coagulum was removed during the centrifuging and when analyzed was found to be high in both calcium and total sulphur.

Discussion

Killing by any method whatsoever irreversibly alters the plant cells. The semipermeability of the protoplasmic membranes is destroyed, dissipating the concentration gradients previously set up within the cell and between adjacent cells. Soluble constituents of the protoplasm may then mix with constituents of the vacuole and those from one cell with those of other cells which may be quite different histologically and in their accumulated salts. With the redistribution of ions within and between cells, some insoluble salts may go into solutions, some salts may be precipitated, and a new exchange equilibrium be established. Fluids expressed from such tissues may then contain quantities of certain ions which are different from the total soluble amounts present in the uninjured cells.

Many organic colloids exert a protective action in preventing the precipitation of compounds whose solubility has exceeded that found for simple solutions. Some similar type of mechanism probably operates within the plant. Evidence shows that heating coagulates most all plant proteins but that freezing does not appear to alter irreversibly their dispersibility in the tissue fluids. Any coagulation which occurs destroys the protective action of these organic colloids and in this way could cause precipitation within the tissues of compounds which formerly were protected.

Coagulation of proteins may reduce the mineral content of the sap by the amount present in the proteins. The relative importance of this effect will depend upon the percentage of the mineral associated with the protein. Besides sulphur and phosphorus, calcium, magnesium, and sodium may also be combined with the protein.

ILJIN (5) suggested that calcium citrate may be precipitated by heating and thus lower the calcium content of saps pressed from heat-killed plants. Data for cotton given in tables I and II show reduction in total sulphur as well as calcium. This suggests that calcium sulphate may have been precipitated by autoclaving. The stoichiometrical reduction of calcium was higher, however, than that for sulphur indicating that calcium was removed by some other means such as precipitation as the citrate or some other insoluble form.

PHILLIS and MASON (9) also found that the calcium content of saps expressed from heated cotton leaves was lower than that expressed from the frozen leaves. This reduction on heating was attributed to the occurrence of base exchange reactions during the heating. Such base exchange reactions could account for small changes in concentration but it is doubtful if they could account for a reduction of over 100 milliequivalents of calcium per liter of sap as shown in tables I and II. Furthermore, no such increases or decreases in any of the other bases were found. If this were a true base exchange reaction, magnesium, sodium, and potassium equilibria would also be expected to undergo proportionate stoichiometric changes. Likewise, it would be difficult to account for the decrease in total sulphur by anion exchange when there was no increase or decrease in the total chlorine content of the saps upon heating.

Pretreatment by freezing or heating allows a large percentage of the plant juices to be pressed from the plant tissues and has been used extensively. Such sap is not comparable to the plant fluids as they exist in the vacuole and cytoplasm of unkilled cells (9) but is a composite of the fluids occurring within these tissues. And, as has been shown, the composition of this composite liquid may be altered by the pretreatment of the tissues prior to pressing. Because of possible precipitation of certain constituents in the sap during heating, killing by severe freezing prior to pressing is the preferable procedure.

Summary

1. The concentration of several mineral constituents in saps expressed from plant materials pretreated by freezing with solid carbon dioxide and by autoclaving were compared.

2. Calcium and total sulphur contents of the saps expressed from autoclaved cotton leaves were lower than those of saps expressed from similar samples killed by freezing.

3. Total sulphur content of saps expressed from autoclaved tomato leaves and stalks was lower than that expressed from similar samples killed by freezing.

4. Precipitation of calcium rather than a base exchange reaction during the autoclaving is indicated.

5. Killing of plant tissues by severe freezing prior to the removal of the fluids by pressing is preferable to killing by heat.

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