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STUDIES ON THE PRESERVATION OF THE ROOTS AND NODULES OF LEGUMES

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Many different methods have been recommended for the preservation of green plants for exhibition or for laboratory use. The most common solutions which have been recommended for the preservation of such material contain ethyl alcohol, formaldehyde, glycerine or sulfurous acid, alone or in combination, and frequently different salts such as copper or zinc are added.

Bailey[3] has published six formulas which were used by F. T. Shutt, Experimental Farm, Ottawa, Canada. All of these solutions contain from one to ten parts of alcohol and since ethyl alcohol is not easily obtained in many laboratories, these formulas cannot be recommended for general use. Another solution which was labeled "a California method" contained glycerine, sulfurous acid, rock salt and saltpeter which was replaced after several months with a solution containing one ounce of sulfurous acid in one gallon of water.

Derr and Lane[5] recommended a solution containing 1 part of alcohol, 1 part of formalin and 15 to 20 parts of distilled water for the preservation of soft fleshy fungi. Keefe[6] used a solution containing 90 cc of 50 per cent alcohol, 5 cc of formalin, 2.5 cc of glyceirne, 2.5 cc of glacial acetic acid, 10 g of copper chloride and 1.5 g of uranium nitrate to preserve plant material which is treated from 3 to 10 days before it is dried and mounted. For yellow and green plants, the copper chloride was reduced to 5 g.

Reid[9] states that sulfurous acid is the best preservative for green, yellow or white vegetation; that formalin or boric acid is best for red or black plant material; and that zinc chloride and alcohol are satisfactory for green or russet colors. Maltby[7] found that the color of plant material preserved with formaldehyde. Errera's salt solution, or alcohol, changes slowly and that dull brown colors appear.

Butler[4] bleached plants with a solution containing 1 per cent of sodium bisulfate acidified with citric acid for 1.5 to 2 hours and then stored the plants in 4 per cent formaldehyde solution. For some types of plant material, the fixing solution contained sulfurous acid and copper sulfate and plants were immersed in it from 3 to 7 days and then stored in a solution containing 1.75 per cent of sulfur dioxide. Another fixing solution was prepared as follows: formaldehyde 15 cc, copper sulfate, 15 g, absolute alcohol 75 cc, and distilled water, 750 cc. Plants were left in this solution 3 weeks and then transferred to an aqueous solution con-

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taining 1.75 per cent sulfur dioxide. Alsterberg[2] recommends the halogen cyanides for the preservation of filamentous algae, protozoans and plant pigments. Swingle[10] has proposed the use of oxyquinoline sulfate to preserve plant tissue.

Adriano and Tabije[1] have studied fifteen different formulas which have been recommended for the preservation of green plant material and two fixing formulas are given which were satisfactory for the preservation of color. Fixing formula *A* contained 1 cc of 40 per cent formalin and 7.5 g boric acid in 1 l of distilled water. Enough copper sulfate was added to give a faint green color. The specimens are placed in the fixing solution for one to two weeks and then transferred to the storage solution. Storage solutions containing from 0.1 to 2 per cent of sulfurous acid gave good results when used for the preservation of fifty-seven kinds of fruits and vegetables.

Perry and Beal[8] studied the concentrations of various substances needed to inhibit and prevent alcoholic fermentation and the relative toxicity of six different substances on the development of mold and yeast was as follows: formaldehyde. sodium benzoate, sodium salicylate, sodium bisulfite, ethyl alcohol and sodium sulfite. Concentrations of at least 15 per cent ethyl alcohol were needed to inhibit yeast and mold growth as compared with 0.4 per cent formaldehyde.

Many patents have been granted on methods for preserving plant material with various chemicals, such as, organic mercury compounds, benzylphenol, isothiocyanates and hexamethylenetetramineborosalicylate. These materials are not readily available in the average laboratory and are not apt to be used if inexpensive reagents which will preserve plant material can be obtained from local sources.

Since the preservation of plant material depends quite largely on the control of bacteria and fungi or decomposition of organic material as a result of enzyme action, any material which will inhibit these processes should be a satisfactory material for use in the preservation of plant tissues. A series of different solutions was prepared, and the roots and nodules obtained from Austrian Winter peas were washed thoroughly to remove soil and were placed in bottles containing the different liquids on May 5, 1933. An observation of these solutions was made on November 25, 1933. The results appear in Table I.

TABLE I. *A comparison of various solutions on the preservation of roots and nodules of Austrian Winter peas.*

No.	Solution used	Condition of roots and nodules
1	.02 N H_2SO_4, .1% $CuSO_4.5H_2O$	Roots and nodules dark brown
2	.02 N HCl	Roots and nodules dark brown
3	.10 N H_2SO_4	Roots and nodules dark brown
4	.02 N H_2SO_4	Roots and nodules brown
5	.10 N HCl	Nodules white, roots slightly yellow
6	.20 N $HC_2H_3O_2$	Nodules white, roots in good condition
7	5% formalin, .1% $CuSO_4.5H_2O$	Nod. slightly brown, roots white
8	5% formalin	Nod. slightly brown, roots white
9	5% formalin, .1% $CuSO_4.5H_2O$, 2.5% glycerine	Nod. slightly brown, roots white
10	5% formalin, .2 N $HC_2H_3O_2$, 2.5% glycerine	Nodules white, roots white
11	5% formalin and .2 N $HC_2H_3O_2$	Nodules white, roots white
12	5% formalin, .02 N H_2SO_4	Nodules white, roots white

The solutions in Table I were arranged according to the condition of the roots and nodules after remaining in the various solutions for 2.5 years. Very little difference occurred between the plant material in solutions No. 1, 2 and 3. The differences between the dilute hydrochloric and acetic acid and many of the formalin solutions were not pronounced. The acidified formalin solutions were distinctly superior to those solutions which contained formalin and no acid. When plant materials are placed in .05 normal solutions of mineral acids, they can be kept for several months without any appreciable physical change.

Comparisons which were made in previous experiments with methyl and ethyl alcohol were not satisfactory. Solutions containing high percentages of methyl alcohol dissolve too much pigment from green material and weak solutions do not preserve the original color of the tissues. Ordinary denatured alcohol usually contains substances that are immiscible in water and cannot be used in the preparation of storage solutions which are diluted with water.

Sulfurous acid is a very satisfactory solution for the preservation of green material if the containers can be sealed so that the gas will not escape. A solution containing 1 per cent of sulfurous acid can be made for less than five cents per gallon for materials and it is not expensive to add fresh solutions to specimen jars if there is any indication that the sulfur-dioxide content of the storage solutions is decreasing.

A convenient method for making a 1 per cent sulfurous acid solution is to dissolve 12.6 g of sodium sulfite in 500 cc of water and add this solution to 500 cc of a solution containing 18 cc of concentrated hydrochloric acid. These stock solutions are very stable and can be kept in separate containers indefinitely. Fresh storage solution can be prepared at any time by mixing equal quantities of the two stock solutions.

Summary—A study was made to determine what solutions could be used to preserve roots and nodules of legumes.

Alcoholic mixtures are expensive and were not satisfactory.

Dilute acid solutions will preserve roots and nodules and also the leaves and stems of many plants for several months and no appreciable change in physical appearance will occur.

Acidified formalin solutions were superior to formalin solutions containing no acid.

Sulfurous acid solutions prepared by mixing sodium sulfite and hydrochloric acid are inexpensive, easy to prepare and roots and nodules of legumes can be kept in these solutions for a long period of time without change. The cost of making the sulfurous acid is about one-half the cost of 5 per cent formalin solution.

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