Preserving Color in Dry Herbarium Specimens Using Calcium Chloride

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ABSTRACT

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The natural color characters of plant tissues were well preserved using granulated calcium chloride ($CaCl_2$) for the quick drying of pressed herbarium specimens.

A well-preserved plant specimen is a very useful record for reference, for accurate comparative work, and for demonstration. Specimens for the herbarium are preserved either dry or in liquid. Large woody specimens like branches of trees are just allowed to dry, whereas fleshy specimens such as fruits and leaves are prepared and preserved in liquid in jars. Most specimens, however, are preserved in dry, pressed form and are usually flat.

The classical method of drying pressed specimens (6,7) has been used for centuries by botanists and plant pathologists. It preserves most botanical and pathological characters, but specimens usually become discolored because of the oxidation of phenolic compounds. This discoloration is a serious disadvantage for the botanist who often needs to see the natural color of flowers and for the plant pathologist who needs to see the natural color of symptoms. The classical drying method is especially unsatisfactory for the plant virologist because most viral disease symptoms involve color changes, eg, mosaic, mottle, chlorotic spots, ring or band patterns, vein yellowing, vein-banding, and color break of flowers. These symptoms are usually lost or become obscure upon drying by the classical method.

Preserving the infectivity of virus inocula using calcium chloride (1–3,5), magnesium perchlorate (4), or freeze drying also preserves the natural color of plant tissues very well. This observation prompted us to experiment with calcium chloride for drying herbarium specimens.

MATERIALS AND METHODS

Fine-granulated, anhydrous calcium chloride ($CaCl_2$) was used in an amount about double the water content of the tissue to be dried.

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The specimen to be preserved was pressed between sheets of paper under a relatively heavy object for several hours. It was then transferred between two new sheets of paper and carefully placed on a flat layer of $CaCl_2$ in a plastic bag on a bench to preserve the flatness of the specimen during drying. For the same purpose, a metallic or plastic screen larger than the sheets of paper was placed on top of the $CaCl_2$ layer, and the paper-protected specimen was placed on top of the screen. Air was removed from the plastic bag containing the specimen and desiccant, the bag was sealed with tape, and a relatively heavy and large object was placed on it. During handling, contact of the tissue with $CaCl_2$ was avoided because prolonged contact caused tissues to turn brown. The specimen was dried on the bench at room temperature or on a tray in the refrigerator for 3–10 days (depending on

Table 1. Natural, fresh color characters of plant specimens after drying with calcium chloride ($CaCl_2$) and by the classical method

Plant	Specimen	Disease	Color retention (%)[a] Classical method	Color retention (%)[a] $CaCl_2$ method
		Trees and shrubs		
Cercis siliquastrum	Leaves	Healthy	NT[b]	85
	Flowers	Healthy	NT	100
Citrus spp.	Leaves	Various psoroses	30	95
Ficus carica	Leaves	Fig mosaic	30	90
Prunus armeniaca	Leaves	Plum pox	20	75
P. cerasus	Leaves	Healthy	NT	85
	Flowers	Healthy	NT	85
P. mahaleb	Leaves	Healthy	NT	90
	Flowers	Healthy	NT	90
P. persica	Leaves	Plum pox	20	80
Pyrus communis	Leaves	Healthy	10	80
	Flowers	Healthy	10	80
	Leaves	Pear ring and band-pattern mosaic	10	80
Vitis vinifera	Leaves	Grapevine fanleaf (mottle)	20	80
	Leaves	Grapevine asteroid mosaic	30	80
	Leaves	Grapevine yellow mosaic	50	90
		Herbaceous plants		
Calendula arvensis	Leaves	Healthy	NT	90
	Flowers	Healthy	NT	100
Cichorium intybus	Leaves	Artichoke yellow ringspot	20	95
Cucurbita pepo	Leaves	Cucumber mosaic	20	80
	Leaves	Watermelon mosaic	20	85
Cynara scolymus	Leaves	Artichoke yellow ringspot	20	95
Cynara sp.	Leaves	Artichoke yellow ringspot	0	70
Geranium sp.	Flowers	Healthy	NT	95
Forsythia sp.	Leaves	Healthy	NT	95
	Flowers	Healthy	NT	100
Jasminum fruticans	Leaves	Healthy	NT	95
	Flowers	Healthy	NT	100
Matthiola incana	Leaves	Turnip mosaic	NT	90
	Flowers	Turnip mosaic	NT	100
Nicotiana tabacum	Leaves	Tobacco mosaic	5	60
	Leaves	Artichoke yellow ringspot	20	95
Papaver rhoeas	Leaves	Healthy	30	90
	Flowers	Healthy	30	70
Silybum marianum	Leaves	Artichoke yellow ringspot	0	70
Sinapis sp.	Leaves	Vein flecking	30	95
Vicia villosa	Leaves	Healthy	30	95
	Flowers	Healthy	30	90

[a] Based on fresh specimen.
[b] Not tried.

the nature of the tissue) before transfer to the herbarium. Using $CaCl_2$ in excess resulted in quicker and better drying.

RESULTS AND DISCUSSION

Color retention was good (60% of original color) to excellent (95–100%) in 38 plant specimens dried by the $CaCl_2$ method (Table 1). In contrast, 23 specimens that were also dried by the classical method showed poor color retention. Results with the classical method were even worse when specimens were dried at temperatures above room temperature.

To date, the $CaCl_2$ method has been used routinely in our laboratory to preserve more than 250 specimens. The method has proved reliable (quality of similar specimens did not deviate) and durable (no discernible loss of color in over a year; virus inocula have been thus preserved in our laboratory for more than 10 yr, with no loss in color of tissues). The method is also convenient and rapid (tissue dried very quickly as compared with the classical method at room temperature).

The $CaCl_2$ method should be useful for phytopathological and botanical herbaria, particularly for preserving the color of flowers, which is an important taxonomic criterion that is usually lost by drying with the classical method.

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