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Preservation of botanical specimens in fluids

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History

Although the history of fluid preservation for zoological specimens can be traced back to Ruysch (1710) and less tangibly earlier still, the history of fluid preservation for botanical specimens starts more recently. Although plants have been preserved in alcohol for sometime (since at least c. 1830), the majority have been preserved dry as herbarium specimens. At Kew, Victor Summerhayes (curator of Orchidaceae) started a fluid-preserved collection during the 1930s and which subsequently expanded to other plant taxa.

Fixation and Preservation Biochemistry

Formalin has been used as a primary fixative to alter slightly the chemical structure of protein molecules, by utilising the electrophilic carbonium/ carbonium ion (as found in formaldehyde), to a more stable structure that is unassailable by bacteria. Most plant material has been fixed in alcohol, formol-acetic-alcohol or (more recently) Kew mixture. Alcohol fixation is known as pseudo-fixation since the material is only dehydrated and guarded against bacterial decay whilst in such fluid. The alcohol additionally denatures some structures found in plants, particularly the tetrapyrole groups found in many natural pigments, such as chlorophyll.

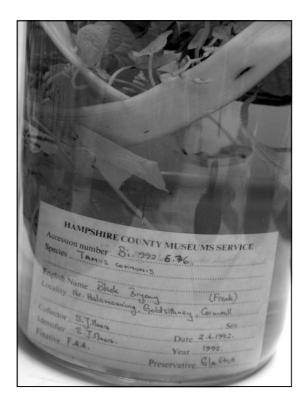


Fig. 1. Black Bryony fixed in FAA and preserved in IMS-glycerine – most (but not all) of the chlorophyll pigment is in solution.

Alcohol is also difficult as a storage medium since it is flammable, it evaporates more rapidly than other preservative fluids and has a lower osmotic pressure than that of the normal fluids found in living plants.

Alcohol does have the benefit of preserving DNA structures intact whereas longer-term preservation in formaldehyde-based preservatives and fixatives will chemically alter the DNA double helix over time. To combat evaporation and slightly reduce the hazard of flammability, humectants, principally glycerol/ glycerine, are added from 5 to 10% in concentration and will also help to prevent specimen embrittlement.

Fluids used

Kew mixture was devised as a plant fixative to complement the preservative Copenhagen mixture and these two are the principal fluids used in botanical collections these days and will also satisfactorily preserve fungal sporocarps.

Note that the formula often varies slightly according to institutional recipes! A more complete list is provided in Bridson & Forman (1992) and Tredwell (2005) provides more information in her leaf-let.

More specialised fluids containing copper salts were used to pseudo-preserve chlorophyll although they were only substitutes and specimens preserved in these fluids often have a blue-green tinge.

Colour-preserving/substitution fluids are mentioned in Moore (in Carter & Walker, 1999) and originally in Wagstaffe & Fidler (1968).

Kew mix (Kew recipe for this fixative): 4-5% of formaldehyde solution (37.6% formaldehyde) 53% IMS (Industrial Methylated Spirit) 5% glycerol 37% water (de-ionised) OR: 1 part 100% formalin (37.6% formaldehyde): 10 parts IMS (absolute): 1 part glycerol: 8 parts deionised water.

Copenhagen mix (preservative):

53% IMS (Industrial Methylated Spirit)
5% glycerol
37% water (de-ionised)
OR: 10 parts IMS: 1 part glycerol: 8 parts deionised water.

Formol-acetic-alcohol (FAA) (fixative):

48ml 100% formalin (37.6% formaldehyde) 160ml IMS (Industrial Methylated Spirit) 8 ml Glacial Acetic acid 320 ml deionised water.

There are other fluids that involve the use of propylene glycol, poly -ethylene glycol, phenoxetol (2-phenoxy-ethanol) &c., so it is difficult to know what to add to a near-dry specimen – if some special fluid was used as a fixative/preservative and without any data in a notebook or on the label, then the standard procedure of using Kew and Copenhagen fluids should be adhered to.



Fig. 2. Parachute mushroom: the diminutive *Marasmius rotula* fixed and preserved in Kew Mixture.

Rehydration

Dried out specimens can either be mounted as herbarium specimens but if required to be returned to a state of fluid preservation, the following procedure can be followed, bearing in mind that any DNA structure will be compromised by the process.

Rehydrate specimen in warm 3-5% Decon-90 solution in deionised water to about 50°C until the specimen appears to be normally flaccid. Rinse in water and if required, trapped air bubbles should be removed under a mild vacuum at this stage. If the specimen is somewhat fragile, it can be fixed in Kew mixture first but must be returned to water before vacuum treatment since solvent and formaldehyde fumes will damage the vacuum pump and contaminate the pump oil.

Specimens may then be fixed in Kew mix overnight and then transferred to Copenhagen mix for storage. Fixation and preservation notes should always be included on the label, especially if these are specialised or new.

Labels

These will be severely disadvantaged if attached to the jar exterior:

Fluids can leach from the jar if moved or if the fluids are glycol or glycerol-based, these can creep out of ground glass and screw-capped jar closures and seep onto the labels making them rather illegible.





Fig. 3. Exterior labels on jars that have become contaminated by fluid seepage and become partly or almost totally illegible!

Labels can also detach and become lost or mixed with those from similar specimens (Fig. 4).

Fig. 4. A detached label – how long until it becomes lost?



Some label papers can also affect the pH of the jar contents; therefore Goatskin Parchment (Arjo Wiggins) is still the recommended label medium for use inside the jar but should not be so large that it obscures the specimen and fluid levels.

Computer-generated labels look really good and are alright as such but the printing may disintegrate within 15 years (pers. comm.): it is currently undergoing experimentation in the USA. For more information and/ or involvement post questions on the NH-Coll website (find via Google). The bottom line is that essential information should still be recorded with pigment ink.

Storage parameters and ideals

Botanical specimens stored in fluids should be away from any light or UV source as they are very susceptible to bleaching. Temperature parameters should be between 10 and 20°C and RH levels should be maintained between 50 and 60%. Appropriate hazard data should be attached to doors and adequate lighting and ventilation should be provided together with an eyewash station.

Acknowledgements

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