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A New Practical Method for Profiling and Topping Up Alcohol Preserved Entomology Collections

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Abstract

Key aspects of recent research into topping up entomology spirit collections are summarised. A new method of profiling alcohol preserved collections is presented and its use as a diagnostic tool is discussed. A novel tool is also presented as a reference table for calculating the concentration of topping up alcohol, which allows the regulation of preservative alcohol concentration within close limits. The method can be used for remedial and routine topping up and can be adapted to the needs of different collections.

Introduction

The first line of defence against evaporation of fluid preservatives must always be the best storage jar affordable; however, all storage jars allow at least some evaporation and will need a regular schedule of inspection, maintenance and topping up.

In order to facilitate these processes a new method of profiling alcohol preserved collections is has been developed which takes into account the volume of preservative present, and its use as a diagnostic tool is discussed. A new approach is taken to topping up which allows the desired concentration to be achieved while taking into account any variation in starting concentrations and volumes. The new topping up method has a number of key features. By analogy with the control of temperature and humidity for purposes of conservation, potentially damaging fluctuations, both in alcohol concentration and in volume, are managed much more closely. Greater weight is given to the volume of preservative present than previous methods for a number of reasons: to prevent the specimen becoming exposed; because it provides an indicator of low alcohol concentration; because low volumes may be an indication of a faulty seal; and because it is important in calculating the correct concentration for topping up. A novel tool is presented, a reference table for calculation of the concentration of topping up alcohol to be added, which makes the method applicable to large collections. The table gives speed and convenience priority over accuracy; however, because of the use of precise monitoring, the results are still accurate within close limits.

A general approach is developed which can be applied to many alcohol preserved collections. The methods proposed are designed for use with collections in modern storage jars, or where replacement and standardisation of jars is possible. The term Alcohol is used here to include ethanol and also mixed alcohols such as industrial methylated spirit (IMS). Alcohol concentration was measured using the Anton Paar DMA 35N digital alcohol meter. This meter was used on the '%ALC/V' setting, which gives a measurement of the equivalent concentration of an ethanol/water mixture in % by volume at 20˚C derived from density at the measuring temperature (Anton Paar, 2000). For best results the environmental conditions within a store should be managed closely, although the method can be adapted to some extent for the different conditions in stores. It is not the purpose of this paper to consider the initial preservation of specimens, and it is assumed that specimens are preserved and equilibrated with their preserving fluid.

The paper is written from experience of working with the large and varied collection of entomological and other terrestrial arthropods preserved in IMS at the Natural History Museum, London. It follows the approach of not discarding alcohol where possible to reduce leaching, and may not be suitable for collections containing fatty material, especially large vertebrates, where replacement of alcohol may be desirable to counter acidification from the decomposition of fats. Only key results of practical value are given here; a full account, including more detailed references and acknowledgments is published in *Collection Forum* (Notton, 2010).

The aims of topping up

The aims of topping up are:

- Firstly to keep the specimen covered with preservative, so: a) it does not dry out, b) it is physically supported, and c) evaporation does not lead to the deposition of salts and other solutes on the exposed portion of the specimen.
- Secondly to maintain the correct concentration of alcohol for preservation. For insects this is generally considered to be 70-80% for general preservation. Lower concentrations can cause distortion of the specimen by absorption of water and autolysis. 50-80% is recommended by Waller and Strang (1996) as the range with the best antiseptic properties, below 50% growth of bacteria and mould become increasingly likely. Higher concentrations can cause tissue distortion and embrittlement.
- Thirdly to keep fluctuations in concentration limited within acceptable boundaries, by analogy with other methods of environmental control. Osmotic pressure increases particularly rapidly for concentrations above 80%, but also rises steadily between concentrations of 0-75%, suggesting that large changes in concentration during topping up should be avoided as a precaution against osmotic stress which may distort the specimen (Waller & Strang, 1996).

Collection profiles – taking account of volume as well as concentration helps to diagnose problems with topping up procedures

Before starting to top up a large collection it is advisable to make a profile of the collection, to find if the aims of topping up are being met. Previous collection profiles have presented concentration as a frequency histogram (Cato, 1990; Pickering, 1997); while this is useful, it records no information about the volume of preservative present. A new method was used instead, plotting concentration of alcohol against the volume of preservative (as a proportion of the jar filled). This also allowed the profile of volumes and any interaction between concentration and volume to be assessed, e.g., if the preservative is more dilute than expected from its volume. A 'target area' of acceptable concentration and volume can be superimposed on the graph and the proportion of the collection in the target area counted and used as an indicator of 'collection health'. An example graph (Fig. 1) made in 2007 shows that: a) topping up has not been applied consistently; b) many jars were not adequately filled; c) many jars were at the wrong concentration. Clearly there was a problem with topping up, as an alcohol meter has been available to all staff since 2002. Many jars were less concentrated than might be expected for their volume. In all probability they were topped up with understrength alcohol (probably 80%) which is known to reduce concentration over time.

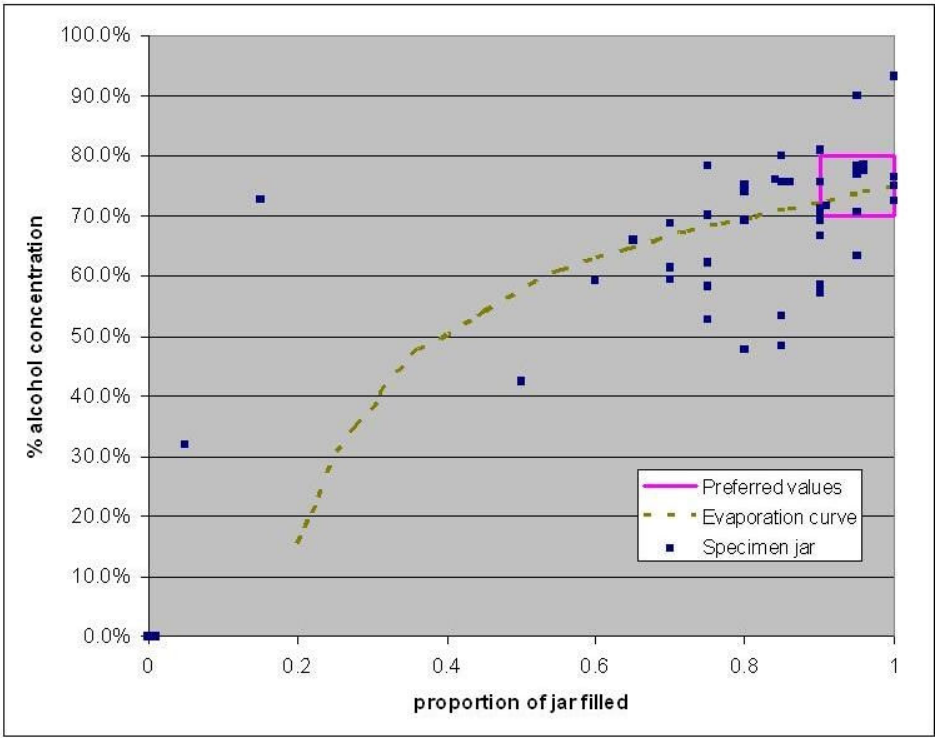


Fig. 1. Profile of Entomology Department alcohol preserved collection, August 2007. Each square represents data for a jar in the collection.

A new & convenient topping up table

Because of the different treatments and conditions that specimen jars may have undergone, different concentrations of topping up alcohol may need to be added to restore them to the desired concentration and volume. A new table was developed to allow this (Fig. 2, overleaf).

The method of using the table is shown simplified in Fig. 3. Read the concentration of the alcohol in the jar using the meter and estimate the proportion of the jar filled with alcohol. On the table read across from the nearest concentration and down from the nearest proportion; where these intersect, the value in the box gives the concentration of alcohol to top up with. Occasionally the alcohol will be too dilute to return it to the desired concentration, and the point of intersection on the table has no value. In this case read left from the point of intersection until there is a box with a value in (arrows). Read up from this box to find the proportion of alcohol, and discard alcohol until this proportion is reached, then top up with 96%.

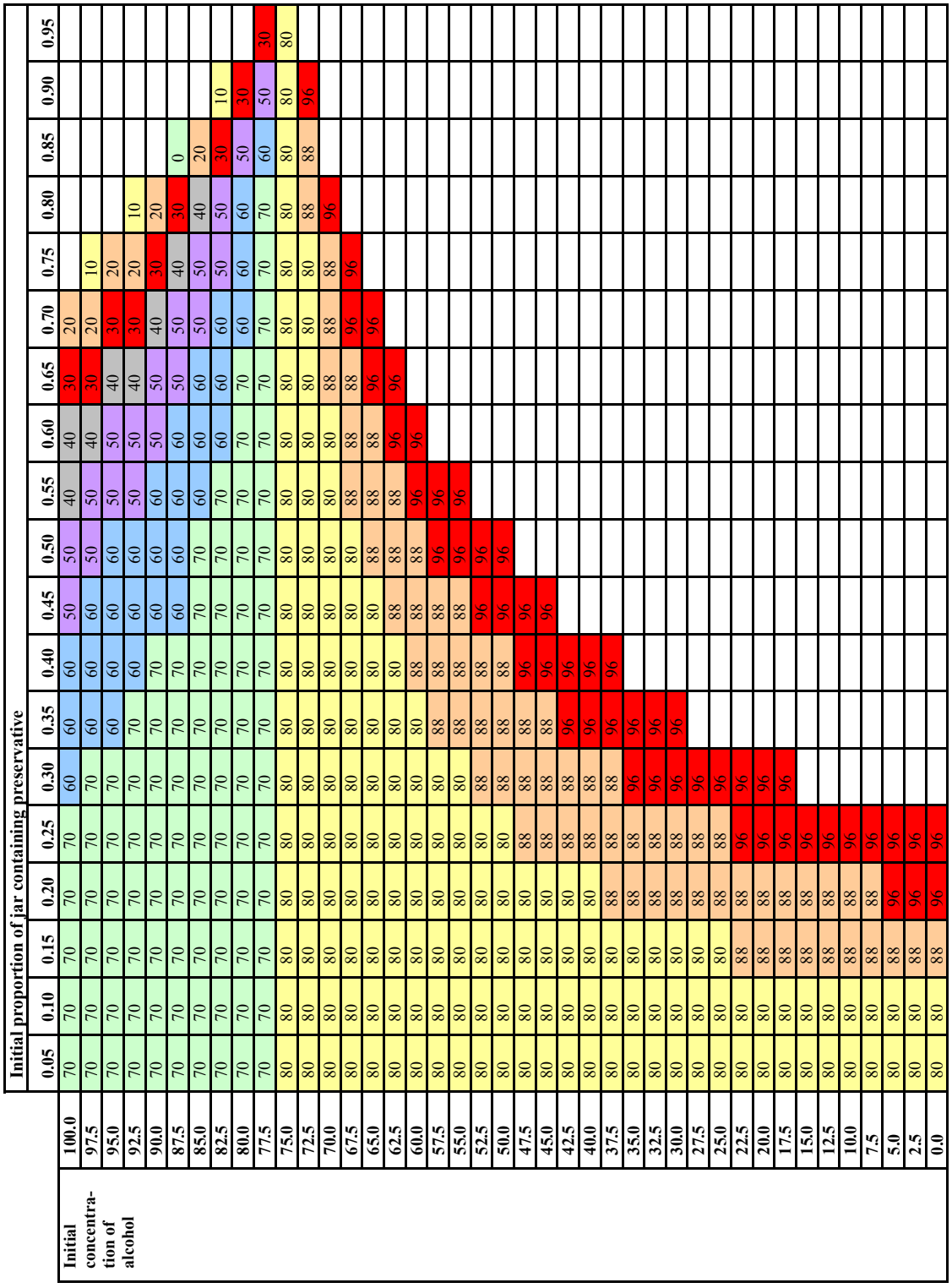
Some general protocols are provided below (Fig. 4) for: a) preparing for topping up; b) remedial topping up for neglected collections; and c) routine topping up for collections which have been regularly topped up.

		Proportion of jar filled				
		0.65	**0.70**	**0.75**	**0.80**	**0.85**
Initial concentration of alcohol	**72.5**	80	80	80	88	88
	70.0	88	88	88	96	
	67.5	88	96	96		
	65.0	96	96			
	62.5	96				

Fig. 3. Illustration of how to find the concentration of alcohol to top up with to get the desired concentration using table 2.

		Proportion of jar filled							
		0.50	**0.55**	**0.60**	**0.65**	**0.70**	**0.75**	**0.80**	**0.85**
Initial concentration of alcohol	**65.0**	88	88	88	96	96			
	62.5	88	88	96	96				
	60.0	88	96	96	←	←	←		
	57.5	96	96						

Fig. 4. Illustration of how to find the concentration of alcohol to top up with in situations where the alcohol is so weak that it cannot be brought to the desired concentration by the addition of 96% alcohol.



Initial concentration of alcohol	Initial proportion of jar containing preservative																			
	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95
100.0	70	70	70	70	70	60	60	60	50	50	40	40	30	20					
97.5	70	70	70	70	70	70	60	60	60	50	50	40	30	20	10				
95.0	70	70	70	70	70	70	60	60	60	60	50	50	40	30	20				
92.5	70	70	70	70	70	70	70	60	60	60	50	50	40	30	20	10			
90.0	70	70	70	70	70	70	70	70	60	60	60	50	50	40	30	20	0		
87.5	70	70	70	70	70	70	70	70	70	70	60	60	60	50	50	40	20		
85.0	70	70	70	70	70	70	70	70	70	70	70	60	60	60	50	50	30	10	
82.5	70	70	70	70	70	70	70	70	70	70	70	70	70	60	60	60	50	30	
80.0	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	60	50	30
77.5	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	80	80	80	80
75.0	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	96	
72.5	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	88	88		
70.0	80	80	80	80	80	80	80	80	80	80	80	80	88	88	88	96			
67.5	80	80	80	80	80	80	80	80	80	80	88	88	88	96	96				
65.0	80	80	80	80	80	80	80	80	80	88	88	88	96	96					
62.5	80	80	80	80	80	80	80	80	88	88	88	96	96						
60.0	80	80	80	80	80	80	80	88	88	88	96	96							
57.5	80	80	80	80	80	80	88	88	88	96	96								
55.0	80	80	80	80	80	80	88	88	88	96	96								
52.5	80	80	80	80	80	88	88	88	96	96									
50.0	80	80	80	80	80	88	88	88	96	96									
47.5	80	80	80	80	88	88	88	96	96										
45.0	80	80	80	80	88	88	88	96	96										
42.5	80	80	80	80	88	88	96	96											
40.0	80	80	80	80	88	88	96	96											
37.5	80	80	80	88	88	88	96	96											
35.0	80	80	80	88	88	96	96												
32.5	80	80	80	88	88	96	96												
30.0	80	80	80	88	88	96	96												
27.5	80	80	80	88	88	96													
25.0	80	80	80	88	88	96													
22.5	80	80	88	88	96	96													
20.0	80	80	88	88	96	96													
17.5	80	80	88	88	96	96													
15.0	80	80	88	88	96														
12.5	80	80	88	88	96														
10.0	80	80	88	88	96														
7.5	80	80	88	88	96														
5.0	80	80	88	96	96														
2.5	80	80	88	96	96														
0.0	80	80	88	96	96														

Fig. 2. Table for calculating the concentration of topping up alcohol needed to return preservative concentration to 75% alcohol.

Preliminary considerations

Before undertaking any topping up:

a) Undertake the health and safety risk assessments for using alcohol based preservatives and obtain appropriate personal protective equipment
b) Decide the concentration of alcohol to store your particular specimens in and the allowed range of fluctuation around this value. For the Darwin Centre store in the NHM this was set at 75 +/- 5 volume % standardised to 20ºC. Decide the volume at which to top up your specimens. This should be based on the normal rate of evaporation for the store (e.g. see the evaporation curve in fig. 1) giving a margin for error before the concentration drops below the acceptable lower limit (b above), and giving a margin for error before any specimens are exposed. For the Darwin Centre store, this was set at 0.9 of the volume.
c) Decide how often to inspect and top up if needed, work out evaporation rates from different kinds of storage jars, both effective and defective. This should be based on how long it takes the worst kind of jar to reduce the volume of preservative to the volume at which to top up (c above). For the Darwin Centre store, past experience suggests annual losses in the region of c.1% volume in jars with an effective seal and 5-10% in those with a defective seal, with some variation depending on jar and seal type – i.e. inspections must be annual at least.
d) Obtain a digital density meter which automatically converts readings to volume % standardised to 20ºC, such as the Anton Paar DMA 35N or equivalent (Anton Paar, 2000).
e) Calculate a topping up table similar to table 2 based on the formula and method described above, and the desired concentration (b above), print it out, preferably in colour, and seal it in a plastic pouch so it will be alcohol resistant.
f) If it is difficult to estimate proportions of the volume (e.g. some designs of jar which taper slightly) make a graduated dipstick for this kind of jar marked off in tenths.
g) Obtain, or make up, verify and clearly label the concentrations of the stock solutions of alcohol.
h) The protocols below may need to be modified, to allow a larger head space in cases where there is a risk of seal breakage from high vapour pressure, for susceptible jar types in stores with sudden temperature fluctuations.
i) Ideally protocols should include the monitoring of pH, however measuring the pH of alcohol solutions is difficult and is best dealt with elsewhere.

Remedial topping up

This is recommended if starting with a neglected collection, or one at the wrong concentration:

a) Make a profile of a proportion of the collection as described above, selecting systematically across the collection, to represent all parts of the collection; this should help estimate the amount of time and materials required and any special problems.
b) Check every jar in turn.
c) Check the jar is not defective and if so, replace it.
d) Check each jar is tall enough so that evaporation between inspections will not leave any specimen exposed; if this is likely, transfer the specimen to a larger jar.
e) Check the concentration of alcohol and the proportion of the jar filled with preservative - do not assume full jars will be at the correct concentration.
f) Calculate the concentration of the alcohol to add using the topping up table.
g) Fill up the jar with alcohol; jars should consistently be filled to the top (leaving c. 10 mm head space to avoid contact between alcohol and the seal), this allows subsequent visual detection of evaporation easier; if a large change of concentration (more than 5%) is needed top up in stages to reduce osmotic stress
h) Make a final check of the concentration, and adjust if needed.
i) Make a record.
j) If specimens are completely dried out, do not try to rehydrate them without good reason. They are usually stable when dry and rehydration will probably cause more damage – leave a note in the jar saying ‘found dehydrated on such and such a date’, and store at humidity and temperature levels appropriate for dried tissue samples (i.e. not necessarily in a spirit store).
k) Jars with very high concentrations may have been preserved for DNA work, if so, clarify the purpose of preservation, and if they need to be kept at high concentration, label them clearly, and preferably transfer them to low temperature storage.

Routine topping up

This is recommended for collections which have recently undergone remedial topping up to the right concentration and volume. Every fourth or fifth time, a complete check is recommended as for remedial topping up:

- Set your timetable for inspection (preliminary d) and stick to it.
- Top up all jars where volume is less than the volume decided above (preliminary c).
- For each jar topped up check to see if seal is defective and jar or seal needs replacing.
- For specimens known or suspected to be at risk of acidification, replace the preservative completely.
- Check the concentration of the alcohol and the proportion of the jar filled with preservative.
- Calculate the concentration of the alcohol to add using the topping up table (once the collection has been stabilised by remedial topping up this should be straightforward as there should be relatively little variation in concentrations and volumes, and the table can be used to provide rules for common situations, e.g. for the Darwin Centre store, if volume reduced by about 10%: top up with 96% if concentration < 73.75%; top up with 88% if concentration > 73.75%).
- Fill up the jar with preservative; jars should consistently be filled to the top (leaving c. 10 mm head space to avoid contact between alcohol and the seal), this allows subsequent visual detection of evaporation easier.
- Check the topped up jars, so the correct concentration is reached.

Final thoughts

Do not underestimate the human factor – topping up can be tedious and stores are often cold, dull and away from regular places of work. Persist to get topping up seen as a priority, and check that it has been done correctly. The use of clear reasoned protocols should help staff appreciate the problems and implement improved collection care, because the benefits will be clearly seen.

Acknowledgements

Gavin Broad, Claire Valentine and Paul Brown; Andries van Dam and Simon Moore for a helpful reviews and discussion; NatSCA for a bursary and the opportunity to present this at the Newcastle Conference.

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