Chapter 34

Selecting Seed Containers for the Millennium Seed Bank Project:

a technical review and survey

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Summary

The need to maintain the low moisture status of seeds destined for long-term storage prompted a review of suitable seed bank containers. A simple and quick testing protocol was developed which utilised the colour change of self-indicating silica gel. The results of tests on eighteen container types, culminated in the choice of five suitable containers for use with the Millennium Seed Bank Project. Different seal compounds and secondary sealing tapes retarded but did not prevent moisture ingress into containers with compromised seals. The use of self-indicating silica gel incorporated within the storage containers is proposed as a long-term indicator for seal performance.

Introduction

Reliable, hermetically sealed containers are essential for long-term seed banks. Failure to use such containers could incur considerable re-drying costs or loss of viability of valuable accessions. In addition, seeds may be wasted due to the need to carry out additional germination tests. Yin Yanping *et al.* (1999) concluded that hermetically sealed containers were especially important for short-lived species. Previous studies (Gomez-Campo, 2002; Freire and Mumford, 1986) have shown that many containers do not seal effectively. Seed within such containers may gradually equilibrate to the moisture status of the storage facility. If the moisture content of stored seed rises just 1%, this may effectively reduce the storage life of the seed by 50% (Harrington, 1972).

This report summarises the main factors to take into consideration when selecting suitable containers for seed banks. It then describes work carried out by the Millennium Seed Bank Project (MSBP) in order to select the most appropriate containers for long-term seed storage at -20°C.

Selecting Suitable Containers for Seed Banks

1. Container Material

Seed banks use a variety of containers made of materials that include glass, metal and plastic (Gomez-Campo, 2002). Provided that a hermetic seal can be achieved, the final choice of material is often dependent on the practical requirements of a particular seed bank.

The experience of the MSBP suggests that plastic containers rarely seal effectively. There is also some concern relating to the potential long-term harmful effects of plasticisers on seed viability.

If glass containers are chosen, consideration must be given to the overall weight with regards to seed bank design. The flooring of cold rooms must be able to bear the loading incurred through the use of glass containers (Linington, 2003 – Chapter 33). Borosilicate glass should be avoided due to potential static problems (possibly due to the insulating properties of such pure glass); sodalime glass does not incur such problems. One disadvantage of glass is the possibility of breakage.

One of the disadvantages of metal containers is the lack of transparency because neither the seed nor any humidity indicator can be seen. Some metal containers are manufactured with seams; these may be a potential source of moisture ingress.

Tri-laminate foil bags are frequently used to contain seeds, but their effectiveness is dependent upon a good heat seal being achieved (constant temperature heat sealers with a sealing bar of at least a 10 mm width are recommended for this purpose). Sharp seeds (e.g., with awns) may puncture the bag. Pre-enclosure in a card envelope can help prevent this. Vacuum sealing can increase the risks of puncturing by seeds and may lead to creasing damage. Only foil designed for long-term storage should be used. Again, foil bags are not transparent, so neither the seed nor any humidity indicator placed inside can be seen.

2. Container Size and Shape/Volume of Seed

A variety of container sizes may be required to hold seed collections, as collections can vary dramatically, both in terms of seed size and number of seeds per collection. This is particularly relevant to wild plant seed banks, due to the diversity of seed size and shape. Within the MSB collections, seed diameters vary a 1000 fold from 100 μm (orchid seed) to greater than 10 cm (palms). Average seed diameter is typically 2–3 mm (J. Wood, pers. com.). For some species, it may be extremely difficult to isolate the seed from its surrounding fruit structures during the cleaning process. In this case, collections are banked as intact fruits which can significantly increase the volume of the collection.

The number of seeds per collection may vary considerably, from tens of seed for very large species, such as palms or very rare ones, to many millions in the case of small seeded orchids. The current average size of MSB collections is 19,470 seeds.

Based on the seeds' physical characteristics and the number of seeds to be banked, the following range of container volumes was selected for the MSB collections: 2; 30; 100; 1,000; and 3,000 cm^3.

The shape of the container is also important. Containers with a wide neck allow easier access for large and irregular shaped seeds. Container shape also affects packing efficiency within the seed bank. Containers with a square cross section pack more effectively than those that are cylindrical.

Finally, labels must be easily read and must adhere well to containers for indefinite periods of time at sub-zero temperatures. Smooth-sided containers are better in this respect.

3. Lid and Seal Construction

There are two routes by which water vapour can enter a container. Firstly, through the material from which the container and lid are manufactured, and secondly, via a poor seal between the container and the lid.

The need to regularly access collections favours the use of containers with screw lids or clamped seals rather than permanent welded glass or metal seals. Due to differences in thermal properties between the lid and the container, screw lids may occasionally loosen during freezing or thawing. This process, referred to as 'backing off', can seriously compromise the seal.

Clamped containers such as storage jars (the best performing containers in experiments 1 and 2, below) or crimped containers such as *Wheaton* vials, are very effective. The constant pressure supplied by the clamp or crimp can overcome differences in thermal expansion or contraction between the lid and the container during freezing or thawing. Unfortunately, such containers may not be readily available in the full range of container size needed for diverse seed collections.

The purpose of a seal is to enhance the hermetic qualities of the container. Seals may be composed of a wide range of substances, including natural rubber, synthetic rubbers, silicone, or waxed card. Both the permeability of the seal and its ability to form a hermetic seal between the lid and the container can vary greatly (as shown by the results of experiments 1 and 2, below).

4. Cost and Re-usability

Finally, cost may be a significant factor in container selection. Most containers can be reused, especially if peelable labels are employed (Table 34.4 shows a comparison of costs for containers used within the MSB).

Materials and Methods

Container Survey

Table 34.1 **Container codes**

Container type	Code	Cap type (material)	Seal type
30 cm^3 glass universal bottle	A	Screw (metal)	Butyl
100 cm^3 aluminium can	B	Screw (metal)	Rubberised paint
100 cm^3 glass *Duran* bottle	C	Screw (plastic)	None
250 cm^3 glass *Duran* bottle	D	Screw (plastic)	None
100 cm^3 glass square *Schott* bottle	E	Screw (plastic)	None
250 cm^3 glass square *Schott* bottle	F	Screw (plastic)	None
500 cm^3 glass square *Schott* bottle	G	Screw (plastic)	None
1,000 cm^3 glass square *Schott* bottle	H	Screw (plastic)	None
5 cm^3 tall glass vial	I	Screw (plastic)	None
5 cm^3 squat glass vial	J	Screw (plastic)	None
2 cm^3 glass vial	K	Screw (plastic)	Waxed card
10 cm^3 aluminium can	L	Screw (metal)	Rubberised paint
20 cm^3 glass *Wheaton* vial	M	Crimped (metal)	Bromobutyl rubber
Tri-laminate foil bag	N	None	None
30 cm^3 glass universal bottle	P	Screw (plastic)	None
3,000 cm^3 glass storage Jar	O	Clamped (glass)	Natural rubber
500 cm^3 glass storage jar	Q	Clamped (glass)	Natural rubber
1,000 cm^3 glass storage jar	R	Clamped (glass)	Natural rubber

Experiment 1. Container Leak Test Utilising Seed Moisture Status

This experiment tested the hermetic qualities of different containers by monitoring changes in seed moisture status over a period of 20 d.

Three replicates of 15 different containers (see Figure 34.1) were filled to 20% by volume with white mustard (*Sinapis alba* L.) seed, previously equilibrated to dry room conditions (18°C/10% RH). The containers were hand sealed in the dry room and then transferred to a relative humidity incubator (LEEC SFC/2CRH) set at 20°C, 95% RH for the duration of the experiment.

The moisture status of the seeds was measured using a hygrometric workstation (Rotronic WA40) consisting of a sample chamber with an integral impedance sensor (with an accuracy of ± 2% RH). Seeds were removed from the container, placed in the sample chamber, and allowed to equilibrate for at least 30 minutes, prior to the equilibrium relative humidity (eRH) being recorded. Seed moisture status was measured at the beginning of the test and then at regular intervals up to and including 20 d. Initial moisture status of the seeds was 11% eRH, and containers that resulted in seed eRH rising above 20% by day 20 were deemed to have failed.

Experiment 2. Container Leak Test Utilising Self-indicating Silica Gel

This experiment tested the hermetic qualities of different containers by monitoring colour changes in self-indicating silica gel over an extended period.

Freshly regenerated (dark blue) cobalt chloride impregnated silica gel crystals (approximately 3 mm diameter) were added to dry-room equilibrated test containers at a rate of one crystal per 30 cm^3 container volume. This equated to approximately 150 mg of silica gel per 100 cm^3 volume of air space. The containers were hand sealed and transferred to a relative humidity incubator as before, for 12 weeks, followed by transfer to the seed bank at -20°C for up to one year. A minimum of ten replicates was used per container type.

The silica gel colour was checked periodically (including 20 d and twelve weeks). If the hermetic seal of the container failed, the silica gel absorbed moisture and the cobalt chloride indicator changed colour from dark blue through light blue to clear and eventually pink. The colour change between light blue and clear occurred at about 20% RH (though this may vary by 1 or 2% RH between batches of indicator). Containers were deemed to have failed the test when 20% or more had leaked by day 20.

The methodology was similar to that used by Gomez-Campo (2002), however the self-indicating silica gel was used at a lower rate (approximately one tenth) and the experiment scored more frequently, thus making the protocol more sensitive and rapid.

Experiment 3. Improving Container Hermetic Qualities Using Sealing Tapes

Results from experiments 1 and 2 indicated that many containers did not seal reliably. This experiment was designed to judge the effectiveness of a variety of sealing tapes, selected for their moisture barrier properties, at improving the sealing of container lids.

Universal bottles with butyl rubber-sealed metal lids that had been equilibrated in a dry room were chosen as the test container. Ten replicates were used for each treatment. Each bottle contained one crystal of freshly dehydrated self-indicating silica gel. The lids of the bottles were hand tightened and then backed off one quarter turn in order to compromise the seal. The tape was applied at a constant pressure for one and one half turns around the circumference of the lid. A control treatment with no tape was included.

Treatments were incubated at 20°C/95% RH for 6 months and checked at 2 d, 4 d and 6 months for indicator colour change. Blue crystals were scored as ‘pass’ and clear or pink as ‘fail’.

Experiment 4. Comparing the Hermetic Qualities of Different Sealing Compounds.

Previous MSBP trials had demonstrated the effectiveness of *Wheaton* vials fitted with bromobutyl seals and crimped metal lids. This experiment examined other possible sealing materials.

20 cm^3 *Wheaton* vials were selected as the test container. Sets of ten replicate containers for each treatment were equilibrated in a dry room and one crystal of freshly regenerated self-indicating silica gel was added to each container prior to closing with colour-coded crimped caps sealed with bromobutyl, chlorobutyl, flurotec, or silicone. The control was left open.

The vials were incubated at 20°C/95% RH and the indicator checked for colour change after 1 d, 1 month and 6 months incubation. Blue crystals were scored as ‘pass’ and clear or pink as ‘fail’.

Results and Discussion

Experiment 1. Container Leak Test Utilising Seed Moisture Status

Hermetically-sealed containers should have maintained the original seed eRH of approximately 11%. However, 100 cm^3 aluminium cans (B), 1,000 cm^3 square *Schott* bottle (H), 5 cm^3 tall glass vial (I), 5 cm^3 squat glass vial (J), and 10 cm^3 aluminium cans (L), all failed to seal adequately (Figure 34.1). The remaining containers passed the test criteria; the 3,000 cm^3 clamped storage jar (O) performing best. Some containers, such as the 30 cm^3 universal bottle with metal lid (A), came close to the fail criteria and further work (not shown) confirmed their poor performance.

The methodology clearly demonstrated the sealing properties of various containers. Measuring the eRH of seeds also meant that the test more closely mimicked seed bank conditions. However, due to the rather lengthy equilibration times, moisture measurement proved time consuming and limited the number of replicates per treatment. In addition, the 20% by volume of mustard seeds held in each container meant that a considerable

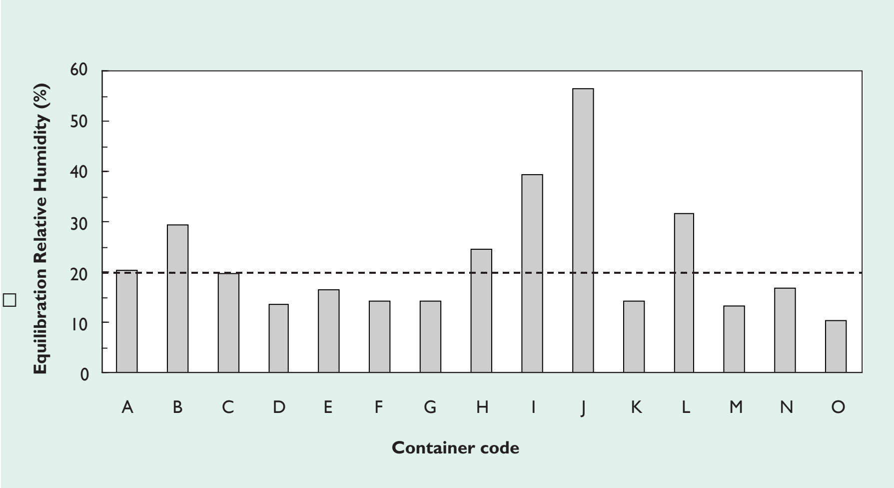

Figure 34.1 A survey of the sealing properties of 15 different seed containers. Results show the equilibrium relative humidity of white mustard seeds following drying in a dry room, packaging and 20 d exposure to an ambient relative humidity of 95% RH (see text). The criterion for containers failing the test was an eRH value of 20% or more (broken line). The containers are labelled as shown in Table 34.1.

amount of water vapour ingress would be required for a measurable increase in seed eRH. Thus in order to test greater numbers of containers, a second more sensitive testing system was developed.

Experiment 2. Container Leak Test Utilising Self-indicating Silica Gel

Five container types passed the test criteria at 20 d. These were the 100 cm^3 square *Schott* bottles (E), 2 cm^3 glass vials (K), 30 cm^3 plastic-capped universal bottles (P), 500 cm^3 storage jars (Q) and 1,000 cm^3 storage jars (R). The 100 cm^3 aluminium cans (B) and 250 cm^3 square *Schott* bottles (F) failed (Figure 34.2). After three months, only four containers retained an adequate seal, the 2 cm^3 vials (K) failing at this point.

The four successful container types were transferred to the seed bank cold room (-20°C/60% RH) and scored after a further 1 year of storage. At that time, the plastic-capped universal bottles began to show evidence of failing at 70%, whilst the 100 cm^3 *Schott* bottles and two sizes of storage jar continued to perform well at 92%, 100% and 100%, respectively.

This improved testing method permitted relatively quick measurement of container sealing qualities and enabled the selection of appropriate containers for the MSB collections.

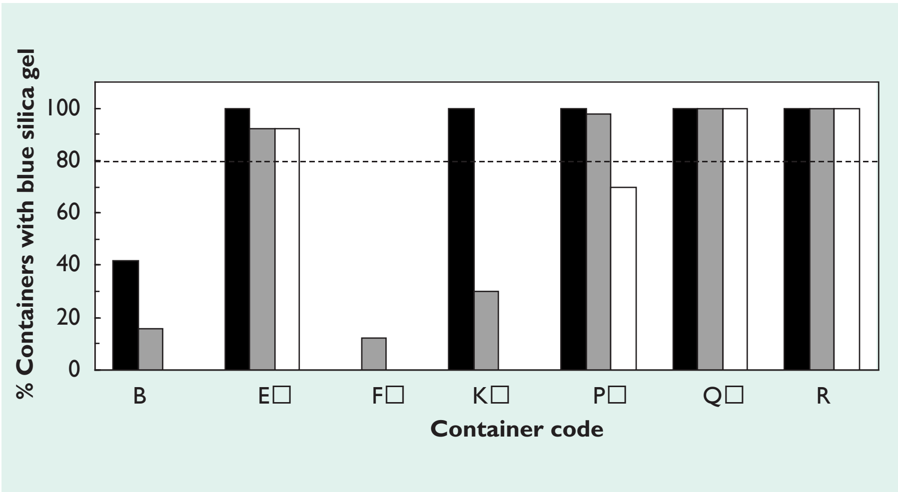

Figure 34.2 A survey of the sealing properties of seven different seed containers. Results show the percentage of containers of each type containing dry (dark blue) indicating silica gel after 20 d (■) and 3 months (▒) exposure of the container to an ambient humidity of 95% RH at 20°C and then transfer to 60% RH at -20°C for 1 year (☐). Containers were deemed to have failed if < 80% (broken line) of the replicates contained blue indicator crystals. The containers are labelled as shown in Table 34.1.

Experiment 3. Improving Container Hermetic Qualities Using Sealing Tapes

All of the tapes ultimately failed with PTFE tape and *Nescofilm* performing worst and PVC insulating tape performing best (Table 34.2).

It is likely that a combination of tape porosity and poor sealing between the contoured lid and the bottle surface led to failure. It may be concluded that some tapes such as PVC insulating tape may reduce the rate of leakage but not prevent it altogether.

Experiment 4. Comparing the Hermetic Qualities of Different Sealing Compounds.

Bromobutyl, chlorobutyl and flurotec are effective as a barrier against water vapour. Silicone does not prevent ingress of water vapour (Table 34.3).

Electron micrographs were taken of three commonly used sealing materials – the natural rubber seal from storage jars, the bromobutyl seal from universal bottles, and a food grade silicone seal (Figure 34.3).

Table 34.2 **Effect of different sealing tapes on hermetic qualities of universal bottles at 20°C/95% RH**

Tape type	% Failure @ 2 d	% Failure @ 4 d	% Failure @ 6 months
No tape (control)	100%	–	–
All-weather PVC insulating tape	0%	20%	90%
Duct tape	30%	80%	100%
Closure plate tape	50%	60%	90%
PTFE tape	100%	–	–
Matt Gaffa tape	20%	40%	90%
Nescofilm	100%	–	–

Comparison between the micrographs (Figure 34.3), shows that the bromobutyl seal is considerably more contoured than the other two seals. This may explain why metal-capped universal bottles seal poorly. However, the surface structure of the natural rubber seal and silicone seal is relatively smooth, yet the silicone seal consistently fails to prevent moisture ingress. The explanation may lie in the constant rate of leakage found between replicates (not shown), suggesting that water vapour can migrate through the silicone itself rather than around a poor seal between lid and container. This is further supported by the results in Table 34.3.

Although the choice of a good sealing compound can greatly improve the hermetic qualities of a container, there is evidence that well designed plastic lids without seals can perform equally well. Glass universal bottles fitted with polypropylene lids (Figure 34.2, container P) out-performed similar bottles with metal caps and butyl rubber seals (Figure 34.1, container A).

Table 34.3 **Effectiveness of different seal types**

Seal type	% Failure @ 1 day	% Failure @ 1 month	% Failure @ 6 months
No seal (control)	100	100	100
Silicone	100	100	100
Bromobutyl	0	0	0
Chlorobutyl	0	0	0
Flurotec	0	0	0

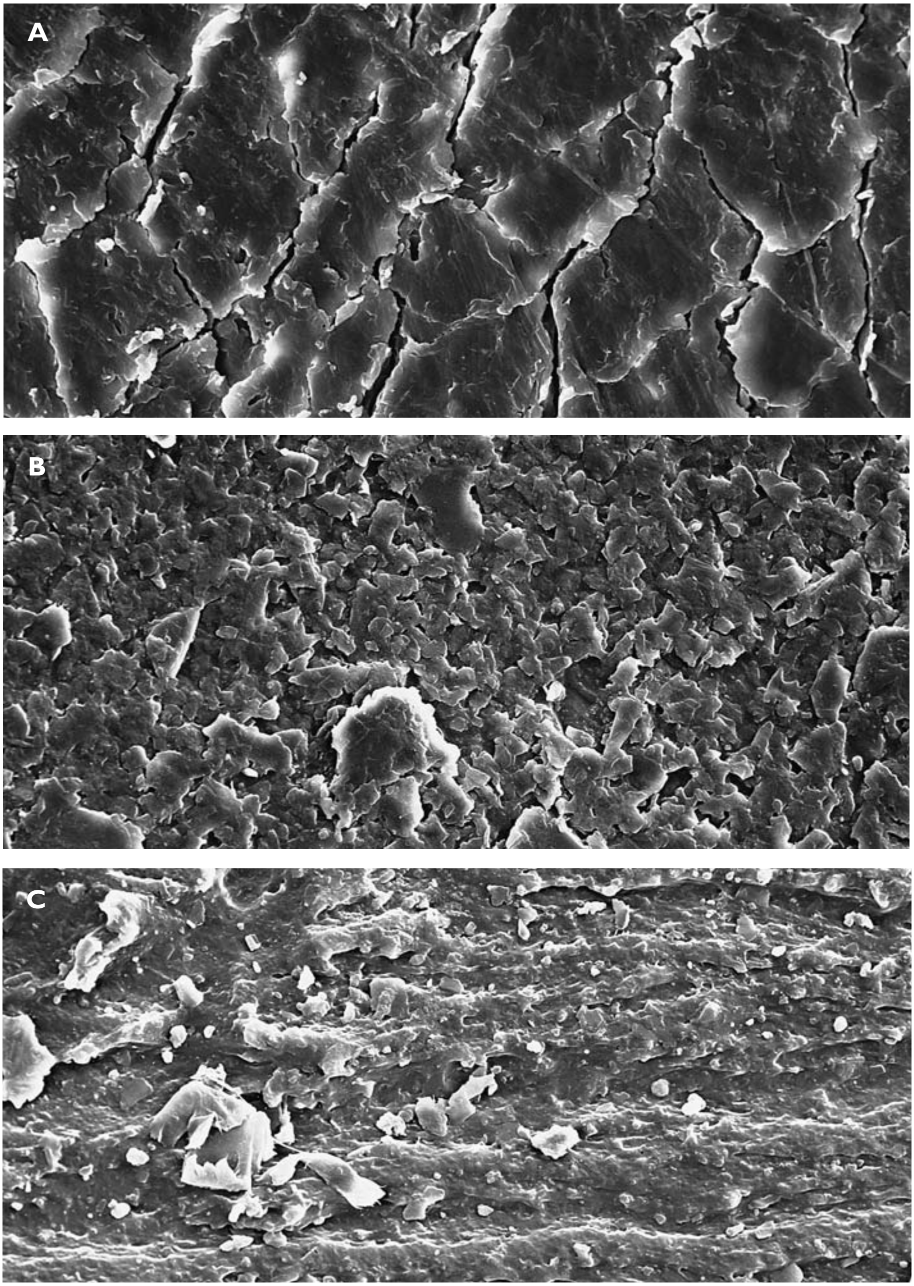

Figure 34.3 Electron micrographs (× 500 magnification) of three seal types

A Natural rubber seal from 3,000 cm^3 glass storage jar (O),

B Food grade silicone rubber seal specially manufactured for 3,000 cm^3 glass storage jar

C Butyl rubber seal from glass universal bottle (A)

Use of Relative Humidity Indicators During Storage

Cobalt chloride is a chemical indicator that is commonly used with silica gel. It is blue when dehydrated and pink when fully hydrated. The colour change from dark blue occurs at about 20% eRH. This form of silica gel indicator is available in transparent, permeable plastic sachets containing 1 g of silica beads. If the sachets are pre-equilibrated to the same eRH as the stored seed ,the silica gel will not cause any further drying of the seed to potentially sub-optimal levels.

Provided the container is transparent, a small quantity of self-indicating silica gel stored with the dry seeds can be utilised as a visible check of container performance. If the container leaks and allows ingress of water vapour, the indicator will change colour. This simple and cheap technique can be employed as a valuable quality control system for long-term seed banks.

It should be noted that the cobalt chloride indicator has been classified as toxic (carcinogenic by inhalation). Care should be taken when handling this product. Other safer indicator systems are currently under evaluation by the MSBP.

Conclusions

Many of the containers tested had poor sealing qualities. Taping the containers slowed down but did not prevent water vapour ingress.

The best performing container was the storage jar, its success being attributed to the use of a natural rubber seal and clamped lid. It is worth noting that at the MSBP the seals are renewed every 10 years as a precaution against ageing of the natural rubber.

Experience within the MSBP has found that tri-laminate aluminium foil/polypropylene bags were also found to seal well provided an effective heat seal is applied (Figure 34.1, container N). These results support those of Freire and Mumford (1986); aluminium cans however, did not perform well in our tests, contrary to their results.

As a result of the studies described here, five types of glass container (Figure 34.4) were selected for long-term seed storage at -20°C. The 2 cm^3 glass vials are ideal for very small seed and two vials will fit in a 30 cm^3 universal bottle for easier storage. The 30 cm^3 plastic-capped universal bottles and the 100 cm^3 square *Schott* bottles are the most commonly used containers, whilst the 1,000 cm^3 and 3,000 cm^3 storage jars allow for the storage of large seeds. All of the containers are easy to label, have wide necks relative to volume and are reusable (see Table 34.4 for details on costs and suppliers).

Figure 34.4 The five selected types of glass container currently in use at the Millennium Seed Bank. From rear to front: 3,000 cm^3 storage jar (O), 500 cm^3 storage jar (Q), 100 cm^3 square Schott bottle (E), 30 ml universal bottle (P) and 2 cm^3 vial (K).

All of the chosen containers prevent ingress of water vapour very effectively under the stringent test conditions used in the study. It is expected that the containers will perform well during long-term storage, however in the event that a container should leak, this would be detected by the 1 g indicating sachets now included with each seed sample. Double packing containers (as for the 2 cm^3 vials) can further reduce the likelihood of leakage.

The use of 1 g sachets of self-indicating silica gel pre-equilibrated to the same relative humidity as that used for drying the seeds is a highly effective and cheap method for monitoring containers during storage.

Table 34.4 **Container specifications and suppliers**

Container description	Supplier	Suppliers code	Unit Cost UK (January 2003)*
2 cm^3 glass vial and plastic lid with PTFE coated seal	Scientific Laboratory Supplies Ltd www.scientific-labs.com	LS32008-1232 LS5360-08	£0.14
30 cm^3 glass universal bottle with polypropylene lid	Scientific Laboratory Supplies Ltd www.scientific-labs.com	LS128044F	£0.19
100 cm^3 square glass *Schott* bottles with polypropylene lid	Fisher Scientific Ltd www.fisher.co.uk	BTF645022Q	£1.53
1,000 cm^3 Storage jars	Fisher Scientific Ltd www.fisher.co.uk	BTF760050T	£2.63
3,000 cm^3 Storage jars	Fisher Scientific Ltd www.fisher.co.uk	BTF760110E	£5.43
20 cm^3 *Wheaton* vials	Fisher Scientific Ltd www.fisher.co.uk	VGA220041K	£0.53
Foil bags	Barrier Foil products Ltd	160 × 240mm 500 × 250mm	£0.22 £0.80

* Note, £1 = US$1.61 (January 2003)

Finally, the silica gel testing protocol as detailed in experiment 2 is a rapid, sensitive and easy way to screen containers. Although this method can be used to assess container performance over a 2–3 week period, it is recommended that containers are also tested at the cold store temperature for several months. Furthermore, due to manufacturing variation, it is recommended that each batch of containers purchased should be tested using the indicating silica gel protocol described.

Acknowledgements

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