# Cleaning seed collections for long-term conservation

Technical Information Sheet 14

Seeds are cleaned to decrease bulk, reduce disease risk, and facilitate future use. Cleaning long-term conservation collections without causing physical damage and reducing seed viability requires care and expertise. This information sheet outlines the seed cleaning techniques used most frequently by the Millennium Seed Bank (MSB).

## Seed diversity

Seeds are dispersed from the parent plant in a variety of ways and forms. Many are released within winged fruits or are blown great distances by feathery 'parachutes'. Others are contained within brightly-coloured fleshy fruits, designed to be eaten by birds. Some seeds develop inside hooked fruits that attach to the fur of passing animals. This diversity of dispersal mechanisms poses challenges when cleaning seeds.

## Seed cleaning principles

Seed cleaning aims to process field-harvested material to a collection of clean, viable plant propagules (seeds or fruits) without incurring damage or loss.

### Box 1: Equipment and materials

- Stainless steel sieves with a range of mesh sizes
- Aspirator
- Rubber bung
- Wire brush
- Small bristle brushes
- Forceps
- Scalpel
- Microscope
- Safety gloves
- Lab. coat
- Dusk mask, dust hood
- Leather gloves
- Large trays
- Paper packets and fasteners for cleaned material
- Vacuum cleaner

Some seeds, for example those from species with dry capsules, are collected directly and require only minimum cleaning. More often, the fruit is collected and seeds need to be extracted.

Cleaning involves:

- removing undesirable bulk (twigs, leaves, etc.) and debris;
- extracting seeds from fruits;
- removing empty and/or insect infested seeds, seeds of other species, insects or inert matter.

Seed cleaning of conservation collections is best done by hand because:

- automated processes produce unacceptable levels of physical damage to seeds;
- automated processes, generally developed for crop seeds, may not be sufficiently flexible to deal with the diversity of seeds and fruits produced by wild species.

Wash fleshy fruits in a sieve to remove fruit pulp, dry slowly under ambient conditions for two weeks (see Technical Information Sheet 04) and then process as dry seeds. Ideally, dry collections in a dry room or a desiccator for several weeks before cleaning, to increase the ease of processing and reduce the risk of physical damage to the seeds.

Examine fruit and seed structures to highlight potential problems (and to give important clues to germination requirements). Use different cleaning techniques, according to the type of seed or fruit (see Box 3).

Figure 1: *Pachycereus* seeds, removed from fruits by hand and sieved to separate debris.

Figure 2: *Sophora* seeds being cleaned by hand.

First, remove as much bulk material as possible by hand (Fig. 1). If live insects are present, dry the collection to below 20% equilibrium relative humidity (eRH) then place at -20°C for at least one week.

For some collections, it may be difficult to ascertain the best method of cleaning. In such cases, it is best to refer to methods used for similar species. It may not always be easy or cost-effective to remove seeds from fruits. Always test new approaches on a small sample first, to avoid damaging the entire collection.

Pursue cleaning to a reasonable end point, taking care to avoid damaging seeds, whilst being as efficient as possible. Physical extraction of seeds from covering structures may be so time-consuming that it becomes more cost effective to store a bulkier collection.

## Seed cleaning techniques

There are four main techniques for cleaning a seed collection:

- Using sieves of different mesh size, and a rubber bung if necessary, to separate seeds from bulk material and smaller debris.
- Using a seed aspirator to remove similarly sized, but lighter/heavier, empty/infested seeds or debris.
- Hand-sorting, involving piece by piece removal of debris and rubbish (Fig. 2).
- Rubber mat and finger rubbing which is most gentle and good to remove pappus.

## Sieving

This is the most commonly used cleaning method. Use a selection of sieves (Fig. 3), depending on the seed/fruit size and the materials that are to be removed. The seed/fruit should be able to fall through the sieve without damage, but the mesh should not be so large that unwanted material also passes through. The last sieve should be small enough to retain the seed and let dust through. If the collection is large, work out the best procedure on a small sample first.

- Place a small quantity of material into the top sieve (the largest of the mesh sizes chosen) and gently rub with a rubber bung if necessary, until most of the seeds have passed through the sieve. Use a mechanical sieve shaker or gently rub with fingers (using rubber gloves) if a rubber bung might cause damage, for example with some species of Compositae.
- Check the fraction remaining in the top sieve for any trace of seed. Use a binocular microscope if necessary. If no seed is found, discard the material.
- Look at the material in the next sieve to see if any seeds have been damaged by the process. If damage is found, select another cleaning method.
- Repeat for each sieve, working down the mesh sizes.
- Ideally, seed will remain in a sieve with material of a similar size, but of a different weight. This fraction can then be separated by aspiration.

## Using an aspirator

Aspirators (Fig. 4) remove lighter material from a collection such as chaff and empty seeds. Use after sieving or as a procedure on its own.

- Place the entire collection, or a sub-sample of the collection, into the hopper of the aspirator.
- Set the air flow so that debris and, where possible, infested and empty seeds will be lifted away from full seeds - some trial and error will be needed to determine the correct flow rate.
- Examine the separated fractions, using a microscope if necessary.
- If seeds are present amongst the 'rubbish', carry out a cut test to determine whether they are full, empty or infested.
- If full seeds are present, return the debris to the aspirator hopper and re-blow using a lower air flow.
- Examine the 'seed' fraction for debris and empty seeds. If necessary, return the material to the aspirator hopper and carry on blowing, checking material as above.
- Once all possible debris and empty/infested seed has been removed, check the final seed fraction. If there is still a significant quantity of debris or empty/infested seeds present, it may be necessary to sort by hand (Fig. 5).
- Use reverse aspiration to remove light seed (e.g. *Betula*) from heavy debris.

## Cleaning fleshy fruits

- Mature seeds within fleshy fruits may lose viability rapidly, so these collections should be dealt with immediately.
- Treat all fruits as potentially poisonous. Wear gloves of a suitable thickness.
- Open fruits with a scalpel, or rub/squash with fingers or a bung.

Figure 3: Sieves of different mesh sizes.

Figure 5: Cleaning by hand sorting.


Figure 4: Zig-zag aspirator.

### Box 2: Cut-test

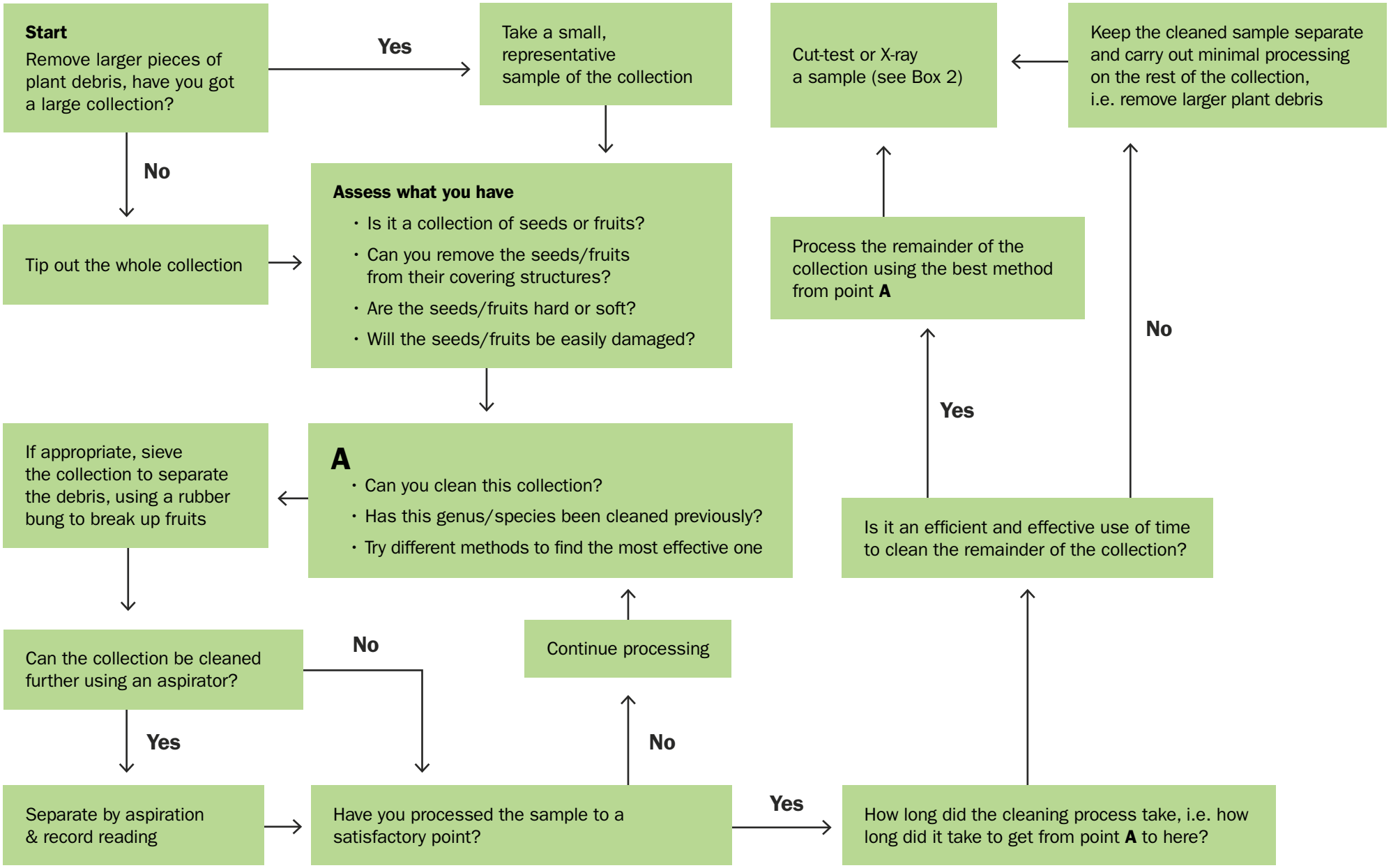
Cut-testing is used to assess the quality of collections after cleaning. It provides an indication of the proportion of empty, poorly developed or insect-infested seeds.

**Table 1:** Representative sub-samples of seeds

| Collection size | Cut-test sample |
|---|---|
| > 2,500 seeds | 50 seeds |
| 1,299 - 2,500 | 20 seeds |
| 259 - 1,299 seeds | 10 seeds |
| < 259 seeds | 5 or 10% of seeds |

- Dissect seeds under a microscope using a scalpel.
- Record the numbers of full, empty and infested seeds, noting anything else you may find of interest, such as the size and positioning of the embryo, and any indication that the seed may not be fully mature. Insect damage often appears as a tunnel of increasing size, usually with a cavity where the larva is found. An exit hole may also be apparent.
- If the cut-test reveals an easily removable fraction of empty or damaged seeds, re-clean the collection.

# Box 3: Seed cleaning process chart for dried material



**Box 4: Other techniques for cleaning dry seeds/fruits**

### Hand Sorting

Collections containing a large quantity of infested seeds, which cannot be separated using an aspirator, may be sorted visually. Only use hand-sorting if it can be completed in a reasonable amount of time.

- Sort large seeds on a smooth flat surface, separating good seeds from bad by eye.
- Use an magnifying glass to sort small-seeded collections.

### Bag crushing

Good for collections with flat pods and for breaking down spiky material. Also useful for collections where sieve and bung is too rough and gloved rolling is unsuitable. Agitate collection in a cloth bag (wear gloves). Check a sample first to ensure material is robust enough to withstand procedure.

- Can be used to process large amounts of material.
- More effective than sieve and bung in removal of 'hairs'.
- Makes subsequent aspiration of collection easier.

### Capsule shaking

Use with Scrophulariaceae, Caryophyllaceae and Crassulaceae collections. Shake capsules to release seeds. Ensure the capsules are completely empty of seed before discarding.

- Good for collections where it would be difficult to separate seed and debris if fruits were crushed.
- Significantly reduces cleaning time.

### Coating sticky seeds in wood ash

Suitable for sticky and oily collections such as some species of Pittosporaceae. Mix sieved wood ash with collection to absorb oil and stop seeds sticking together. Sieve off any excess ash. The collection can then be aspirated.

- The ash does not scratch equipment and is not harmful to the seeds.

- Scrape out seeds into a sieve with a mesh size small enough to retain the seeds. This operation may be carried out under cool, running water to facilitate seed removal.
- Wash away any mucilage with warm (never hot) water.
- Allow seeds to drain on a nylon mesh or sieve, and then dry slowly under ambient conditions for at least 2 weeks before transferring to a dry room. Make sure that the collection is clearly labelled.
- Do not place wet seeds on paper towels or newspaper as they will be difficult to remove once dry.
- After drying, remove any remaining debris using cleaning procedures for dry seed.

## After cleaning

- Label all collections.
- Record how long it took to process the collection and include the final cut-test results (see Box 2).
- Place the cleaned collection in a dry room or desiccator.
- Clean working areas carefully, to prevent cross-contamination.

Figure 6: Delicate, wind-dispersed *Asclepias* seeds, being cleaned under a dust hood.

## Microseeds

Microseeds need to be cleaned and handled very carefully. Please refer to separate information.

## Health and Safety

Some collections may be excessively dusty or contain fungal spores. Wear a lab. coat and follow good laboratory practice at all times.

- Grinding, crushing and sieving may release large amounts of dust. Carry out cleaning processes in dust-extraction cabinets (Fig. 6) or wear a protective mask.
- Treat all seed collections as potentially poisonous. Do not process if you have a known allergy to any materials.
- Clearly label and be aware of any collections with irritant properties. If irritant hairs are found, wear protective gloves and carefully clean any equipment after use.


## Acknowledgements

J. Terry, V. Sutcliffe, N. Mills & S. Gattiker, RBG Kew.


## Further reading


Terry J., Probert R.J. and Linington S.H. (2003). Processing and maintenance of the Millennium Seed Bank collections. In: R.D. Smith, J.B. Dickie, S.H. Linington, H.W. Pritchard and R.J. Probert (eds), Seed Conservation: turning science into practice. Royal Botanic Gardens, Kew, UK.


## Equipment specifications*

| Description | Model/Product | Supplier |
|---|---|---|
| Sieve set | • 5.6mm, 3.35mm,2.36mm, 1.4mm, and 600µm sieves<br>• Base pan<br>• Brush<br>• Rubber bung<br>• Stainless steel sieve lid | Fisher Scientific Ltd www.fisher.co.uk |
| Dust-control cabinet with modified lower front panel and light | Powder handling workstation with HEPA filtration | Bigneat Ltd www.bigneat.com |
| Agriculex seed aspirator with acrylic catcher | Agriculex CB1 | Agriculex Inc. www.agriculex.guelph.org |
| Zig-Zag seed aspirator | Zig-Zag type 1 | Strong Seed https://strongseed.org/sorting/zz-gravity-seperator |

*Please note that the above equipment is used by the Millennium Seed Bank and has been chosen carefully using our many years' experience. The list of suppliers is for guidance only and does not represent an endorsement by the Royal Botanic Gardens, Kew. The manufacturer's instructions must be followed when using any of the equipment referred to in this Information Sheet.