

Seed cleaning and cut testing practical exercise

Objective

To become familiar with the strengths and weaknesses of basic seed cleaning techniques and varying suitabilities to a range of harvested material. You will also be introduced to the technique of cut-testing seeds as a means of evaluating the quality of seed collections.

Seed Cleaning Introduction and Principle.

To prepare seeds for storage, it is necessary to remove them from other plant material and any foreign bodies which are present. The variety of seeds presented necessitates employing a number of differing techniques. These will vary according to the type of seed/fruit i.e. wet or dry fruit, size, robustness etc. Dry seeds/fruits are stored in the dry room before processing, desiccation often improving the ease with which they can be cleaned. In general, a selection of variously-sized sieves is initially employed to release the seeds, followed by the use of an appropriate aspirator to remove debris; hand sorting may be used as a final resort. Wet fruits need more immediate attention, being washed in a sieve to remove fruit pulp then dried and processed as for dry seed. There are often collections where these techniques are inappropriate, or it is difficult to ascertain the best method of cleaning.

Objectives: Removal of wet fruit/seed head/capsule material, empty and/or insect infested seeds.

- **Reasonable** reduction of plant bulk by extracting the smallest plant propagule from the larger plant structures, **without** incurring any damage or losses to the collection.
- Remove (or record, if not possible) contamination of collection by other seed species, insects or inert matter.

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

1. automated processes inevitably give levels of seed damage during processing, which are unacceptable for conservation purposes.
2. automated processes are not flexible enough for the diversity of material handled by a seed bank conserving wild species.
3. cleaning equipment takes a long time to clean between seed lots

Cleaning is pursued to a reasonable end point. If further cleaning will result in damage to the collection, the final result will have to be less than perfect. Also, physical extraction of seeds from covering structures may be so time consuming that it becomes more cost effective to store a bulkier collection. (In these cases, make a note that there may be extraction/sampling problems when removing samples from the bulk for germination or despatch.)

This practical will cover a selection of the most commonly used seed cleaning techniques used by the Millennium Seed Bank but it can never be an exhaustive source of information. The underlying principles, as detailed above, are the most important thing. Once these are understood, the technician may employ whatever methods and tools are at his or her disposal in order to achieve the desired end result.

You may be cleaning to a less high standard if you are not intending your seed is for long term storage or if you are cleaning seed mixtures but the principles will remain the same.

Materials

- Dissecting microscopes
- Dissecting kit
- Pre-collected plant material
- Seed cleaning equipment

Notes on the cleaning techniques employed are detailed in Appendix 1.

Wet fruits

Wet, fleshy material should be dealt with immediately by squashing berries etc. through sieves and washing with warm water. The extracted seeds are then spread in a thin layer (not on paper) and dried down to "safer" moisture contents.

Sticky seeds

These can be cleaned more easily if coated in a material such as wood ash or unscented talcum powder.

Dry material can be extracted and cleaned using several techniques:

- 1) Gentle crushing/grinding using a rubber bung and suitable size of sieve (separation of seed, etc. from bulk material).
- 2) Hand sorting (individual, piece by piece removal of debris, seeds with obvious infestation etc.).
- 3) Gloved rolling on a rubber mat. A more gentle technique than sieve and bung and very effective with 'fluffier' or fragile seeds.
- 4) Weight separation using a seed aspirator (removal of lighter/heavier elements from collection i.e. debris or empty/infested seeds).
- 5) Bag crushing. Agitation/friction of the collection within the confines of a cloth bag. Useful on collections where sieve and bung is too rough and gloved rolling is unsuitable.
- 6) Capsule shaking. Used mainly on collections such as Scrophulariaceae, Caryophyllaceae and Crassulaceae where debris is very difficult to separate from seed if crushed, and the ripe seed are easily removed from the capsule by shaking.

Appendix 1

Wet fruits. (Example: *Skimmia japonica*: Rutaceae)

- Process immediately upon arrival.
- Immature fruits may need ripening first.
- Warm, not hot, water.
- Do not spread wet seeds on paper towels or newspaper because they stick to them when dry. Use a plastic or cloth mesh.
- Spread seeds in thin layer. Turn if necessary while drying.
- Equilibrate to ambient conditions before placing in drying room or silica gel dryer.
- Once dry, process as for dry seeds.

Sieve and bung. (Example: *Eryngium maritimum*: Umbelliferae)

- Select appropriate sieve sizes.
- If collection is large, work out best procedure on a small sample first.
- Remove large debris first by hand.
- Ensure seed will not be damaged during rubbing.
- Rubbing will produce dust – wear mask or use cabinet.
- Check each fraction for good seed before discarding.

Hand sort.

Only to be used when all other methods prove unsuitable.

- How long will it take?
- Do the results justify the effort?

Gloved rubbing. (Example: *Distephanus polygalifolius*: Compositae)

- For collections where sieve and bung is too destructive.
- Very often used on Compositae (Asteraceae) and Poaceae.
- Remove as much large material as possible first by hand.
- Ensure seed will not be damaged during rubbing.

Aspirating. (Example: *Albizia boinensis*: Leguminosae Mimosoidae)

- May be used after sieving/gloved rubbing or as a procedure on its own.
- Will remove lighter material such as chaff, empty seeds.
- Reverse aspirating can be used to remove light seed from heavy debris.
(*Betula*)
- Experiment with a small sample to find best conditions to use.
- Check 'debris' carefully for filled seeds before discarding.
- Fractions can be re-combined and re-aspirated if wrong conditions are used.

Bag crushing. (Example: Asclepiadaceae)

- Check a sample first to ensure material is robust enough to withstand procedure.
- Proves more effective than sieving and binging in removal of 'hair'.
- Good technique for flat pods.
- Aspiration of collection then easier.
- Can deal with larger amounts of material than sieve and bung.
- Good in breaking down spiky material (wear gloves).
- Can also generate dust so take precautions.

Foot crushing. (Example: Acacia schnaffneri: Leguminosae)

- Useful for fruits very tough to crush with sieve and bung.
- Need to check for damage on a sample.
- Care as lot of dust can be generated.
- Heavier tread can cause damage.
- Can then sieve and aspirate as normal.

Capsule shaking. (Example: Verbascum, Lychnis)

- Used particularly on Scrophulariaceae and Caryophyllaceae.
- Especially for collections where seed and debris are difficult to separate when crushed.
- Cleaning time significantly reduced.
- Must ensure the capsules are completely empty of seed before discarding.

Oily or Sticky seeds. (Example: Pittosporum colensoi [oily], Juniperus [sticky])

- Coating seeds in wood ash/talcum powder stops them sticking together.
- Sieve wood ash first.
- This enables seeds to be aspirated.
- The ash does not scratch the equipment and is not harmful to the seeds.
- Useful for oily species such as Pittosporum as the ash absorbs the excess oil.

Mixed collection.

How many seed types are there?

- Which is the correct type?
- Is there any morphological variation to take account of?
- How are we going to clean this?

Morphological variation. (Example: *Calendula officinalis*, Compositae.)

- Disc and ray florets produce morphologically distinct seeds.
- May appear to be a mixed collection.
- Can be seen in families such as Compositae (Asteraceae) and Umbelliferae (Apiaceae).
- May produce issues relating to cleaning and germination testing.
- If in doubt, keep everything and grow material for verification.

Cones. (Example: *Banksia*, *Pinus*)

- Hold cones with scales opening away from you.
- Use metal tongs and safety glasses and hold cone over container to catch loose seeds.
- Use blowtorch so heat does not go inside cone scales and does not overheat any one area.
- When cones are open, place in dry room to allow opening to continue and seeds to fall out.

Cut Testing - Examining Seed Structure

Cut testing is the method used to determine seed quality after cleaning. An examination of fruit and seed structure will highlight potential problems and can also give important clues to germination requirements.

Seeds are destroyed during the process and some of the finer detail, especially in small or hard coated seeds, may be lost.

Equipment and materials.

- Cleaned seed material.
- Binocular microscope.
- Dissecting equipment.

Procedure

- Randomly select seeds to be dissected.
- Cut open under a microscope.
- Determine numbers of full, empty and infested seeds, noting anything else you may find of interest.

Questions to ask:-

Is the seed endospermic or non-endospermic?

What does the embryo look like (if it can be seen)?

Where is the embryo?

Are there insect damaged/empty seeds/immature seeds?



Cleaning Exercise - Initial Visual examination

Examine the material you are provided with, using a dissecting microscope if necessary, and attempt to answer the following questions:

What has arrived from the field? Is it a seed or a fruit?

What do you wish to bank or sow? (i.e. loose seeds, fruits, pods?) and how will you achieve this? Experiment with a sample of the collection and decide what the most practical final state is.

When answering the above, consider the following points, using cut tests under the microscope where appropriate:

Is the collection contaminated with seeds from other species?

Is the seed going to be lost or damaged easily during cleaning?

Are there obvious empty/aborted/infested seeds?

Does the seed appear fully mature?

Following the initial evaluation, clean the seed collection using the most appropriate technique (see above).

Health and Safety:

- Treat all seed collections as potentially poisonous.
- Good laboratory practice must be followed.
- Protective clothing should be worn.
- Clearly label and be aware of any collections with irritant properties and wear protective clothing.
- Be aware of any collections to which known operatives are allergic.
- Where collections require processing think about protection of yourself and others from dust. I.e. facemask, goggles etc.
- Think about disposal of plant and seed waste carefully. Consider introduction of alien species, diseases etc.
- Always clean working areas carefully, both after each collection is cleaned (to prevent cross contamination), and at the end of each cleaning session. Ensure the floor area where you are working is also hoovered.
- Remove lab coat when leaving the cleaning room and wash hands.
- Beware of sharp objects such as scalpels, spiny seed and some types of plant material.

Materials and costs:

- Sieves cost in the region of £50 each and are widely available.
- Agriculex seed blower costs around £1000
- Selecta 'zig zag' seed blower costs around £5000