MILLENNIUM SEED BANK PROJECT Kew

Identifying desiccation-sensitive seeds

Kate Gold and Fiona Hay, Seed Conservation Department

The first stage in developing an *ex-situ* conservation strategy for a particular plant species is to determine seed storage behaviour. Seed desiccation sensitivity may limit the conservation of some species, particularly trees and shrubs from wet tropical habitats. This information sheet provides practical guidance on identifying desiccation sensitive seeds.

Seed storage behaviour refers to the capacity of seeds to survive desiccation:

- Desiccation tolerant, or orthodox, seeds can be dried, without damage, to low moisture content (mc). Seed longevity increases with reductions in mc and temperature in a quantifiable and predictable way.
- Desiccation sensitive, or recalcitrant, seeds do not survive drying to any large degree, and are thus not amenable to long-term storage, although the critical moisture level for survival varies among species.
- Intermediate seeds tolerate drying to around 8% mc. They generally lose viability more rapidly at low temperature and do not withstand storage at -20°C.

How do you know if seeds are desiccation sensitive?

- RBG Kew's Seed Information Database:

  This database currently contains information on seed storage behaviour for more than 10,600 species.

- Desiccation tolerance screening:

  Protocols, e.g. Hong & Ellis (1996), to determine seed storage behaviour are based on drying seeds to two or three different moisture levels and assessing percentage germination. To date, about 540 species with desiccation-sensitive seeds have been identified by the Millennium Seed Bank, using such methods.

Most of these are trees and shrubs; very few herbaceous species produce desiccation-sensitive seeds.

If very few seeds are available, a 100-seed test (see box overleaf) can provide a preliminary indication of desiccation tolerance.

- Seed morphology:

  A good indication of desiccation sensitivity is provided by seed morphology. With the exception of palms, desiccation sensitive seeds tend to be large with thin seed coats. A recently developed model (see box overleaf) uses seed weight and seed coat ratio (SCR = seed coat mass : whole seed mass) to predict the likelihood of woody species possessing desiccation sensitive seeds.

- Seed provenance:

  This can also provide clues about the likelihood of desiccation tolerance.

  - Tropical rainforest species are more likely to produce desiccation sensitive seeds.

Above: *Washingtonia filifera*, a palm species proven to be desiccation tolerant using the 100-seed test

- Savannah species are more likely to produce desiccation tolerant seeds.
- Palms from dryland habitats nearly always have desiccation tolerant seeds.
- In drier habitats, species which shed their seeds during the wet season are more likely to have desiccation sensitive seeds than species that shed seeds during the dry season.
- Contrary to common belief, most aquatic species produce orthodox seeds.

Below: Longitudinal section of an *Aesculus hippocastanum* seed, a desiccation sensitive species - note the thin seed coat in comparison to seed size

Predicting desiccation sensitivity in woody species

(adapted from Daws *et al.*, 2006)

- Collect a sample of fruits at natural dispersal; remove and clean seeds, but do not remove the woody endocarp surrounding seeds formed in berries or drupes.
- Dissect a minimum of eight individual seeds (dispersal unit) into their component parts: endocarp/testa and embryo/endosperm.
- Dry at 103°C for 17 hours (ISTA, 2007) and determine dry weight.
- Calculate Seed Coat Ratio (SCR) as follows:

$$\text{SCR} = \frac{\text{dry weight of the covering structures (endocarp and testa)}}{\text{dry weight of the total dispersal unit}}$$

- Use the following equation to predict the likelihood of desiccation sensitivity (P):

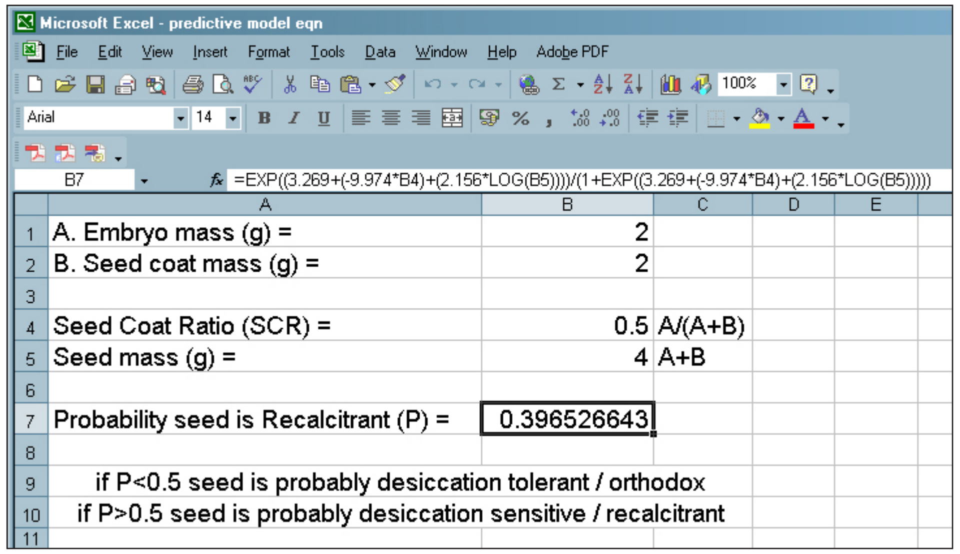
$$P = \frac{e^{3.269 - 9.974a + 2.156b}}{1 + e^{3.269 - 9.974a + 2.156b}}$$

where **a** is SCR and **b** is $\log_{10}$ (seed dry weight) in grams. This calculation can easily be carried out by inserting the following formula into a spreadsheet such as Excel (typed on one line):

=EXP((3.269+(-9.974*B4)+(2.156*LOG(B5))))/(1+EXP((3.269+(-9.974*B4)+(2.156*LOG(B5)))))

If P is greater than 0.5, species are likely to be desiccation sensitive.

Below: Excel screen shot, showing formula for desiccation sensitivity calculation



Further reading

Daws M.I., Garwood N.C. and Pritchard H.W. (2006). Prediction of desiccation sensitivity in seeds of woody species: a probabilistic model based on two seed traits and 104 species. Annals of Botany 97: 667-674 (open access article).

Daws M.I., Garwood N.C. and Pritchard H.W. (2005). Traits of recalcitrant seeds in a semi-deciduous tropical forest in Panama: some ecological implications. Functional Ecology 19 (5): 874–885.

Hay F.R., Probert R.J., Marro J. and Dawson M. (2000). Towards the *ex-situ* conservation of aquatic angiosperms: a review of seed storage behaviour, pp. 161–177. In: M. Black, K.J. Bradford and J. Vazquez-Ramos (eds), Seed Biology: advances and applications. Commonwealth Agricultural Bureaux International, Wallingford, UK.

Hong T.D. and Ellis R.H. (1996). A protocol to determine seed storage behaviour. IPGRI Technical bulletin No.1. International Plant Genetic Resources Institute, Rome, Italy.

ISTA (2007). International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.

Leist N. and Krämer S. (2003). Working sheets on tetrazolium testing, 1st edition 2003. International Seed Testing Association, Bassersdorf, Switzerland.

Liu, K., Eastwood, R.J., Flynn, S., Turner, R.M., and Stuppy, W.H. 2008. Seed Information Database (release 7.1, May 2008) http://www.kew.org/data/sid.

Pritchard H.W., Daws M.I., Fletcher B., Gaméné S., Msanga H. and Omondi W. (2004). Ecological correlates of tropical dryland seed desiccation tolerance. American Journal of Botany 91 (6): 863-870.

Pritchard H.W., Wood C.B., Hodges S. and Vautier H.J. (2004). 100-seed test for desiccation tolerance and germination: a case study on eight tropical palm species. Seed Science and Technology 32 (2): 393-403.

Tweddle J.C., Dickie, J.B. Baskin C.C. and Baskin J.M. (2003). Ecological aspects of seed desiccation sensitivity. Journal of Ecology 91 (2): 294-304.

Equipment specifications

Description	Model/Product	Supplier
Hygrometer with docking station and clamp	AW-DIO sensor with HygroPalm 3 display unit	Rotronic Instruments (UK) Ltd. www.rotronic.com
Ventilated oven with service tube	Genlab 100 litre (Mino/100 dig/f), vertical style: 467-063	Jencons PLS www.ecomcat.com
Cooled incubator with auto-defrost cycle	LMS 250A free standing incubator	LMS Ltd. www.lms.ltd.uk

Please note that the above equipment is used by the Millennium Seed Bank Project 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.

Millennium Seed Bank Project, Wakehurst Place, Ardingly,
West Sussex RH17 6TN, UK

www.kew.org/msbp

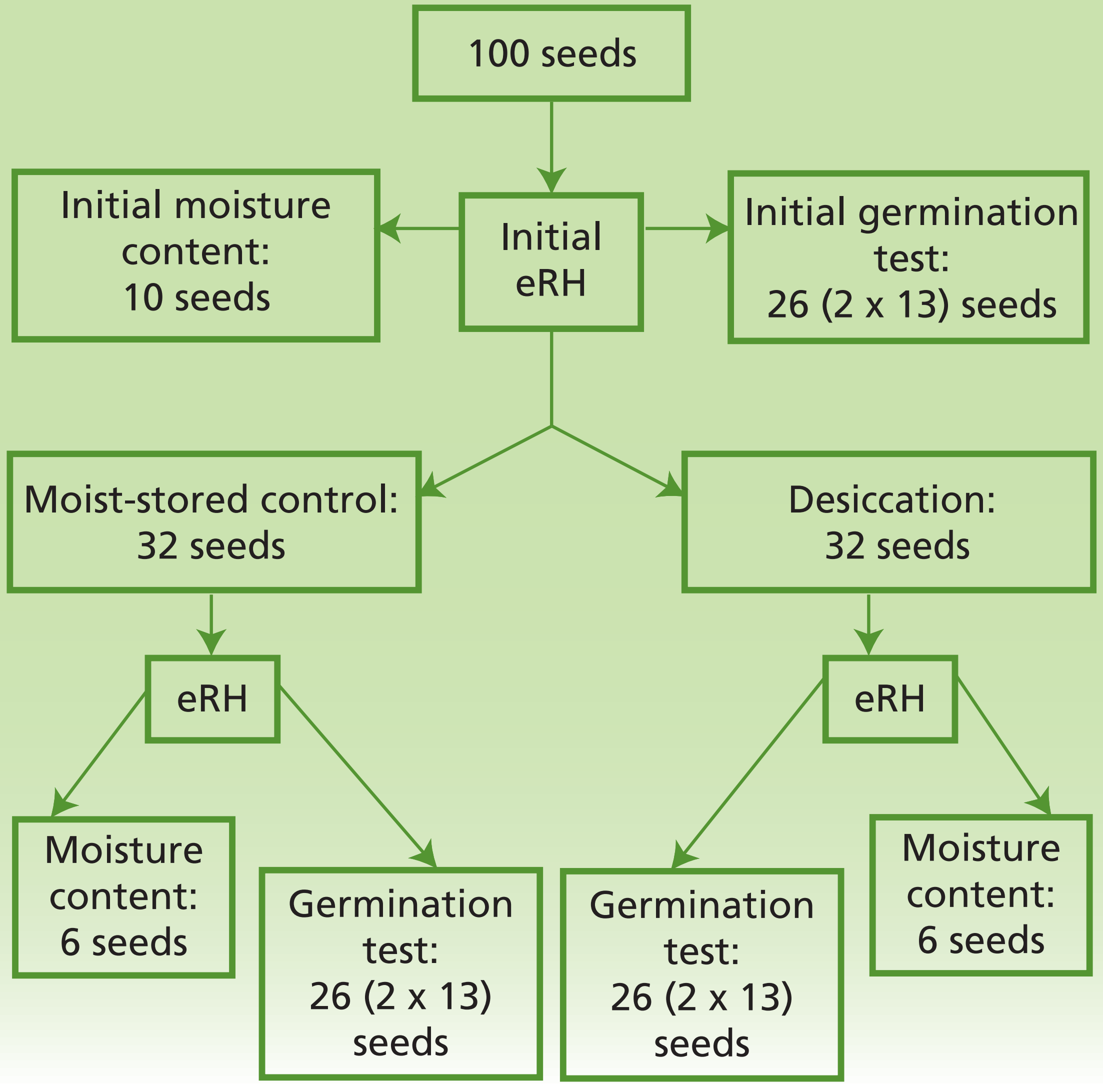
Supported by The National Lottery® through the Millennium Commission

wellcome trust

100-seed test for desiccation tolerance

(adapted from Pritchard *et al.*, 2004)

- Determine the initial equilibrium relative humidity (eRH) of the seed lot with a hygrometer, using sufficient seeds to fill the sample chamber (see Technical Information Sheet_05).
- Determine the moisture content (ISTA, 2007) of 10 individual seeds.
- Carry out an initial germination test, at an optimum temperature for the species, on two samples of 13 seeds.
- Dry 32 seeds by mixing them with an equal weight of silica gel in a suitable sealed container. At the same time, place 32 control seeds at high humidity in a sealed container, using moistened vermiculite or filter paper for example, to maintain humid conditions. Hold both samples in an incubator at 15°C (for temperate species) or 25°C (for tropical species).
- For the desiccated sample, change the silica gel and weigh the seeds every 1-3 days, depending on seed size. As the seeds dry and their weight decreases, the frequency of weighing can be reduced. When the seeds reach a constant weight at equilibrium, usually after 2-3 weeks, determine eRH. The sample should be dried to around 15% eRH. Take 6 seeds to determine the moisture content after desiccation.
- The container holding the moist-stored control seeds should be opened every 1-3 days to allow aeration. Remove the control sample from moist storage, at the same time as terminating the desiccated sample. Determine eRH and take 6 seeds to determine moisture content of the control sample, as above.
- Carry out germination tests on two samples of 13 seeds from both the desiccated sample and the moist-stored control sample.
- Plot germination progress curves (% germination x incubation period) for initial germination of fresh seeds, germination after desiccation and germination after moist storage (see graphs, right). Note that this method will distinguish between desiccation tolerant and desiccation sensitive species, but will not determine species classed as Intermediate.



False Negatives

Desiccation tolerance screening can sometimes produce misleading results. Seeds that germinate after drying must be desiccation tolerant, but seeds that do not germinate are not always desiccation sensitive.

Failure to germinate may be due to:

- Seed immaturity
  Immature seeds are desiccation sensitive. The majority of orthodox seeds acquire full desiccation tolerance shortly before natural dispersal. Ensure that you only use fully ripe seeds in desiccation tolerance screening tests.
- Non-optimal germination conditions
  If seeds are thought to be dormant, use an appropriate dormancy breaking treatment such as warm/cold stratification or mechanical scarification. Also ensure that appropriate environmental cues are applied, including alternating temperatures, light, nitrate or smoke treatment. Carry out a cut-test (see Technical Information Sheet_14) on any ungerminated seeds at the end of the germination test. Fresh, firm seeds are more likely to be dormant than desiccation sensitive. Carry out a tetrazolium viability test to identify dormant seeds (see box below).

Tetrazolium viability test

(ISTA, 2007)

This test is used to determine whether an ungerminated or partially germinated seed is still alive (viable) or is dead.

- Allow a seed sample to imbibe, leaving the seeds overnight at 20°C in a petri dish over a water bath for example, then carefully dissect each seed to expose the embryo.
- Immerse the seeds in 1% buffered triphenyl tetrazolium chloride (TTC or TZ) solution in the dark for 1-2 days.
- Cut the seeds and examine by eye or using a microscope.
- Living tissue will stain various shades of red, due to the action of dehydrogenase enzymes, whilst dead material remains unstained.

- Incipient germination
  As seeds begin to germinate, they lose tolerance to desiccation. If seeds are held in moist conditions for too long they may start to germinate. Carry out desiccation tolerance screening as soon as possible after collection.

Below: 100 seed test for desiccation tolerance - typical germination progress curves of
(a) a desiccation tolerant species, and
(b) a desiccation sensitive species