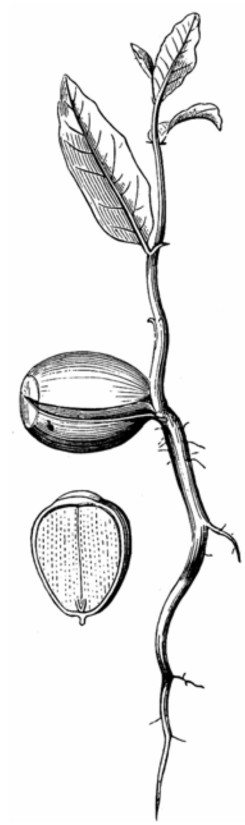
SEED STORAGE GUIDELINES

For California Native Plant Species



The five most important factors affecting seed longevity are:

1. Seed type
2. Seed quality
3. Integrity of the protective seed coat
4. Seed moisture content
5. Storage environment

Inside each seed is a living plant embryo that even in a state of dormancy breathes through the exchange of gases and is constantly undergoing metabolic (aging) processes. The natural lifespan of a seed is influenced by several factors including: permeability of the seed coat, dormancy, seed physiology, and the storage environment. Seeds of many of our native plants and weedy alien species have dormant embryos and hard seed coats, a condition that retards germination and consequently enhances longevity. The presence and degree of seed dormancy and subsequent metabolic rate varies considerably between species and thus influences their natural lifespan. For most species from temperate and arid climates reducing and maintaining a low seed moisture content, storing seeds at moderate to low temperatures, and taking precautions not to damage seeds during cleaning and handling, slows down the metabolic process and thereby increases their longevity in storage.

Seeds are generally categorized into the following types: [1]

- **Orthodox.** Seeds that can be dried, without damage, to low moisture contents, usually much lower than those they would normally achieve in nature. Their longevity increases with reductions in both moisture content and temperature over a wide range of storage environments.
- **Recalcitrant.** Seeds that do not survive drying to any large degree, and are thus not amenable to long term storage.
- **Intermediate.** Seeds that are more tolerant of desiccation than recalcitrants, though that tolerance is much more limited than is the case with orthodox seeds, and they generally lose viability more rapidly at low temperature.

Recalcitrant seeds are not only desiccation-sensitive, but also metabolically active. In contrast, orthodox seeds, owing to their dry state, are metabolically quiescent.[2]
One can estimate a species' natural potential for storage tolerance by:

1 RBG KEW Seed information Database, http://www.kew.org/data/sid/storage.html

2 Science 7 January 2005: Vol. 307. no. 5706, pp. 47 – 49 Patricia Berjak, Protector of the Seeds: Seminal Reflections from Southern Africa

1. *Seed size*. Large seeds often have a high moisture or oil content and are generally recalcitrant in their storage behavior.
2. *Seed physiology*. A heavy impervious seed coat even on large seeds, as is often found on desert legumes and lupines, promotes long-term seed viability.
3. *Climate and habitat* conditions in which the species grow. Seeds from plants adapted to tropical or riparian habitats, due to a semi to permanent water source and/or consistently mild and reliable growing conditions, may not require long term seed viability for survival. Conversely, plants from desert, temperate and Mediterranean climates, where environmental conditions suitable for germination are often infrequent, are more likely to produce seeds capable of surviving for long periods.
4. *Life cycle*. Annuals and perennials are more dependent on a persistent soil seed bank than woody and long-lived shrub and tree species.
5. *Ecological associations*. Plants that are early successional colonizer species that may occur only after a disturbance and species that depend on other plants for their development must maintain viability until a suitable host plant is available.

SEED LONGEVITY IN THE NATURAL ENVIRONMENT

For many plant species a substantial portion of their population exists below the soil surface in the form of a soil seed bank. Under the right conditions seeds of many plant species can survive for extremely long periods. The following examples of extreme longevity illustrate the potential for long term seed viability.

In 1966, seeds of the Arctic lupine *Lupinus arctica* were recovered from a rodent burrow six meters below the frozen silt surface and later successfully germinated. Along with the seeds a collared lemming skull was found in the burrow. Since this lemming species disappeared from the region 10,000 years ago it was proposed that the seeds were also this age. Plants of the sacred lotus *Nelumbium nuciferum* were grown from seeds whose fruits were carbon dated at more than 1,200 years old. These seeds were discovered in an ancient peat bed in Pulantien, Liaoning Province, China. In Denmark , Odum (1965) extracted and germinated seeds of *Chenopodium album* and *Spergula arvensis* from soil samples collected beneath buildings. These seeds were estimated by the dates of the structures to be over 1700 years old.[3] In 2005 2,000 year old seeds of the Judea date palm *Phoenix dactylifera* were germinated and produced healthy plants. These seeds were discovered in 1970 in an archeological dig in Israel. After the discovery the seeds were stored away in a desk and there remained until they were successfully germinated. The age of the seeds was determined by carbon dating of the seeds from the same lot that produced the plant.[4] In 2006 seeds were germinated and healthy plants produced from 200 year old seeds of an *Acacia* species that were discovered in a merchant’s notebook that had been stored since 1803 in the Tower of London.[5]

While these seed longevities are interesting, they are the exception rather than the rule. Under most circumstances even the hardiest of seeds that are shed into their natural environment face a host of hazards including degradation from exposure to harsh

3 Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Carol C. & Jerry M. Baskin, pages 145-148, 1998 Academic Press, 666 pp

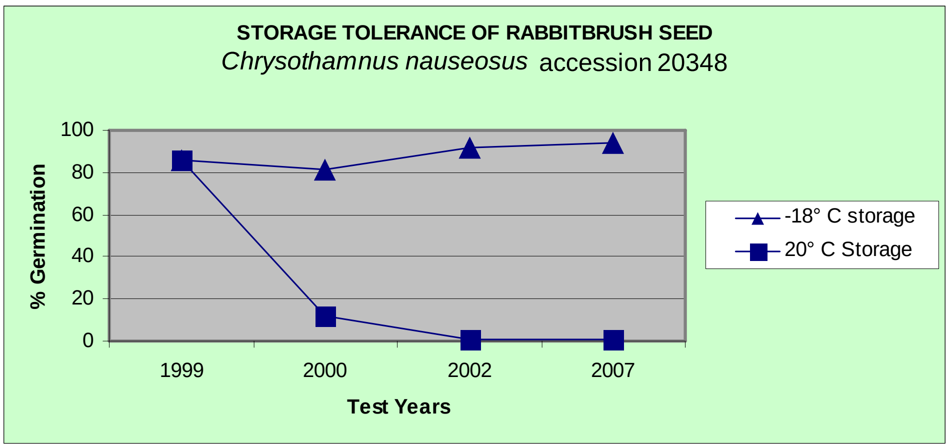
4 BBC News, Monday, 13 June, 2005, 01:21 GMT 02:21 UK, http://news.bbc.co.uk

5 http://news.bbc.co.uk Monday, 13 June, 2005, 01:21 GMT 02:21 UK

environmental conditions, predation, and the chance distribution of ending up in places wholly inappropriate for germination and establishment.

SEED LONGEVITY UNDER CONTROLLED CONDITIONS

Seed banking, the practice of maintaining seeds under safer and more controlled conditions, has proven to be extremely effective in slowing down the aging process thereby maintaining viability and seed vigor over extended periods. Rabbitbrush (*Chrysothamnus nauseosus*.) is an example of one plant species whose seeds lose viability rapidly[6] but whose viability can be greatly extended under controlled conditions in an ex-situ seed bank.[7]



The practice of seed preservation is as old as agricultural practices but systematic collections and storage facilities have been a development of the 20th century. Today there are an estimated 1300 seed or gene banks around the world containing over 6 million seed accessions. Seed banking from a species conservation perspective has many benefits as well as some negative consequences. Establishing a seed storage program, i.e. maintaining viability in seed lots so that they will be available when needed, is generally more cost effective than making field collections for annual propagation needs. Seed production in nature is frequently unreliable and adequate seed may not be available in the year that it is needed. Having a system in place to properly handle and store seeds allows one to take advantage of good crop years. Especially for annual and some perennial species, propagating from seed lots made over multiple years from one population increases the genetic diversity represented in the plants and any subsequent regenerated seed. Maintaining germplasm in seed banks serves as a hedge against extinction for threatened wild plant species, their populations, or cultivated landraces of agricultural plants.

For some plant species, using relatively fresh rather (that which is less than one year old) gives superior germination over stored seed. Viability of a seed lot declines over time and though old seed may germinate, the resulting seedlings may have reduced vigor and fail to establish as well as seedlings from fresh seed. While seed storage takes up relatively

6 Monsen and Stevens 1987

7 M. Wall 2007; Comparative germination study on room stored vs. frozen Chrysothamnus nauseosus seed; RSABG, unpublished data

little space, an adequate seed storage program requires considerable time, materials and energy. Additionally, horticultural selection during propagation events can potentially have negative effects on the genetic make up of regenerated seed lots. Finally, conserving plants in ex-situ seed banks over long periods of time removes them from natural selection processes. In the case of annuals and short-lived perennials, it may be questioned whether the plants that result from seeds stored in long-term storage collections (e.g. greater than 30 years) are really the same entity once they are grown out and reintroduced into the wild.

Principles of seed storage

In the natural environment and when stored at ambient room conditions, seeds respond to constantly changing relative humidity and temperatures. Maintaining seeds under controlled conditions lowers metabolic activity, thereby reducing the aging process and increasing longevity of the seed lot. For most seeds, a cool and dry environment is preferred and for orthodox seeds the cooler and drier the greater the longevity that can be achieved. Harrington's rule[8] states that:

1. Each 1 percent reduction in moisture content doubles the life of the seed.
2. Each 10 degree F reduction in temperature doubles the life of the seed.

Orthodox seeds can be dried down to very low moisture content (1-14 percent) and frozen. There is some current discussion as to the optimum storage seed moisture content. The FAO/IPGRI Genebank Standards (1994) recommends drying seeds to low moisture contents (3–7% fresh weight, depending on the species) and storing them in hermetically-sealed containers at low temperature, preferably -18˚C or cooler. This is achieved by drying seeds to equilibrium at 10–15% RH and a temperature of 10°–25°C.[9] There is some concern, however, that drying seeds to these levels, combined with storage at very low temperatures, can be damaging for some species and can actually shorten storage life.[10] According to Dr. Christina Walters at the National Center for Genetic Resource Preservation (NCGRP), the optimum seed moisture content should be based on the storage temperature and her current recommendations suggest that the optimum water content for seed storage increases with decreasing storage temperatures (i.e. the greater the temperature difference between the drying and storage temperatures, the higher the allowable RH for drying).[11] The following chart from the referenced publication shows the recommended drying conditions for orthodox seeds stored in moisture proof containers.

Drying Temperature (°C)	Drying RH for Storage at 15° C	Drying RH for Storage at 5° C	Drying RH for Storage at -18° C
25	28	33	46
15	20	26	38
5	14	20	32

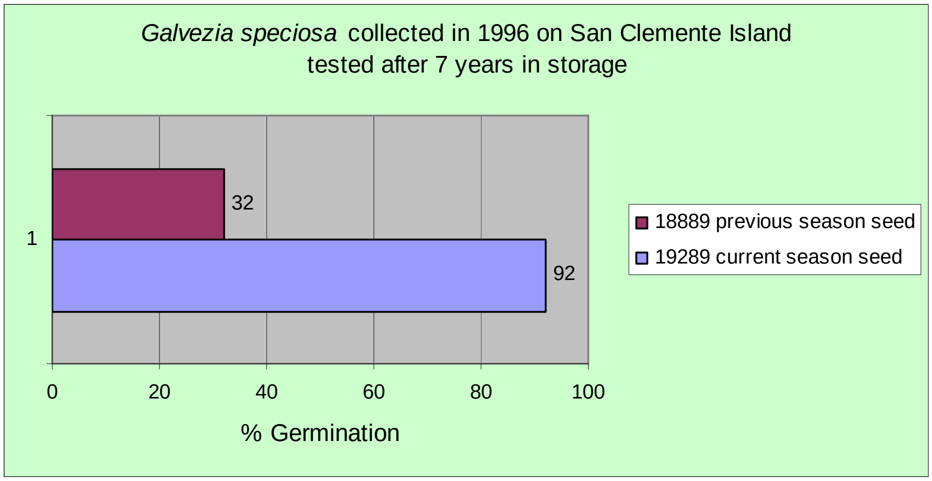
8 "Harrington's Rule" Principles and Practices of Seed Storage, U.S. Department of Agriculture, 1978

9 Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D and Larinde M. 2006. Manual of seed handling in genebanks. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy

10 Christina Walters, 2004, Appendix 2 in Ex Situ Conservation Supporting Species Survival in the Wild. Guerrant, Havens and Maunder, Island Press

11 Christina Walters, 2004, Appendix 2 in Ex Situ Conservation Supporting Species Survival in the Wild. Guerrant, Havens and Maunder, Island Press

In general, the sooner orthodox seeds are dried down and placed into storage the greater their longevity. Seeds harvested prematurely may have shorter shelf lives than seeds harvested when fully mature. This may be because immature embryos are not fully tolerant of desiccation and so are damaged when dried. Seeds harvested at an immature stage of development should be dried slowly, preferably within their fruiting structures and at higher humidity than matured seed collections.[12] Old seeds from previous season's fruits can also have a shorter storage life and/or viability. The following germination test results demonstrate storage tolerance variation on previous season (March) and current season (August) seed collections from two separate San Clemente Island populations.



Recalcitrant seeds of temperate species (oaks and walnuts) can be maintained from between 0.5 and 2 years when kept moist and at a temperature just below the minimum temperature needed for germination. Seeds can be stored in moist peat, vermiculite, or Sponge Rock. A fungicide is frequently applied to the seeds to control microbial activity.

Intermediate seeds will generally not tolerate freezing and therefore should be stored at 5° C or above. Because they are more tolerant of drying than recalcitrant species, they can be dried down to lower RH values (40 - 60 percent) which will increase storage life. Moisture can be controlled with desiccants and/or with fungicides. For all storage situations seeds should not be exposed to light.

To determine seed type the seeds can be tested prior to storage. Seeds that tolerate desiccation (show no loss in viability) to 5% moisture content or below (values in equilibrium with 10–15% RH at 20°C) are likely to show orthodox seed-storage behavior. Seeds that tolerate desiccation to about 10–12% moisture content (values in equilibrium with 40–50% RH at 20°C), but whose viability is reduced when subjected to further desiccation to a lower moisture content are likely to show intermediate seed storage behavior. Seeds that are killed by desiccation to 15–20% moisture content (values in equilibrium with >70% RH at 20°C) are likely to be recalcitrant.[13] Information on storage behavior of a wide range of species is available at *www.rbgkew.org.uk/data/sid*.

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12 Christina Walters, 2004, Appendix 2 in Ex Situ Conservation Supporting Species Survival in the Wild. Guerrant, Havens and Maunder, Island Press

13 Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D and Larinde M. 2006. Manual of seed handling in genebanks. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.

SEED STORAGE AT RANCHO SANTA ANA BOTANIC GARDEN

The oldest seed collections from the Garden are currently at the National Center for Genetic Resource Preservation as part of the Fritz Went and Philip Munz long term seed longevity experiment.[14] Seeds of more than 100 different native species from a wide range of families and habitats were supplied by the Garden for Dr. Went's 1947 experiment. The seeds were dried down to very low moisture content and placed into glass tubes that were then sealed under vacuum. The seeds were divided into 20 sets with the experiment intended to run for 360 years or until 2307. In 1997, as part of her Master's Thesis, Teri Christensen conducted the fifty year viability testing.[15] Some of the results of her review of the collection are presented here in Appendix 1.

When the Garden moved to Claremont in 1951 the Garden's seed collections were stored in glass mason jars and housed in the stone building at the bottom of the east slope of the Mesa. In 1988, to extend the longevity of the Garden's seed collections, an upright refrigeration unit and a chest style freezer were purchased and installed in the stone house.[16] At this point the horticultural seed collections were transferred from the glass mason jars into double sealed plastic storage bottles and placed into the refrigerator at 5 ^{o} C / 41 ^{o} F. The endangered species collections following 2 to 3 weeks storage at 12% relative humidity were packaged and sealed in Crystal Springs© storage pouches and placed into chest style freezers at -18 ^{o} C / 0 ^{o} F. It was customary during this period for seed collections of rare but non-listed plant species to be split between the Horticulture collections and the Endangered Species Program collections. In 1995, both the cold storage and the freezer units were moved into the new Fletcher Jones Seed Storage facility. In 1998 additional chest style freezers were installed and the horticultural collections were dried to equilibrium at 12% RH over calcium sulfate desiccant and placed into storage at -18^{o} C. The rare, threatened and endangered species collections were re-dried and transferred from the "Crystal Springs" storage pouches to the newer and sturdier Barrier Foil© laminate seed storage pouches. Thus over this time some of the oldest seed collections have been stored at a wide range of temperatures from room temperature (16^{o} - 27 ^{o} C), refrigerated (5^{o} C) to frozen (-18^{o} C). Appendix 2 lists germination results from tests conducted on a number of our older seed collections.

The Seed Program currently maintains the seed collections in (4) 23 cu. ft. freezers and (1) 14 cu. ft. refrigerator. As the program continues to grow a larger capacity walk in freezer unit will be needed.

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14 F.W. Went and P.A. Munz, April 27, 1947. A Long Term Test of Seed Longevity, El Aliso; Vol. 2, No. 1.

15 Teri Christensen, May 2000, Germination of 91 Native Species after 50 Years in Vacuum Storage, University of Northern Colorado, Department of Biological Sciences

16 RSABG Annual Report, 1987 - 1988

Appendix 1: 50 year test results on selected species from the Went and Munz Long Term Test of Seed Longevity for seeds stored in vacuum

		Test Year ~ % Germ		
Family	Went and Munz Test Species	1947	1967	1997
Asteraceae	Achillea millefolium (borealis)	98	98	62
Chenopodeaceae	Allenrolfea occidentalis	5	40	60
Chenopodeaceae	Atriplex hymenolytra	0	10	20
Philadelphaceae	Carpenteria californica	100	35	32
Fabaceae	Cercidium microphyllum	40	56	97
Asteraceae	Chaenactis glabriuscula var. glabriuscula	33	32	30
Polygonaceae	Chorizanthe staticoides	22	22	36
Asteraceae	Cirsium occidentale	77	68	90
Onagraceae	Clarkia (Godetia) amoena var. lindleyi	85	89	88
Onagraceae	Clarkia (Godetia) amoena	96	92	68
Onagraceae	Clarkia (Godetia) bottae	nd	90	82
Onagraceae	Clarkia (Godetia) cylindrica	90	80	56
Onagraceae	Clarkia (Godetia) dudleyana	90	96	100
Onagraceae	Clarkia elegans	100	95	88
Onagraceae	Clarkia (Godetia viminea) purpurea ssp. viminea	92	90	36
Asteraceae	Coreopsis bigelovii	58	63	8
Asteraceae	Coreopsis maritima	nd	93	86
Crossosomataceae	Crossosoma californicum	72	37	44
Asteraceae	Encelia actoni	13	7	0
Polygonaceae	Eriogonum arborescens	7	59	58
Asteraceae	Eriophyllum lanatum	nd	22	20
Papaveraceae	Eschscholzia caespitosa	55	85	34
Papaveraceae	Eschscholzia californica	78	75	0*
Asteraceae	Geraea canescens	7	3	12
Polemoniaceae	Gilia achilleifolia	92	78	38
Polemoniaceae	Gilia chamissonis	72	94	92
Polemoniaceae	Gilia tricolor	96	94	0*
Asteraceae	Lasthenia (Baeria) maritima	49	90	54
Asteraceae	Lasthenia glabrata	36	70	0
Asteraceae	Layia platyglossa	20	14	6
Polemoniaceae	Linanthus grandiflorus	92	97	6
Polemoniaceae	Linanthus montanus	80	94	0*
Fabaceae	Lotus scoparius var. scoparius	7	0	10
Fabaceae	Lupinus succulentus	13	38	24
Asteraceae	Malacothrix arachnoidea	25	29	16
Loasaceae	Mentzelia lindleyi	4	62	10
Lamiaceae	Monardella lanceolata	95	96	94
Hydrophyllaceae	Nemophila maculata	30	80	90
Onagraceae	Oenothera deltoides	52	91	52
Scrophulariaceae	Penstemon heterophyllus	nd	54	82
Scrophulariaceae	Penstemon spectabilis	12	50	0*
Hydrophyllaceae	Phacelia ciliata	43	99	30
Hydrophyllaceae	Phacelia parryi	82	99	30
Hydrophyllaceae	Phacelia tanacetifolia	23	76	52
Hydrophyllaceae	Phacelia viscida	55	97	80
Lamiaceae	Prunella vulgaris (Brunella)	nd	98	94
Platanaceae	Platanus racemosa	45	63	4
Lamiaceae	Salvia columbariae	nd	64	0*
Iridaceae	Sisyrinchium bellum	23	49	0*

An * indicates that no vacuum existed ~ nd = no data

Appendix 2: Seed germination test results from selected RSA collections

Accession Num	Lot & Test Num	NAME	Store Date	Years in Storage	%GERM
15405	781*1	Aquilegia formosa	1986	12	66
15405	781*2	Aquilegia formosa	1986	21	52
14262	847*4	Berberis haematocarpa	1977	25	72
14529	886*1	Carex spissa	1981	26	42
14620	1632*1	Carpenteria californica	1980	10	8
14620	1632*3	Carpenteria californica	1980	12	49
14620	1632*6	Carpenteria californica	1980	23	66
14702	901*1	Ceanothus cuneatus	1983	23	31
15875	1641*1	Clarkia amoena ssp. huntiana	1987	2	47
15875	1641*2	Clarkia amoena ssp. huntiana	1987	20	57
15877	1643*1	Clarkia arcuata	1987	2	15
15877	1643*2	Clarkia arcuata	1987	20	80
15884	1650*1	Clarkia concinna ssp. concinna	1987	2	8
15884	1650*2	Clarkia concinna ssp. concinna	1987	14	65
15884	1650*3	Clarkia concinna ssp. concinna	1987	16	75
15610	1686*1	Cordylanthus maritimus ssp. maritimus	1987	3	40
15610	1686*2	Cordylanthus maritimus ssp. maritimus	1987	4	61
15610	1686*3	Cordylanthus maritimus ssp. maritimus	1987	20	72
15327	228*1	Coreopsis maritima	1985	16	79
14732	1689*1	Cupressus bakeri	1982	20	54
14732	1689*2	Cupressus bakeri	1982	24	63
15164	1048*1	Dodecatheon clevelandii ssp. insulare	1983	24	94
14216	1703*1	Dudleya nesiotica	1979	12	96
14216	1703*2	Dudleya nesiotica	1979	21	83
14216	1703*3	Dudleya nesiotica	1979	28	96
15207	1094*1	Echinocereus engelmannii	1985	19	32
15593	1098*1	Elymus glaucus	1987	18	38
15105	1706*1	Eriastrum densifolium ssp. sanctorum	1986	5	82
15105	1706*2	Eriastrum densifolium ssp. sanctorum	1986	14	79
15105	1706*3	Eriastrum densifolium ssp. sanctorum	1986	21	66
15358	229*1	Eriogonum giganteum var. giganteum	1983	14	54
15358	229*2	Eriogonum giganteum var. giganteum	1983	22	43
15358	229*3	Eriophyllum staechadifolium	1987	15	60
19262	2182*1	Ferocactus viridescens	1984	23	60
15292	1186*1	Fraxinus velutina	1984	23	0
14951	1291*1	Hyptis emoryi	1983	24	63
19219	1624*1	Lupinus excubitus var. austromontanus	1981	25	82
14665	1398*1	Lupinus longifolius	1982	25	100
14936	1425*1	Malosma laurina	1983	15	64
14936	1425*2	Malosma laurina	1983	24	68
14552	1918*4	Mimulus aridus	1981	18	47
14552	1918*5	Mimulus aridus	1981	22	53
14940	63*1	Salvia apiana	1983	24	43
14674	1929*1	Solanum wallacei	1982	23	49
14417	1791*1	Sphaeralcea ambigua var. rosacea	1980	27	81

Number of years in storage represents the germination test year minus the store date.
Shaded groupings indicate a tread over time in storage; increasing, decreasing or stable.

Additional References:

Seed Conservation - Turning Science into Practice. **Smith, R.D., Dickie, J.B., Linington, S.H.,Pritchard, H.W., Probert, R.J. (eds), 2003 Kew: Royal Botanic Gardens.**