Selbyana 14: 89–104

ORCHID SEED STORAGE: HISTORICAL PERSPECTIVE, CURRENT STATUS, AND FUTURE PROSPECTS FOR LONG-TERM CONSERVATION

H. W. PRITCHARD*

Jodrell Laboratory, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK

P. T. SEATON

Kidderminster College of Further Education, Hoo Road, Kidderminster, Worcs., DY10 1LX, UK

ABSTRACT. For the limited number of species studied, the storage characteristics of orchid seeds are classified as 'orthodox' in the sense that seed longevity is enhanced by reducing moisture contents (from around 20%, wet basis, to 5%) and decreasing storage temperatures (from 62°C to 0°C). When stored dry at 5–8°C, the time taken for viability to fall to 50% can be 8–14 years, (assuming high initial seed quality). However, seeds of *Cattleya aurantiaca* exhibit reduced longevity at 5°C when stored at equilibrium moisture contents both above and below 5%, indicating that the optimal water status for these seeds during storage is approximately 30% r.h. (or a water potential of ca. −150 MPa). *C. aurantiaca* seeds at 2.2 to 5.6% moisture content (up to 31% r.h.) generally exhibit reduced longevity at −18°C compared to 5°C, and this sensitivity to sub-zero storage has been shown by differential scanning calorimetry to coincide with the presence of the seed lipids in a transitional conformation state. Similar results in the literature for 'dry' seeds of other tropical orchids stored at −10°C suggest that the long-term conservation of such species under conventional seed bank conditions (i.e., about −20°C and 5% moisture content) is problematical. In contrast, dry seeds of two temperate species *Dactylorhiza fuschii* and *Orchis morio* germinated after 6–7 yr storage of sub-zero temperatures. The results are discussed in relation to seed longevity in species from other plant families.

With an estimated 23,000 species (Atwood 1986) the Orchidaceae constitute ~7 to 10% of the world's flowering plants. Many of these are threatened with extinction in the immediate future, due largely to the rapid contraction of habitats (Raven 1976). The problem is particularly acute in the tropics, and demands prompt action if a disastrous narrowing of the genetic base is to be averted.

Although in situ conservation through the establishment of suitable biosphere reserves may be considered the most desirable long-term strategy, in the short-term it is essential that ex situ methods be utilized to preserve material suitable for later reintroduction into the wild. Indeed it would seem likely that some orchid species may only exist in cultivation in the future.

Ex situ methods of conservation consist of the maintenance of species in cultivation, including their clonal propagation, and the storage of pollen and seed. Seed storage is the most attractive option, allowing both preservation and easy distribution of material with a broad genetic base, and financial and manpower savings over other alternatives (e.g., approximately 50,000 seeds of many orchid species can be stored in only a 2 cm^3 capacity container). Accordingly, the International Orchid Commission has recommended that a world-wide network of orchid seed banks should be established (Greatwood 1984). Here we assess the validity of this recommendation based on our review of the published data and supplemented by our own new data on orchid seed storage physiology.

This paper is divided into 3 sections: Historical Perspective of the development of seed storage studies; Current Status of the interaction between seed moisture content and storage temperatures on orchid seed longevity, and Future Prospects for research activity.

HISTORICAL PERSPECTIVE

Seed storage at 0°C and above (TABLE 1)

The first reported comments on orchid seed storage and longevity appear to have been those of Jancke (1915). Jancke recommended the equilibration of seed from dehisced capsules at room temperature and subsequent cool, dark storage in strong paper-bags or test-tubes stoppered with cotton-wads or corks. Under such conditions seeds "will usually be capable of germination after a year of storage . . ." but ". . .

* To whom all correspondence should be addressed.

TABLE 1. Orchid seed viability after storage at temperatures of 0°C and above.

Species (and hybrid code no.)	Time in store[1]	Storage conditions: Temperature (°C)	Storage conditions: Moisture status[2]	Seed viability (%)[3]: Initial	Seed viability (%)[3]: After storage	Reference
Cattleya and *Laelia*	1 yr	Cool—n.s.	Air-dry	n.s.[4]	n.s.—"usually still capable of germination"	Jancke 1915
Cattleya trianae	3 yr	Room—n.s.	Room conditions	n.s.	40	Knudson 1924
Cattleya	8 yr	8	Over anhydrous calcium chloride	n.s.	n.s.—"some seed still viable"	Knudson 1934
Cymbidium	4 yr	8	Over anhydrous calcium chloride	n.s.	n.s.—"marked loss of viability"	Knudson 1934
Brassavola digbyana	10 yr	8	Over anhydrous calcium chloride	n.s.	1	Knudson 1940
Brassocattleya (B151)	10 yr	8	Over anhydrous calcium chloride	n.s.	80	Knudson 194
Brassocattleya (B67)	14 yr	8	Over anhydrous calcium chloride	n.s.	40	Knudson 1940
Brassocattleya (B143)	10 yr	8	Over anhydrous calcium chloride	n.s.	75	Knudson 1940
Brassocattleya (B204)	8 yr	8	Over anhydrous calcium chloride	n.s.	2	Knudson 1940
Brassocattleya (B155)	10 yr	8	Over anhydrous calcium chloride	n.s.	80	Knudson 1940
Brassocattleya (B167)	10 yr	8	Over anhydrous calcium chloride	n.s.	80	Knudson 1940
Brassocattleya (B179)	9 yr	8	Over anhydrous calcium chloride	n.s.	80	Knudson 1940
Brassocattleya (B230)	9 yr	8	Over anhydrous calcium chloride	n.s.	60	Knudson 1940
Brassocattleya (B238)	8 yr	8	Over anhydrous calcium chloride	n.s.	4	Knudson 1940
Cattleya hybrid (B209)	8 yr	8	Over anhydrous calcium chloride	n.s.	80	Knudson 1940
Cattleya hybrid (B255)	7 yr	8	Over anhydrous calcium chloride	n.s.	70	Knudson 1940
C. gigas × *C. gaskelliana*	14 yr	8	Over anhydrous calcium chloride	n.s.	1	Knudson 1940
C. mossiae × *C. gigas*	8 yr	8	Over anhydrous calcium chloride	n.s.	6	Knudson 1940
C. gigas × *C. mendelii*	8 yr	8	Over anhydrous calcium chloride	n.s.	5	Knudson 1940
C. shroederae × *Laelia purpurata*	8 yr	8	Over anhydrous calcium chloride	n.s.	60	Knudson 1940
Cymbidium hybrids	8 yr	8	Over anhydrous calcium chloride	n.s.	2 to 5	Knudson 1940
Cypripedium hybrid	10 yr	8	Over anhydrous calcium chloride	n.s.	50	Knudson 1940
L. purpurata × *C. schroederae*	8 yr	8	Over anhydrous calcium chloride	n.s.	35	Knudson 1940
Laeliocattleya hyeana × *C. gigas*	14 yr	8	Over anhydrous calcium chloride	n.s.	45	Knudson 1940
Lycaste skinneri × *Bifrenaria harrisoniae*	10 yr	8	Over anhydrous calcium chloride	n.s.	20	Knudson 1940
Cattleya luegeae	16 d	4	n.s.	n.s.	n.s.—"differences in average number of seedlings . . . are probably not significant. . ."	Burke and Northen 1948

Editor's note: Please note the following information, for *Selbyana* 14.

ERRATUM
Orchid seed storage: Pritchard and Seaton
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In Table 1 the following species details apply on Pages 92–3.

Species (and hybrid code no.)	Time in store	Storage conditions		Seed viability (%)		Reference
		Temperature (°C)	Moisture status	Initial	After storage	
Listera ovata	2 wk	Room-n.s.	Room conditions	21	3	) Ronse 1989, after) van Waes, 1984
Listera ovata	4 wk	4	With silica gel	21	5	)
Calanthe	4 mo	1–4	n.s.	n.s.	7	) Arditti 1992, after) Tcherevtchenko
Cymbidium	4 mo	1–4	n.s.	93	8	) and Kushnir 1986.

TABLE 1. Continued.

Species (and hybrid code no.)	Time in store[1]	Storage conditions: Temperature (°C)	Storage conditions: Moisture status[2]	Seed viability (%)[3]: Initial	Seed viability (%)[3]: After storage	Reference
Cattleya luegeae	16 d	22	n.s.	n.s.	n.s.—"differences in average number of seedlings . . . are probably not significant. . ."	Burke and Northen 1948
Brassocattleya (B167)	22 yr	8	Over anhydrous calcium chloride	n.s.	10	Knudson 1953
Brassocattleya (B179)	21 yr	8	Over anhydrous calcium chloride	n.s.	10	Knudson 1953
Cattleya hybrid (B209)	20 yr	8	Over anhydrous calcium chloride	n.s.	30	Knudson 1953
Cattleya hybrid (B255)	19 yr	8	Over anhydrous calcium chloride	n.s.	8	Knudson 1953
Cattleya schroederae × *Laelia purpurata*	20 yr	8	Over anhydrous calcium chloride	n.s.	5	Knudson 1953
Paphiopedilum hybrid	10 yr	8	Over anhydrous calcium chloride	n.s.	5	Knudson 1953
Phalaenopsis hybrid	10 yr	8	Over anhydrous calcium chloride	n.s.	30	Knudson 1953
Brassolaeliocattleya sp.	91 d	Room—n.s.	Room conditions	88	0	Kano 1965
Brassolaeliocattleya sp.	511 d	Room—n.s.	In desiccator	88	58	Kano 1965
Brassolaeliocattleya sp.	511 d	0	In desiccator	88	69	Kano 1965
Dendrobium sp.	64 d	Room—n.s.	Room conditions	95	0	Kano 1965
Dendrobium sp.	484 d	Room—n.s.	In desiccator	95	2	Kano 1965
Dendrobium sp.	484 d	0	In desiccator	95	60	Kano 1965
Dendrobium phalaenopsis	20 d	25–28	Over anhydrous calcium chloride	100	70	Limartha 1975
Phalaenopsis amabilis	20 d	25–28	Over anhydrous calcium chloride	100	70	Limartha 1975
Eulophia alta	2 mo	2	23% MC	68	43	Pritchard 1985b
Eulophia alta	6 mo	2	5% MC, over silica gel	75	75	Pritchard 1985b
Dactylorhiza fuchsii	6 d	62	Pre-equilibration at 15% rh and 15°C	69	4	Pritchard 1985a
Cattleya aurantiaca	1 yr	20	2.2 and 3.7% MC	94	0	Seaton 1985
Cattleya aurantiaca	1 yr	20	5.6% MC	94	1	Seaton 1985
Dactylorhiza maculata	6 mo	4	50–70% rh	n.s.	95	Dijk 1987
Gymnadenia conopsea	6 mo	4	50–70% rh	n.s.	n.s.—"a few percent germinated"	Dijk 1987
Platanthera chlorantha	52 wk	4	With silica gel	66	2	Dijk 1987, after van Waes 1984
Anacamptis pyramidalis	4 wk	Room—n.s.	Room conditions	42	3	Ronse 1989, after van Waes 1984
Anacamptis pyramidalis	12 wk	4	With silica gel	42	10	Ronse 1989, after van Waes 1984
Aceras anthropophorum	2 wk	Room—n.s.	Room conditions	28	2	Ronse 1989, after van Waes 1984

TABLE 1. Continued.

Species (and hybrid code no.)	Time in store[1]	Storage conditions: Temperature (°C)	Storage conditions: Moisture status[2]	Seed viability (%)[3]: Initial	Seed viability (%)[3]: After storage	Reference
Aceras anthropophorum	12 wk	4	With silica gel	28	2	Ronse 1989, after van Waes 1984
Dactylorhiza maculata	52 wk	Room—n.s.	Room conditions	80	11	Ronse 1989, after van Waes 1984
Dactylorhiza maculata	52 wk	4	With silica gel	80	28	Ronse 1989, after van Waes 1984
D. sambucina	52 wk	Room—n.s.	Room conditions	n.s.	22	Ronse 1989, after van Waes 1984
D. sambucina	52 wk	4	With silica gel	n.s.	36	Ronse 1989, after van Waes 1984
Epipactis atrorubens	1 wk	Room—n.s.	Room conditions	64	11	Ronse 1989, after van Waes 1984
Epipactis atrorubens	4 wk	4	With silica gel	64	5	Ronse 1989, after van Waes 1984
E. helleborine	2 wk	Room—n.s.	Room conditions	29	4	Ronse 1989, after van Waes 1984
E. helleborine	8 wk	4	With silica gel	29	2	Ronse 1989, after van Waes 1984
Gymnadenia conopsea	12 wk	Room—n.s.	Room conditions	88	3	Ronse 1989, after van Waes 1984
Gymnadenia conopsea	24 wk	4	With silica gel	88	5	Ronse 1989, after van Waes 1984
Listera ovata	2 wk	Room—n.s.	Room conditions	21	3	
Listera ovata	4 wk	4	With silica gel	21	5	
Cattleya aurantiaca (seedlot 2)	6 yr	5	2.2% MC	94	0	Seaton and Hailes 1989
(seedlot 2)	6 yr	5	3.7% MC	94	36	Seaton and Hailes 1989
(seedlot 2)	6 yr	5	5.6% MC	94	70	Seaton and Hailes 1989
(seedlot 2)	6 yr	5	6.5% MC	94	61	Seaton and Hailes 1989
(seedlot 2)	6 yr	5	10.4% MC	94	57	Seaton and Hailes 1989
(seedlot 2)	6 yr	5	14.1% MC	94	1	Seaton and Hailes 1989
Cattleya aurantiaca (seedlot 1)	7 yr	5	5.6% MC	80	28	Seaton and Hailes 1989
(seedlot 1)	7 yr	5	3.7% MC	80	1	Seaton and Hailes 1989
(seedlot 1)	7 yr	5	2.2% MC	80	0	Seaton and Hailes 1989
Calanthe	4 mo	1–4 n.s.		n.s.	7	Arditti 1992, after Tchereutchenko and Kushnir, 1986

TABLE 1. Continued.

Species (and hybrid code no.)	Storage conditions			Seed viability (%)[3]		Reference
	Time in store[1]	Temperature (°C)	Moisture status[2]	Initial	After storage	
Calanthe	4 mo	1–4 n.s.	n.s.	n.s.	7	Arditti 1992, after Tcheremeutchenko and Kushnir, 1986
Cymbidium			n.s.	93	8	Thornhill and Koopowitz 1992
Disa uniflora	10 wk	4–5	without desiccant	n.s.	24 (of original)	Thornhill and Koopowitz 1992
Disa uniflora	10 wk	6	without desiccant	n.s.	75 (of original)	Thornhill and Koopowitz 1992
Disa uniflora	10 wk	6	with 'Drierite' desiccant	n.s.	90 (of original)	Thornhill and Koopowitz 1992

[1] d, days; wk, weeks; mo, months; yr, years.
[2] MC, moisture content (wet basis); rh, relative humidity.
[3] Values given are for germination, except for the data adapted from van Waes (1984) where triphenyl tetrazolium chloride staining was used as the viability test.
[4] n.s., not specified.

storage periods in excess of 1 year are unlikely to yield viable seeds." It is likely that by 'cool' conditions Jancke meant a cool room rather than refrigeration which only became available later.

The validity of Jancke's advice subsequently came from numerous sources. While *Cattleya trianae* retained 40% viability after 3 yr storage under room temperature conditions, "such a high germination of old seed is unusual" (Knudson 1924, 1934). In contrast, *Brassolaeliocattleya* and *Dendrobium* species seed was completely inviable after only 2 to 3 mo storage under room conditions (Kano 1965) and seed of eight European terrestrial orchids retained between only 2% and 22% (histochemical stain) viability after 2 to 52 wk storage at room temperature (Ronse 1989 after van Waes 1984).

Seed longevity at room temperature was improved by a reduction in seed moisture status: dry *Brassolaeliocattleya* and *Dendrobium* seeds lost 30% and 93% of their viability respectively after 17 mo storage (Kano 1965). However, *Cattleya aurantiaca* seed was nearly completely inviable after 1 yr at 2.2% to 5.6% moisture content and 20°C (Seaton 1985) and dry seeds of *Dendrobium phalaenopsis* and *Phalaenopsis amabilis* both lost 30% viability in only 20 days at 25–28°C (Limartha 1975). Not surprisingly the longevity of dry *Dactylorhiza fuchsii* seed at an elevated temperature of 62°C was limited to a few days (Pritchard 1985a).

Alternatively, increased longevity was achieved through a reduction in temperature rather than moisture content by refrigeration (2 to 6°C; approximately 50–70% r.h.). This resulted in a relatively small loss in seed viability for *Eulophia alta* (Pritchard 1985b) and *Dactylorhiza maculata* (Dijk 1987) after 2 to 6 mo storage. Also, *Cattleya aurantiaca* seeds at 6.5% and 10.4% moisture content (i.e., 52% to 79% r.h.—see FIGURE 1) lost only 33% and 37% viability in 6 yr storage at 5°C (Seaton & Hailes 1989).

The largest improvement in orchid seed longevity came from the combination of cool, refrigerator temperatures with dry storage conditions. This was evident nearly 60 years ago when it was found that some viability was maintained in *Cattleya* seed after 8 yr storage (Knudson 1934). Shorter term benefits have also been shown for seeds of *Brassolaeliocattleya* and *Dendrobium* species (Kano 1965), *Eulophia alta* (Pritchard 1985b) and eight species of European orchids (Ronse 1989, after van Waes 1984) stored for varying periods up to 17 mo. However, comprehensive data on the long-term storage potential associated with the use of cold, dry conditions came from the pioneering studies of Knudson on *Cattleya* hybrids and a few other species. These studies demonstrated varying lev-

TABLE 2. Orchid seed viability after storage at temperatures below 0°C.

Species	Time in store[1]	Storage conditions: Temperature (°C)	Storage conditions: Moisture status[2]	Seed viability (%)[3]: Initial	Seed viability (%)[3]: After storage	Reference
Cattleya loddigesii × *C. schroederiana*	2 hr	−5 to −10 (following freezing to −78 for about 3 min)	Vacuum dried, bathed in coconut liquid	n.s.[4]	n.s.—"all but one tube . . . germinated"	Svihla and Osterman 1943
Laelia anceps × *Cattleya trianaei*	2 hr	−5 to −10 (following freezing to −78 for about 3 min)	Vacuum dried, bathed in coconut liquid	n.s.	n.s.—"all but one tube . . . germinated"	Svihla and Osterman 1943
Cattleya luegeae	16 d	−23	Air dry	n.s.	n.s.—"germination . . . not strikingly impaired . . ."	Burke and Northen 1948
Cybidium (two kinds, n.s.)	n.s.	n.s.	Dry freezing in vacuum (0.05–0.1 mm Hg)	About 90% & 25%	n.s.—"showing about the same percentage . . ." as control	Dungal 1953
Cattleya Remy Chollet × *Laeliocattleya* Hyperion	465 d	−79	Desiccated—n.s.	n.s.	n.s.—germinated "well"	Ito 1965
Cattleya Suzanne Hye × *C.* Bob Betts	209 d	−79	Desiccated—n.s.	n.s.	n.s.—germinated "well'	Ito 1965
Dendrobium nobile	365 d	−79	Soaked in 80–100% glycerine	n.s.	n.s.—germinated "well'	Ito 1965
Laeliocattleya Corisande × *Brassolaeliocattleya* Norman's Bay 'Royal Bride'	465 d	−79	Desiccated—n.s.	n.s.	n.s.—germinated "well'	Ito 1965
Acampe papillosa	2 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
Arundina graminifolia	2 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
Catasetum dilectum	2 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	<20	Bowling and Thompson 1972
Cattleya loddigesii	2 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
C. luteola	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972

TABLE 2. Continued.

Species	Time in store[1]	Storage conditions: Temperature (°C)	Storage conditions: Moisture status[2]	Seed viability (%)[3]: Initial	Seed viability (%)[3]: After storage	Reference
Coelia triptera	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
Coelogyne fimbriata	3 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
Cycnoches haagii	2 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	<20	Bowling and Thompson 1972
Dendrobium moschatum	2 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
Doritis pulcherrima	2 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	<20	Bowling and Thompson 1972
Epidendrum nocturnum	6 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
E. pallidiflorum	3 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	50–75	Bowling and Thompson 1972
E. patens	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	50–75	Bowling and Thompson 1972
E. sceptrum	3 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
Eulophidium maculatum	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
Jumellea sagittata	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
Maxillaria valenzuelana	6 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972

TABLE 2. Continued.

Species	Time in store[1]	Storage conditions: Temperature (°C)	Storage conditions: Moisture status[2]	Seed viability (%)[3]: Initial	Seed viability (%)[3]: After storage	Reference
Oncidium ampliatum	3 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
Pleione bulbocodioides	3 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
Polystachya affinis	6 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
P. albescens	6 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
P. cultriformis	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
P. fusiformis	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
P. vulcanica	6 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	<20	Bowling and Thompson 1972
Sophronitis coccinea	3 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	20–50	Bowling and Thompson 1972
Spathoglottis plicata	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
Stanhopea bucephalus	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	50–75	Bowling and Thompson 1972
S. tigrina	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	<20	Bowling and Thompson 1972
Zygopetalum intermedium	7 mo	−10	Pre-dried over anhydrous calcium chloride	n.s.	>75	Bowling and Thompson 1972
Calanthe discolor × *C. sieboldii*	1 yr	−15 to −17	Dry—n.s.	72	20	Hasegawa *et al.* 1978
C. discolor	1 yr	−15 to −17	Dry—n.s.	35	5	Hasegawa *et al.* 1978

TABLE 2. Continued.

Species	Time in store[1]	Storage conditions: Temperature (°C)	Storage conditions: Moisture status[2]	Seed viability (%)[3]: Initial	Seed viability (%)[3]: After storage	Reference
Encyclia vitellinum	35 d	−40	Pre-dried with 'Drierite'	n.s.	n.s.—"comparable . . . quantity of germination . . . to control . . ."	Koopowitz and Ward 1984
Disa uniflora	≥15 min	−196	11% MC	61	56	Pritchard 1984
Eulophia alta	≥15 min	−196	11% MC	52	47	Pritchard 1984
E. stenophylla	≥15 min	−196	5% MC	68	70	Pritchard 1984
E. streptopetala	≥15 min	−196	11% MC	63	58	Pritchard 1984
Gymnadenia conopsea	≥15 min	−196	5% MC	45	46	Pritchard 1984
Orchis coriophora	≥15 min	−196	5% MC	43	43	Pritchard 1984
O. morio	4 × 15 min	−196	5% MC	62	82	Pritchard 1984
Phalaenopsis equestris	≥15 min		11% MC	84	79	Pritchard 1984
Satyrium nepalense var *ciliatum*	≥15 min	−196	11% MC	58	55	Pritchard 1984
S. n. var. *nepalense*	≥15 min	−196	11% MC	62	62	Pritchard 1984
Vanda pumilla	≥15 min	−196	11% MC	94	97	Pritchard 1984
Cattleya aurantiaca (seedlot 2)	50 d	−18	2.2% MC	94	2	Seaton 1985; Seaton and Hailes 1989
(seedlot 2)	90 d	−18	3.7% MC	94	10	Seaton 1985; Seaton and Hailes 1989
(seedlot 2)	1 yr	−18	5.6% MC	94	96	Seaton 1985; Seaton and Hailes 1989
Cattleya aurantiaca (seedlot 1)	10 d	−18	2.2% MC	80	8	Seaton 1985; Seaton and Hailes 1989
(seedlot 1)	80 d	−18	3.7% MC	80	25	Seaton 1985; Seaton and Hailes 1989
Cattleya (seedlot 1)	200 d	−18	5.6% MC	80	10	Seaton 1985; Seaton and Hailes 1989
Orchis morio	1 yr	−10	n.s.	n.s.	"very good germination	Ronse 1989, after Reinecke 1989

[1] min, minutes; hr, hours; d, days; mo, months; yr, years.
[2] MC, moisture content (wet basis).
[3] Values given are for germination.
[4] n.s., not specified.

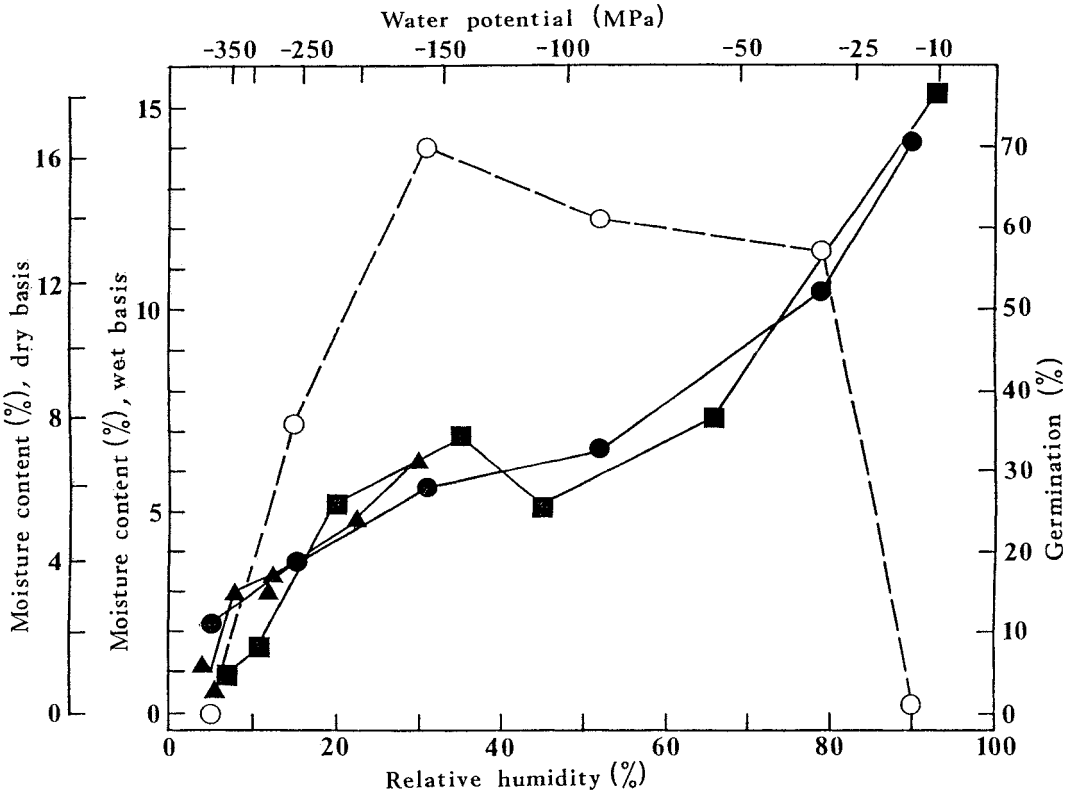

FIGURE 1. Comparison of the relationship between equilibrium moisture content (%) and relative humidity (%) at 21°C (●) with seed germination (%) after 6 years storage at 5°C (○) for seeds of *Cattleya aurantiaca* (adapted from Seaton & Hailes 1989). The same sorption relationship is shown for a 1990 seedlot for which the equilibrium moisture content (■) or equilibrium relative humidity were determined (▲). Also presented is a scale indicating the relationship between relative humidity and seed water potential (MPa) at 21°C.

els of viability after up to 22 yr storage at 8°C over anhydrous calcium chloride (Knudson 1940, 1953). Such longevity remains unsurpassed today.

A limitation of the early and also current studies is the failure to record the initial viability of the seedlot before storage (TABLES 1, 2). Such data are essential for the assessment of the rate of viability loss. Nonetheless, for the five seedlots which Knudson tested in 1940 and again in 1952, the time taken for seed viability to fall to 50% germination was 10 to 14 yr: the variation in longevity probably reflected differences in the initial viabilities of the seedlots. Similarly, the time taken for two seedlots of *Cattleya aurantiaca* to reach 50% viability when stored at 5°C and 5.6% moisture content appeared to be dependent on the initial seed quality (Seaton & Hailes 1989): ~4 years in the lower quality seedlot and 8 years for the higher quality seedlot (TABLE 1).

While cool, dry conditions are considered optimal for orchid seed storage, studies on the relationship between seed longevity and moisture content of the seed are few (Pritchard 1985b, Seaton 1985, Seaton & Hailes 1989). Due to the extremely small size of seed of most orchid species (about 1.5 μg for a single seed of *Cattleya aurantiaca* (Harrison 1973)) impracticably large quantities of seed are necessary to determine seed moisture contents accurately, unless a seven place balance is available. However, such determinations have shown that seed longevity of *Eulophia alta* is considerably improved at 2°C when the seed moisture content is 5% rather than 23% (Pritchard 1985b). Also, *Cattleya aurantiaca* seed displayed an increase in longevity at 5°C when seed moisture content was decreased from 14% to 5%, but further drying to 3.7–2.2% reduced longevity at 5°C and 20°C (Seaton & Hailes 1989).

Seed Storage at Temperatures below 0°C (TABLE 2)

Survival of orchid seeds following exposure to freezing temperatures was first demonstrated for a *Cattleya* hybrid and *Laelia* × *Cattleya* hybrid which had been vacuum dried at −5°C to −10°C

in the presence of coconut liquid, following ~3 min pre-freezing at −78°C (Svihla & Osterman 1943). In a similar study, but in the absence of a bathing fluid, *Cymbidium* seeds were successfully subjected to dry freezing under vacuum for an unspecified time period (Dungal 1953). Also, brief exposure of up to 1 hr in liquid nitrogen temperature (i.e., −196°C) was not damaging for seeds of 10 orchid species across 7 genera (Pritchard 1984). *Orchis morio* seeds even exhibited increased germination after four freezing treatments, and protocorm growth rate was unaffected. In addition, leaf production was unhindered in *Orchis coriophora* and *Disa uniflora* (Pritchard 1984).

Short-term (~2 wk) storage of air-dry *Cattleya luegeae* seeds at −23°C resulted in germination which was "not strikingly impaired" by the treatment, compared with storage at 4°C and 22°C (see TABLE 1) (Burke & Northen 1948). Likewise, storage of 5 wk at −40°C did not reduce the "quantity of germination" of *Encyclia vitellinum* seed (Koopowitz & Ward 1984), and 1 yr at −10°C was possible for seeds of *Orchis morio* (Ronse 1989, after Reinecke 1989). Moreover, seeds of 29 species across 20 genera tolerate dry storage at −10°C for 2–7 months with varying levels of viability retention (Bowling & Thompson 1972).

As a result of these short-term successes at −10°C to −40°C, it appeared that the long-term conservation of orchids as seed stored at conventional seed bank temperatures would be possible. However, *Calanthe discolor* × *C. sieboldii* and *C. discolor* seed stored at −15°C to −17°C for 1 year lost 52% and 30% viability, respectively (Hasegawa *et al.* 1978). This report provided the first indication that dry seed of some species may be relatively short-lived when stored at conventional seed bank temperatures. Subsequently, it was noted that the seedlots stored at −10°C by Bowling and Thompson (1972) were inviable when retested 8 yr later (Pritchard 1986). Finally, more evidence that dry seed (2.2% to 5.6% moisture content) may have reduced longevity at −18°C as compared with 5°C (see TABLE 1) was provided for *Cattleya aurantiaca* (Seaton 1985, Seaton & Hailes 1989).

In contrast, while the response of seeds to dry storage at about −20°C for 1 yr varied between species, desiccated or glycerine soaked seeds of three *Cattleya* hybrids and *Dendrobium nobile* all germinated "well" after 209–465 days storage at −79°C (Ito 1965).

CURRENT STATUS

Seed Storage Characteristics

The distinguishing features of orthodox seeds are desiccation tolerance and an increase in longevity accompanying a decrease either in storage temperature (from 90°C to −13°C) or seed moisture content (from 25% to 1.8%, wet basis) (Roberts & Ellis 1989, Dickie *et al.* 1990). From the extended seed longevity that is evident after drying from 23% to 5% moisture content and after cooling from 62°C to 0°C (TABLE 1), it appears that seeds from the orchid species investigated to date are essentially orthodox in their storage characteristics.

Cattleya aurantiaca seed exhibited increased longevity at 5°C when moisture content was reduced from 14% to around 5%. A further reduction in moisture content decreased longevity not only at 5°C but also at −18°C and 20°C (Seaton & Hailes 1989). This critical level of hydration approximates to a relative humidity of 30% (FIGURE 1). This relationship between seed moisture content and relative humidity was constructed after equilibration of the seeds for 4–7 days over silica gel (<5% r.h.) and saturated salt solutions yielding relative humidities from 7% to 90% (Greenspan 1977, Weast 1971–1972). After equilibration, seed moisture content was determined gravimetrically following heating at 103°C for 17 hr (International Seed Testing Association 1985), or at 80°C to constant weight. A majority of determinations utilized 2 mg dry matter weighed on a balance with a resolution of 0.1 μg. Alternatively, a single 60 mg dry matter aliquot of seed was desorbed from 31% to less than 5% r.h. At each sampling time the seed equilibrium relative humidity was directly determined using a Michell Series 4020 dewpoint hygrometer operating at 21°C. Seed wet weight was recorded immediately after withdrawal from the hygrometer and the seed moisture content subsequently related to the seed equilibrium relative humidity following dry weight determination after oven drying.

Orthodox seeds of pea are susceptible to imbibitional injury if soaked in water after drying to below 8% moisture content (Ellis *et al.* 1990a), a moisture level which is equivalent to about 30% relative humidity (Vertucci 1990a). This suggests that rehydration injury during the surface sterilization of *C. aurantiaca* seeds could confound the longevity response. In this respect, the morphology of orchid seeds may be important.

Like many other orchid species, *C. aurantiaca* seeds are fusiform in shape with a centrally located prolate spheroid embryo (Arditti *et al.* 1979). Our measurements indicate that the volumes of dry *C. aurantiaca* seed and embryo are 0.81 $mm^3 \times 10^{-3}$ and 0.46 $mm^3 \times 10^{-3}$ respectively. The embryo occupies approximately 57% of the seed volume, and accordingly, any water uptake during surface sterilization should occur in the vapor phase across the air space. As vapor phase humidification precludes imhibi-

TABLE 3. Orchid seed germination after long-term storage at sub-zero temperatures.

Species (code no.)	Storage conditions			Seed germination (%)[3]		Reference
	Time in store[1]	Temperature (°C)	Moisture status[2]	Initial	After storage	
Orchis morio (250785)	6 yr	−20	Pre-equilibration at 15% rh and 15°C	62	74	Pritchard (unpubl)
Dactylorhiza fuchsii (220884)	6.8 yr	−20	Pre-equilibration at 15% rh and 15°C	73	15	Pritchard (unpubl)
D. fuchsii (120985)	5.9 yr	−196	Pre-equilibration at 15% rh and 15°C	57	15	Pritchard (unpubl)
D. fuchsii (110886)	5.9 yr	−196	Pre-equilibration at 15% rh and 15°C	54	41	Pritchard (unpubl)

[1] yr, years.
[2] rh, relative humidity.
[3] Values given are for protocorm formation from ≥200 full seeds of *D. fuchsii* or *O. morio* sown on KC or Norstog medium respectively and incubated for ≥12 weeks in the dark (Pritchard, 1985b).

tional injury in dry pea seeds (Ellis *et al.* 1990a), it seems unlikely that rehydration injury contributes to the reduced longevity of *C. aurantiaca* seeds at relative humidity less than 30%.

The low moisture content limit to the logarithmic relations between moisture content and longevity of orthodox seeds coincides with a water potential of about −350 MPa (Roberts & Ellis 1989) or 10% relative humidity (Ellis *et al.* 1989). However, reduced longevity was observed for *C. aurantiaca* seed stored at −18°C to 20°C at equilibrium relative humidities less than 31% r.h. (Seaton & Hailes 1989). This relative humidity is equivalent to a water potential of about −150 MPa (FIGURE 1) based on the equation:

$$\psi_w = (RT/\bar{V}_w) \ln(\%r.h./100)$$

where R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the absolute temperature, and $\bar{V}_w$ is the partial molar volume of water (i.e., 18 cm^3 mol^{-1}) (Nobel 1970).

A similar longevity response has been noted recently in coffee seeds stored over a similar temperature range at water potentials lower than −90MPa (i.e., less than 50% r.h.) (Ellis *et al.* 1990b). Like *C. aurantiaca* seeds, coffee seeds exhibit an accelerated rate of viability loss when stored dry at sub-zero temperatures compared with warmer temperatures: specifically, 5°C for *C. aurantiaca* (Seaton & Hailes 1989) and 15°C for coffee (Ellis *et al.* 1990a). These responses in coffee have led to the suggestion that an 'intermediate' category of storage behavior exists between orthodox and recalcitrant seeds. (Note: recalcitrant seeds are those killed when their moisture content is reduced below some relatively high value (Roberts 1973).) It was evident for coffee that considerable variation between seedlots existed in desiccation sensitivity and longevity under cool, dry storage conditions (Ellis *et al.* 1990b, 1991), suggesting that a distinction between fully orthodox and 'intermediate' categories of seed storage behavior is not clear. Moreover, the uncertainty about classifications within the orthodox seed storage category is confounded by the response of *Araucaria columnaris* seeds. They display desiccation sensitivity below about 10% moisture content similar to that seen in some coffee seedlots, but do not exhibit a concomitant reduction in longevity (Tompsett 1984). Consequently, we suggest at this stage that *C. aurantiaca* seeds be considered as essentially 'orthodox' but unlike other orthodox seeds such as those of soybean and lettuce. Optimum long-term storage conditions remain to be identified.*

* However, we now have evidence of orthodox long-term storage at freezing temperatures for dry seeds of

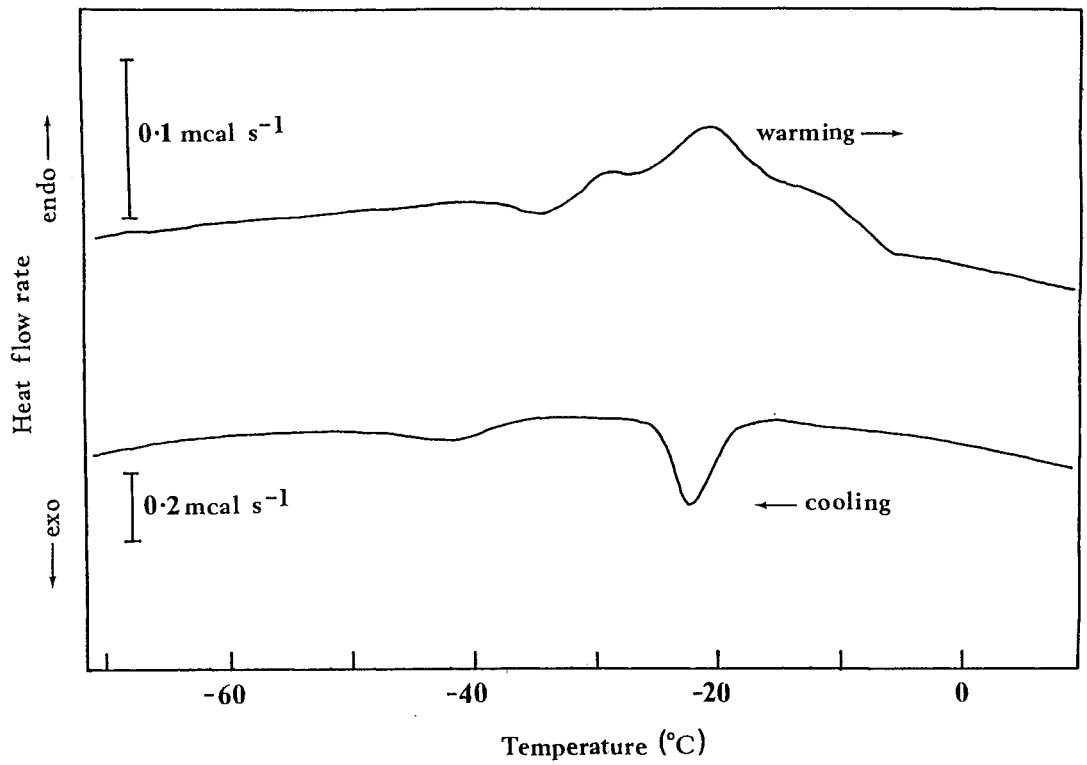

FIGURE 2. Differential scanning calorimetry thermograms for *Cattleya aurantiaca* seed pre-equilibrated to 31% relative humidity and cooled and warmed at 20°C min^{-1}.

Comparative Seed Longevity

It is apparent from TABLE 1 that under certain conditions orchid seeds can be stored for many years. We addressed one question: how long-lived are *C. aurantiaca* seeds under ideal conditions in comparison to the seeds of other angiosperm species? A comparison was made between the longevity of *C. aurantiaca,* a herbaceous perennial, lettuce (*Lactuca sativa*), a herbaceous annual) and smooth-leafed elm (*Ulmus carpinifolia*), a woody perennial. Storage conditions of 5°C and 31% r.h. were used in this analysis.

The influence of moisture content and temperature on seed longevity in lettuce and smooth-leafed elm have been determined (Dickie *et al.* 1990). From these constants it was possible to estimate how long it will take for seed viability to fall by a fixed proportion (e.g., by 1 probit. One probit is equivalent to one standard deviation of the mean distribution of seed deaths in time and, in real terms, represents the time taken for seed viability to fall from 84% to 50%).

At 31% r.h. the equilibrium moisture content was close to 5% (wet basis) for lettuce (Roberts & Ellis 1989, Vertucci 1990a) and 6.5% for smooth-leafed elm (Tompsett pers. comm.). Using these moisture contents in the viability equation (Ellis & Roberts 1980), we estimate that lettuce and smooth-leafed elm will take >20 and 4 yr respectively to lose 1 probit of viability at 5°C. By comparison, approximately 7 yr were required for an equivalent loss in viability in *C. aurantiaca* seeds. Despite the optimal storage conditions for this orchid species, their seeds were ~3× shorter-lived than lettuce but 2× longer-lived as smooth-leafed elm.

Thermal Transitions in Dry Seeds

As with dry *C. aurantiaca* seeds, a similar sensitivity to storage at sub-zero temperatures was noted for isolated embryos of oil palm at around 10% moisture content, and all viability was lost during only 1 mo storage at −18°C (Grout *et al.* 1983). Detailed histochemical and anatomical studies have revealed that both *C. aurantiaca*

two temperate species (TABLE 3). In *Orchis motio* seed no loss in viability was observed after 6 yr storage at −20°C; in *Dactylorhiza fucshii* seed, where some losses in viability occurred under these conditions, such losses were reduced by storage in liquid nitrogen (i.e. −196°C).

(Harrison 1973) and oil palm embryos (Alang 1981) are abundant in protein and lipid. Because ultra low temperature treatment induced changes in the embryo lipid reserves of *Setaria lutescens* (Jordan *et al.* 1982), we hypothesized that lipid transitions at sub-zero temperatures might be responsible for the low temperature response of dry *C. aurantiaca* seeds. In our initial studies, differential scanning calorimetry (DSC) has been used to investigate the conformational changes in the seed lipids at sub-zero temperatures.

In 1990 seeds were harvested, cleaned, equilibrated to 31% relative humidity and stored at 6°C in polypropylene vials by the method of Seaton and Hailes (1989) and Seaton and Pritchard (1990a, 1990b) until required.

A 3.64 mg sample of seeds was sealed in an aluminum pan and subjected to the following thermal treatment: cooling at -20°C min^{-1} from 25°C to -40°C, crash cooling to -140°C (at about 140°C min^{-1}), rewarming to 60°C at 20°C min^{-1} and cooling again to -140°C at 20°C min^{-1}. All operations were conducted with a Perkin-Elmer DSC-2 equipped with liquid nitrogen sub-ambient accessory, with machine calibration, data acquisition and manipulation as reported by Sutton (1991). The moisture content of the sample was estimated gravimetically after heating of the punctured pan for 17 hr at 103°C and found to be 6.1%.

The DSC thermogram presented in FIGURE 2 shows two exothermic events during cooling: a relatively sharp peak at approximately -20°C and a broader, flatter peak at -35°C. A third deflection of the thermogram was observed at -90°C which was also evident at the same temperature during warming (data not presented). Also during warming, a broad, complex endotherm with numerous component peaks was observed over the temperature range -35 to around -5°C.

The lowest moisture content at which freezable water is detectable in seeds varies with the chemical composition of the seed. In lipid-rich soybean the threshold value is, on a dry weight basis, about 23% for the cotyledons, compared to 26% for pea cotyledons which are less oily (Vertucci 1989b). On a relative humidity basis, freezable water is not detected in seeds after equilibration to less than approximately 80% r.h. (Vertucci 1990b). Thus, the thermal events recorded during cooling and warming of *Cattleya aurantiaca* seeds previously equilibrated to 31% r.h. cannot be ascribed to the freezing of water.

The transitions related to the lipid component of the seed as similar responses in the DSC have been observed for dry soybean and sunflower seeds, and in their extracted oils (Vertucci 1989b). Determination of the lipid content of a 0.36g sample of *C. aurantiaca* seed by the hexane extraction method (British Standards Institution 1982) revealed a content of 29% (dry basis). While only a single determination was performed on approximately 100,000 seeds, the close similarity between the water sorption isotherm for *C. aurantiaca* (FIGURE 1) with that for lettuce (37% lipid, Vertucci 1990a) indicate that the determination was reasonably accurate.

It is known that dry, lipid-rich seeds of some species exhibit a reduction in germination index (i.e., percent germination × radicle length) when rapidly cooled to liquid nitrogen temperatures, i.e., -196°C, and this response has been linked to a glass transition, or vitrification, of the lipid component at around -90°C (Vertucci 1989a). However, the damaging effects of storage of dry *C. aurantiaca* seed at -18°C cannot be ascribed to lipid vitrification as the temperature excursion was insufficiently low. It appears then that the reduced longevity in these seeds may be associated with the occurrence of the lipids in a specific conformational state: transitions taking place over the temperature range -35 to -5°C during warming (FIGURE 2). Storage of the seeds at a temperature a few degrees above that at which the lipids have completed their conformational state changes appears to offer the best longevity in these seeds (i.e., 5°C > 20°C > -18°C) (Seaton & Hailes 1989). The mechanism by which the state of the lipid bodies might influence seed longevity remains unknown.

FUTURE PROSPECTS FOR LONG-TERM CONSERVATION

It is not possible at this stage to comment conclusively on future prospects for the long-term conservation of orchid species as this would be based on empirical observations made on a limited number of species (TABLES 1, 2, 3): more comprehensive long-term study is required. Preferably, these species should reflect the natural variation that exists within the family based on taxonomy (i.e., representatives from the five subfamilies), habit (i.e., terrestrial or epiphytic) and geographical provenance (i.e., tropical or temperate origin).

The data for *C. aurantiaca* (Seaton & Hailes 1989) and other tropical orchids (Bowling & Thompson 1972, Pritchard 1986) do suggest that the long-term storage of such species under conventional seed bank conditions (i.e., ca. 5% moisture content and -20°C) is problematical. Whether these difficulties reflect a real difference between the seed storage physiology of tropical and temperate orchid species or relate to seed handling and storage imperfections is still uncertain in this regard. Comparative long-term

studies on seed longevity at sub-zero storage temperatures which are not coincidental with lipid transitions in the seed would be invaluable.

Provisional recommendations on the medium term storage of orchid species i.e., 30% r.h. and 5°C, have been made on the basis of the evidence from *C. aurantiaca* over a limited range of storage conditions (Seaton & Pritchard 1990a, 1990b). However, the precise inter-relationship between seed longevity, temperature and moisture content have not been produced. The generation of the 'viability constants' which describe these inter-relationships will be invaluable not only for the design of future orchid seed storage facilities but also for a more comprehensive evaluation of longevity of orchid seed compared with seeds of other families.

Although seeds of some temperate species store well at 15% r.h. it is not yet clear whether longevity might be improved initially and then subsequently reduced as relative humidity is decreased from 30% to 15% and lower (see FIGURE 1). What is evident however, is that the drying of seeds with desiccants such as anhydrous calcium chloride and silica gel may reduce seed equilibrium relative humidities below that optimal for storage. For example, freshly regenerated silica gel desiccant has a relative humidity $<5\%$ (see FIGURE 1). (Note: the presence of pink crystals with blue could mean that the relative humidity is close to 20%; and anhydrous calcium chloride has been measured at about 10% r.h., rising to approximately 30% when saturated with water.) In this relative humidity range below 30% it remains to be seen whether rehydration injury must be eliminated through the use of a humidification treatment prior to seed surface sterilization.

Finally, it is evident from TABLE 1 that an important factor in the practical storage of any seedlot is its initial viability (Knudson 1940, 1953, Seaton & Hailes 1989). Further, research is required to correlate seed harvesting with the time of highest seed viability.

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