CHAPTER 3

Storage

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The importance of seed storage to mankind has been recognized since prehistoric times (Priestley 1986). Most seed storage practices have developed empirically, often as a result of a combination of chance observation and pragmatic decisions on what can be achieved most easily by way of environmental control or modification. In agriculture, for example, the driving force for such empirical knowledge has been the simple practical requirement to store seeds reliably from harvest to the next production season. In silviculture, similarly, advice on storing tree seeds has long been available. For example, one and a half millenia ago it was recommended with regard to chestnut (probably *Castanea mollissima*) that "the fresh seeds should be stored in damp soil placed in the house, and during transport the seeds should be packed in a leather bag, because chestnut seeds will die when exposed to sun and wind" (Ssu-Hsieh Chia 535).

In discussing tree seed storage, we need to consider four principal factors: seed survival (i.e. viability, the ability of a seed to remain alive so that, when required, it will germinate and produce a seedling capable of autotrophic growth); storage environment; storage duration; and the species (actually five factors if variation among seed lots within a species is also considered).

One structure for such an essay would be to emphasize the duration of seed storage required. It is often convenient to classify such requirements into three, somewhat arbitrary, categories: short-term storage, typically from a matter of a few days to perhaps 6 or 9 months; medium-term storage, typically 1 to 5 years (for example, in order to carry stocks of seeds to avoid seed shortages after poor seed-production years); and long-term storage, typically 10 to very many more years (principally for genetic resources conservation). However, long-term seed storage is currently feasible only for species with certain characteristics. Consequently, we believe it is helpful to structure this essay around the principal differences in seed storage behavior among species. While most readers of this essay will be concerned solely with the seeds of trees, literature on other species is also cited here where helpful, since the principal differences among species in seed storage behavior that have been identified apply to all species of higher plants.

CLASSIFICATION OF SEED STORAGE BEHAVIOR

Although far from complete, the world literature includes several comprehensive compendia which provide information on tree seed storage (table 1). Some compendia on seed storage behavior were based on the duration of seed survival (whether air-dried or in soil or water) without considering the role of

Table 1

Collation of Several Compendia of Information on Seed Storage of Tree Species

Reference	Range of Species	Comments
Ewart (1908)	2,371 species of weeds, crops, and trees	Information on seed survival in relation to storage duration in soil, water, air-dry storage (mostly open storage at ambient temperature)
Dent (1948)	244 species of forest trees of India	Information on seed survival in relation to storage duration (mostly open storage at ambient temperature)
Holmes and Buszewicz (1958)	170 species of temperate forest trees	Information on seed survival in relation to storage duration and environment
Altman and Dittmer (1972)	123 species of weeds, crops, and trees of temperate climate	Information on seed survival in relation to storage duration and environment
Harrington (1972)	857 species of weeds, crops, and trees of temperate climate	Information on seed survival in relation to storage duration and environment
Wang (1974)	48 species of temperate forest trees	Information on seed survival in relation to storage duration and environment
King and Roberts (1979)	77 species (mostly trees) with recalcitrant seed storage behaviour	Information on seed survival in relation to storage duration and environment of recalcitrant seeds
Hofmann and Steiner (1989)	211 species (mostly trees) with recalcitrant seed storage behaviour	Updated list of species with recalcitrant seeds
Gordon (1992)	40 genera of temperate forest trees	Advice on medium-term tree seed storage environments
Tompsett and Kemp (1996)	120 species within 29 genera of tropical forest trees	Summary of authors' results of studies in seed desiccation and/or storage
Hong and others (1996)	6,914 species within 2,069 genera and 251 families including crops, and trees all over the world	Classification of seed storage behavior with information on seed survival in relation to desiccation and storage

environmental factors on seed longevity (e.g. Altman and Dittmer 1972, Ewart 1908, Harrington 1972). It was later found that certain species' seeds classified initially as "microbiotic" by Ewart (1908) or "short-lived" seeds (e.g. Harrington 1972) can in fact be stored for long periods of time under more suitable conditions. Therefore, later compendia have classified species on the basis of seed desiccation tolerance and/or the response of seed survival or longevity to environment (Hong and others 1996, King and Roberts 1979, Tompsett and Kemp 1996).

In this article we consider seed survival and longevity in the context of the response to environment, three different, discrete categories having been identified: orthodox seed storage behavior (Roberts 1973); recalcitrant seed storage behavior (Roberts 1973); and intermediate seed storage behavior (Ellis and others 1990a).

ORTHODOX SEED STORAGE BEHAVIOR

Orthodox seeds can be dried without damage to low moisture contents and, over a wide range of environments, their longevity increases with decreases in seed storage moisture content and temperature in a quantifiable and predictable way (Roberts 1973). The latter is defined by the seed viability equation

$$v = K_i - p / 10^{K_E - C_W \log_{10} m - C_H t - C_Q t^2}, \qquad (1)$$

where v is probit percentage viability after p days in storage at m percent moisture content (w.b.), t°C, K_i is a constant specific to the seed lot, and K_E, C_W, C_H and C_Q are species viability constants (Ellis and Roberts 1980). The constant K_I indicates the initial viability (in probits) of the seed accession when placed in storage. The value of K_I may vary considerably among different seed lots within a species, for example as a result of differences in seed production environment, time of harvest, or genotype (e.g. Ellis and others 1993).

The constants C_H and C_Q together describe the response of seed longevity to temperature. The particular form of the relation between longevity and temperature is a negative curvilinear semi-logarithmic relation whereby the relative benefit to longevity of each 10 °C reduction in temperature (i.e. Q_{10}) declines, the cooler the temperature (Dickie and others 1990, Ellis and Roberts 1981). A comparison of contrasting species, which included one tree species, found that the values of the temperature terms C_H and C_Q did not differ significantly among species (Dickie and others 1990). Similarly, the values of C_H and C_Q determined for four forest tree species (*Liquidambar styraciflua* L., *Pinus elliottii*, *Pinus taeda*, and *Platanus occidentalis*) by Bonner (1994) were 0.0306-0.0508 and 0.000328-0.000976, respectively, and thus close to the common estimates for diverse species of 0.0329 and 0.000478, respectively, determined previously (Dickie and others 1990). This suggests that seed longevity in all orthodox species may well show a similar quantitative response to storage temperature.

At one temperature, the gradient of the negative logarithmic relation between seed moisture content (m) and longevity provides the value of the constant C_W. Thus the value of this constant quantifies the effect of moisture content on longevity. The value of C_W differs considerably among species. For example, the values of C_W collated by Hong and others (1996) for 53 species from 24 families vary from 0.983 for *Pinus elliottii* (Bonner 1994) to 6.305 for *Sorghum bicolor* (Kuo and others 1990). In general, species having seeds with a high starch content, e.g. cereals, have high values for C_W (around 6) but in oily seeds it is much lower, e.g. between 3.5 and 4 for onion (*Allium cepa*) and soyabean (*Glycine max*). Since tree seeds are generally rich in oil in the embryo and/or endosperm (Tompsett and Kemp 1996), tree species tend to have lower values for C_W, e.g. 0.983 for *Pinus elliottii* (Bonner 1994) and 4.23 for Norway maple (*Acer platanoides*) (Dickie and others 1991). In one comparison of estimates of C_W, the mean value for forest tree species was 2.8 compared with a mean value of 4.72 for herbaceous species (Tompsett 1994). One consequence is that to obtain the same relative increase in longevity it is necessary to dry orthodox tree and oily seeds more than starchy seeds from a given initial moisture content.

As a consequence of the negative logarithmic relation between seed moisture content and longevity, the relative benefit to longevity becomes greater for each successive reduction in moisture content. For example, based on the viability parameters estimated for *Ulmus carpinifolia* seeds by Tompsett (1986), the effect of a reduction from 7 percent to 5 percent moisture content provides about a 2.7-fold increase in longevity whereas a reduction from 5 percent to 3 percent in seed moisture content increases seed longevity about 4.5-fold. There appears, however, to be a common response of longevity to equilibrium relative humidity among contrasting crop species, at least, whereby longevity increases by a factor of about 2.2 for each 10 percent reduction in equilibrium relative humidity (Ellis and others 1990c, Zanakis and others 1993). However, we do not yet know whether this generalization for seeds of arable and horticultural crops also applies to tree seeds.

There are two limits to the negative logarithmic relation between seed moisture content and seed longevity (Roberts and Ellis 1989). One is an upper limit, above which seed longevity in hermetic storage is no longer reduced with further increases in moisture, and above which in aerated storage seed longevity increases with further increase in moisture content (Roberts and Ellis 1982). The other is a lower limit, below which further reduction in moisture content no longer increases longevity in hermetic storage (Ellis and others 1988, 1989, 1990b, 1990c, 1992). The upper moisture-content limit appears to be 11 to 12 percent in pine (*Pinus elliottii* and *Pinus taeda*) (Bonner 1994), 22 percent in elm (*Ulmus carpinifolia*) (Tompsett 1986) and *Araucaria columnaris* (Tompsett 1984a), and between 15 percent in lettuce (*Lactuca sativa*) (Ibrahim and Roberts 1983) to about 26 percent in durum wheat (*Triticum durum*) (Petruzzelli 1986) for crop species. Despite wide variation among species in terms of moisture content, these values coincide with a water potential of about -14 MPa (Roberts and Ellis 1989, Zewdie and Ellis 1991), i.e. the upper moisture-content limit to the viability equation occurs when seed moisture contents are in equilibrium with about 90 percent relative humidity (r.h.) at 20 °C. The lower limit varies substantially between crop species, e.g. between about 6 percent moisture content for pea (*Pisum sativum*) and mungbean (*Vigna radiata*), and about 2 percent for sunflower (*Helianthus annuus*) (Ellis and others 1988, 1989). However, these variant moisture contents coincide with 10 to 12 percent equilibrium relative humidity at 20 °C (Ellis and others 1988, 1989, 1992) or with a seed water potential of about -350 MPa at this temperature (Roberts and Ellis 1989). We do not yet know whether this lower limit also applies to forest tree seeds.

In open storage (i.e. where seeds are exposed directly or indirectly to ambient relative humidity; e.g. in a paper bag, cloth bag, or thin, unsealed polythene bag which permits seed moisture content to be influenced by ambient relative humidity) at ambient temperature the longevity of orthodox seeds varies considerably. For example, viability of *Salix* spp. cannot be maintained beyond 10 days in open storage (Brinkman

1974b) while seeds of *Cassia multijuga* were capable of 100 percent germination when tested after 158 years in open storage at room temperature in a Mediterranean climate (Becquerel 1934). For the maintenance of viability in long-term storage, *Salix* spp. require hermetic storage at low moisture contents and cool temperatures, e.g. sub-zero temperatures and moisture contents in equilibrium with 10 to 30 percent r.h. (relative humidity) (Zasada and Densmore 1977). Clearly, seeds of *Cassia multijuga* do not need such precise control of the storage environment in order to maintain viability in storage over the long term.

Provided estimates of the viability constants are available, the viability equation can be applied to determine suitable air-dry storage environments for a given duration of storage for a particular orthodox species. Since the same storage life can be achieved by different combinations of storage temperature and seed moisture content–the precise values depending upon species–there can be considerable flexibility. Since the relative benefit to longevity from reducing temperature becomes less the lower the storage temperature, while the relative benefit to longevity from reducing moisture content becomes greater the lower the moisture content (within the wide range of environments over which the viability equation applies) (Ellis and Roberts 1980), it is often more cost-effective to reduce seed moisture content than to lower storage temperature. This approach is of particular importance for seed storage centers where refrigeration to zero and sub-zero temperatures cannot be provided. In such situations it has been recommended that the seeds be dried to moisture contents in equilibrium with 10 to 12 percent r.h. at 20 °C and then stored hermetically at ambient or (preferably) cooler temperatures (Ellis and others 1989). This low-technology approach has been described by some as “ultra-dry” seed storage (International Board for Plant Genetic Resources 1992). Results from a 5-year investigation have shown that the longevity of ultra-dry seed at ambient temperatures is greater than that of crop seeds stored at 5 to 6 percent moisture content, at least for several crops (Ellis and others 1996). Similarly, over a much longer duration, 90 percent germination of seeds of barley (*Hordeum vulgare*) was reported when tested after 110 years in ultra-dry storage (3.1 percent moisture content) at 10 to 15 °C (Steiner and Ruckenbauer 1995). However, to date, ultra-dry seed storage has not been applied to tree seeds to our knowledge.

Successful storage of orthodox tree seeds over the long term under the FAO/IPGRI preferred conditions, i.e. 3 to 7 percent moisture content and -18 °C (Food and Agriculture Organization/International Plant Genetics Resources Institute 1994), has been realized for more than two decades in the Royal Botanic Gardens’ Kew Seedbank (Linington 1994) and for about 7 years at the International Institute of Tropical Agriculture (Ng and others 1993). For example, seeds of *Rhus verniciflua* and *Acacia tortilis* stored in the Kew Seedbank since 1970 and 1974, respectively (Linington 1994), showed 100 percent germination in recent tests (Hong and others 1996).

Long-term storage of orthodox tree seeds has also been achieved by cryopreservation (or cryostorage) at ultra-low temperatures from -80 °C to -196 °C with liquid nitrogen (Pence 1991b, Stanwood 1985, Touchell and Dixon 1993, Wang and others 1993). For example, no loss in viability was reported for *Pinus ponderosa*, *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heterophylla* following 3 years of cryopreservation in liquid nitrogen (Stanwood 1985). An essential first step in seed cryopreservation is the determination of optimum (safe) moisture contents for each orthodox species, particularly those with oily seeds. Wang and others (1993) collated information on the cryopreservation of orthodox tree seeds; it suggests that moisture contents between 3.8 and 11 percent are safe for at least short periods of time (from 4 days to 3 years). However, a loss in viability of about 10 percent following 14 days storage in liquid nitrogen at 6 to 8 percent moisture content was reported for seeds of certain tree species (Wang and others 1993). One possible cause of problems with liquid nitrogen seed storage is the requirement to optimize both the rate of cooling and the subsequent rate of warming; these must be determined empirically (Meryman and Williams 1981). Pretreatment with cryoprotectants sometimes improves seed survival in liquid nitrogen, but in some circumstances can be damaging (Touchell and Dixon 1993). Mechanical injury to seed by direct immersion into liquid nitrogen causing loss in viability has also been reported for some species (Pritchard and others 1988, Wang and others 1993). Finally, despite suggestions of infinite seed longevity by cryopreservation in liquid nitrogen, there is no firm evidence that reducing seed storage temperatures much below -20 °C results in further significant benefits to longevity, at least for tree seeds (Stanwood 1985; Tompsett 1986, 1994). For example, Tompsett (1986) found similar longevity for *Ulmus carpinifolia* seed stored at -13 °C and -75 °C.

For the maintenance of viability over the medium term, i.e. a period of 2 to 10 years or so, hermetic storage in a wide range of contrasting environments can be successful. For example, with relatively high moisture contents (in the air-dry range) combined with cool temperatures, e.g. 8 to 10 percent moisture content and 0 to 10 °C for most leguminous trees (Albrecht 1993), or low moisture contents combined with relatively warm temperatures, e.g. 3 to 7 percent moisture content and 10 °C to ambient temperatures, e.g. Barton (1961) reported 33 percent germination for *Ulmus americana* L. seeds following 4 years of hermetic storage at 3 percent moisture content at room temperature. Ultra-dry seed storage may be applicable to tree seeds for medium-term storage at ambient temperatures. For example, Joseph (1929) and Brinkman (1974a) recommended that seeds of *Betula* spp. be stored at moisture contents as low as 0.6 to 1 percent at room temperature. For practical advice on medium-term tree seed storage see Gordon (1992).

In addition to air-dry storage over a very wide range of different environments, successful storage of orthodox seeds over the short-term, from a few months to 1 or 2 years, can also be achieved by moist storage at cool temperatures. This approach is commonly used for tree species that exhibit strong dormancy such as those adapted to temperate climates (i.e. temperate latitudes and high altitudes of the Tropics) since prechilling during storage can remove dormancy. For example, several orthodox tree species of temperate climates, such as *Carya* spp., *Chionanthus virginicus*, *Euonymus* spp., *Hamamelis virginiana*, *Ilex* spp., *Juglans* spp., *Juniperus* spp., *Liriodendron tulipifera*, *Nyssa* spp., *Picea* spp., and *Taxus* spp. can be stored moist at 3 to 5 °C (Schopmeyer 1974). Similarly, orthodox seeds of several species that are native to high altitudes in the Tropics and show considerable dormancy can also be stored moist at 3 to 5 °C. For example, seeds of *Prunus africana*, native to high mountains between 900 m and 3400 m in Africa (Albrecht 1993), and *Michelia champaca* L. and *Michelia compressa*, native to high mountains of Asia (Lin and Wu 1995), can be stored moist at 4 °C for 1 year (Albrecht 1993, Lin and Wu 1995). In addition, seeds of some pioneer forest trees that have an absolute requirement for light in order to germinate can also be stored moist in the absence of light at ambient temperature for long periods of time. For example, seeds of *Piper aequale* can be maintained for 7 years fully imbibed in darkness without loss in viability (Vázquez-Yanes and Orozco-Segovia 1996).

RECALCITRANT SEED STORAGE BEHAVIOR

Recalcitrant seeds cannot be dried without damage (Roberts 1973) and so their longevity does not respond to air-dry environment in the manner described by the seed viability equation (1). When freshly harvested recalcitrant seeds are dried, viability is at first slightly reduced as moisture is lost, but then begins to be reduced considerably at a certain moisture content, termed the "critical moisture content" (King and Roberts 1979) or "lowest safe moisture content" (Tompsett 1984b). If drying continues further, viability is eventually reduced to zero.

Critical moisture contents for loss of viability on desiccation vary greatly among the recalcitrant species (Chin 1988, King and Roberts 1979), among cultivars and seed lots (Chin 1988, King and Roberts 1979), and depending upon the stage of seed maturity at time of collection (Finch-Savage and Blake 1994, Hong and Ellis 1990). The critical moisture content may also vary with the method of seed drying (Farrant and others 1985, Pritchard 1991, Pritchard and Prendergast 1986). The values of the "lowest safe moisture content" vary between extremes of about 23 percent for cocoa (*Theobroma cacao*) (Mumford and Brett 1982) to 61.5 percent for *Avicennia marina* (Farrant and others 1986). Despite this variation, these moisture contents are equivalent to a relatively narrow band of relative humidities of 96 to 98 percent, or seed water potentials of about -1.5 MPa to -5 MPa (Dickie and others 1991, Poulsen and Eriksen 1992, Pritchard 1991, Roberts and Ellis 1989, Tompsett and Pritchard 1993).

There is no satisfactory method of maintaining the viability of recalcitrant seeds over the medium and long term. Since they cannot be dried neither can they be stored at subzero temperatures, because they would be then killed by freezing injury resulting from ice formation. The longevity of recalcitrant seeds is short, from a few weeks to a few months for species adapted to tropical environments (King and Roberts 1979), and up to about 3 years for several species adapted to temperate environments (Suszka and Tylkowski 1981, 1982). However, if the optimum storage environments are carefully determined, longevity of several tropical recalcitrant seeds can be extended to 3 years, at least for species of *Symphonia globulifera* (Corbineau and Côme 1989).

The principle of successful moist seed storage for recalcitrant seeds is that seeds must be maintained at moisture contents close to that at which they are shed, with continuous access to oxygen; these circumstances minimize seed deterioration since repair mechanisms can operate (Villiers 1975). Under these conditions (high seed moisture and available oxygen), however, seeds tend to germinate. Clearly it is also essential that the conditions should prevent or at least delay germination. It is therefore easier to store recalcitrant species with dormant seeds (either primary dormancy or induced dormancy) than with nondormant seeds under such conditions. For nondormant seeds, as shown by most tropical tree seeds at maturity (King and Roberts 1979), low temperatures can reduce the rates of both seed deterioration and germination provided they remain above the value that results in chilling damage or the lower value at which ice crystallization occurs. For example, chilling injury occurs at between 5 and 10 °C for *Shorea roxburghii*, 5 and 12 °C for *Symphonia globulifera* and *Hopea odorata* Roxb. (Corbineau and Côme 1988). Determination of the optimum temperature for imbibed seed storage is required for each recalcitrant species. A suggested protocol for determining these values has been described elsewhere (Hong and Ellis 1996). The optimum temperature appears to be that at which nondormant seeds remain alive but are unable to germinate, i.e. the base temperature for germination (Corbineau and Côme 1988, Pritchard and others 1996). For example, the optimum storage temperatures determined in this way are 10 °C for *Shorea roxburghii*, 12 °C for *Mangifera indica*, and 15 °C for *Hopea odorata* and *Symphonia globulifera* (Corbineau and Côme 1988). However, recalcitrant seeds of species adapted to temperate climates (including high altitudes in the Tropics) show considerable dormancy which requires long periods of prechilling at 2 to 5 °C to overcome. Furthermore, seeds of many temperate species with recalcitrant seed storage behavior have the ability to germinate at 2 to 5 °C, and thus moist storage of such recalcitrant seeds at 0 to 10 °C may result in germination during storage. In such cases, germination may be prevented by reducing seed moisture content slightly (by about 5 percent below that of fresh seeds), or either reducing the storage temperature to below the optimum prechilling temperature (e.g. 0 to -3 °C) (Suszka 1978) or increasing it to the base temperature for germination of dormant seeds (Pritchard and others 1996). For example, the viability of seeds of sycamore (*Acer pseudoplatanus*) and *Quercus robur* can be maintained for 3 years at 24 to 32 percent and 40 to 45 percent moisture content, respectively, at -1 to -3 °C (Suszka 1978, Suszka and Tylkowsky 1981, Tylkowsky 1989), and horsechestnut (*Aesculus hippocastanum*) for 3 years if moist dormant seeds are stored at 16 °C, although in this case subsequent prechilling is required to remove dormancy (Pritchard and others 1996).

In practical terms, species with recalcitrant seeds can therefore be divided into two subcategories (Bonner 1990, Hong and Ellis 1996): those adapted to tropical climates, and those adapted to temperate climates (i.e. temperate latitudes, or high altitudes in the Tropics). In general, the viability of recalcitrant seeds can be maintained (albeit only for limited periods in aerated conditions) at moisture contents just less than fully imbibed, i.e. about 2 to 5 percent below those of fresh seeds, or in equilibrium with 98 to 99 percent r.h., at optimum storage temperatures which vary from about 7 °C to 17 °C among species adapted to tropical climates, and between about -3 °C and 5 °C among many of those adapted to temperate climates (Hong and others 1996). Maintaining recalcitrant seeds fully imbibed or subimbibed, with continuous aeration, and at the same time preventing germination and fungal contamination, is difficult. Aeration can result in loss of seed moisture, and respiration can quickly deplete oxygen. The storage medium is therefore very important for recalcitrant seeds. It should fulfill two functions: first, to maintain seed moisture constant at high values; second, to allow diffusion of sufficient oxygen to the moist seeds. The storage of moist recalcitrant seeds in damp charcoal, sawdust, or moist sand is generally reported to be more efficient than storage in polyethlene bags. Storage at or near the harvest moisture content in media such as sawdust (at 16 percent moisture content) or perlite (at 0 to 4 percent moisture content) within suitable containers, such as open-weave sacks or bags, placed in a high-humidity room has been recommended (Tompsett and Kemp 1996). Coating recalcitrant seeds with alginate or alginate with ABA was also reported to extend seed lifespan by a factor of up to 3 or 4 (Pammenter and others 1997).

Cryopreservation of zygotic embryos has been reported to be successful for a number of tree species that show orthodox, intermediate, or recalcitrant seed storage behavior (Engelmann and others 1995b). For successful cryopreservation, excised embryos or embryonic axes must survive desiccation below the threshold freezable moisture content (Hor and others 1990) of about 18 to 33 percent (see Hong and others 1996), below which value there is no freezable water for ice formation in ultra-low temperatures. Embryos and embryonic axes of some recalcitrant species are able to survive desiccation to lower moisture contents than are the whole seeds (Chandel and others 1995, Chin 1988, Finch-Savage 1992a, Normah and others 1986). For example, fresh seeds (36 percent moisture content) of *Hevea brasiliensis* tolerated desiccation to 20 percent moisture content, but no seeds survived further desiccation to 15 percent moisture content (Chin and others 1981). However, after desiccation to 16 percent moisture content followed by 16 hours cryopreservation in liquid nitrogen, 87 percent and 69 percent, respectively, of the excised embryonic axes (55 percent moisture content) survived (Normah and others 1986). Unfortunately, embryos of many recalcitrant seeds are damaged by desiccation before seed moisture content is reduced below those values at which ice crystallization no longer occurs, and so the cryopreservation of excised embryos of these species results in death, either by ice formation for embryos at moisture contents above the threshold freezable moisture content, or by desiccation to moisture contents below that threshold (Hor and others 1990).

Fast drying (also termed “flash” drying) may allow excised embryos to survive desiccation to lower moisture contents than those dried more slowly within intact seeds (Finch-Savage 1992a, Normah and others 1986, Pammenter and others 1991, Pritchard 1991, Pritchard and Prendergast 1986). Excised embryos or embryonic axes can be dried quickly in a laminar airflow cabinet at room temperature (e.g. Normah and others 1986) or under a flow of compressed air at about 9 liters min^{-1} (Pammenter and others 1991). After flash drying of embryonic axes extracted from mature seeds of *Landolphia kirkii* reduced moisture content from 67 percent to 23 percent in 30 minutes, 90 percent of the excised embryonic axes were able to germinate (Pammenter and others 1991). Fast drying with silica gel or with an aseptic air current has allowed excised embryonic axes to survive desiccation to a lower value than that achieved by vacuum drying (Fu and others 1993). The stage of maturity of the seeds from which the embryos are excised is an important factor influencing survival following desiccation: embryos excised from mature seeds not only survived desiccation to lower moisture contents than immature embryos, but also survived subsequent cryopreservation in liquid nitrogen (Chandel and others 1995, Engelmann and others 1995b). Fast freezing can allow desiccated embryos to better survive cryopreservation in liquid nitrogen (Berjak and Pammenter 1997). In contrast, slow freezing with immature excised embryos of cocoa has been reported to be more successful than fast freezing (Pence 1991a). The specimen to be frozen must be as small as possible, and pretreatment of embryos with cryoprotectants, such as DMSO, proline, sucrose, and glycerol (Assy-Bah and Engelmann 1992, Dumet and Berjak 1997, Pence 1991a), are important factors influencing the survival of excised embryos (or embryonic axes) in liquid nitrogen.

Results reported for *Aesculus* spp. (Pence 1990, 1992), *Araucaria hunsteinii* (Pritchard and Prendergast 1986), *Artocarpus heterophyllus* Lam. (cited by Engelmann and others 1995b), *Castanea sativa* (Pence 1990, 1992), *Citrus hystrix* (Normah and Serimala 1997), *Coffea liberica* (Hor and others 1993, Normah and Vengadasalam 1992), *Cocos nucifera* (Assy-Bah and Engelmann 1992, Chin and others 1989), *Dimocarpus longan* (Fu and others 1990, 1993), *Hevea brasiliensis* (Normah and others 1986), *Landolphia kirkii* (Vertucci and others 1991), *Quercus* spp. (González-Benito and Perez-Ruiz 1992; Jorgensen 1990; Pence 1990, 1992), *Trichilia dregeana* (Dumet and Berjak 1997) and *Theobroma cacao* (Pence 1991a) have shown the feasibility of the cryopreservation of excised embryos or embryonic axes of recalcitrant seeds. However, others have reported a lack of success using the same species. For example, experiments with excised embryos of *Artocarpus heterophyllus* (Dumet and Berjak 1977), *Landolphia kirkii* (Dumet and Berjak 1977), and *Quercus robur* (Poulsen 1992, Chmielarz 1997) found that none survived cryopreservation in liquid nitrogen.

INTERMEDIATE SEED STORAGE BEHAVIOR

Although the term "intermediate seed storage behavior" was introduced only at the beginning of this decade, this third category of seed storage behavior was hinted at more than eight decades ago. Elliott (1912, cited by Baldwin 1942) divided temperate forest tree seeds into three classes: (i) those that can be dried, (ii) those that can survive partial drying, and (iii) those that can rarely be dried at all. The first and third classes are more or less equivalent to the orthodox and recalcitrant categories of seed storage behavior defined in detail by Roberts (1973), respectively; the second class is equivalent to the "intermediate seed storage behavior" defined by Ellis and others (1990a).

The definition of intermediate seed storage behavior is based on the response of longevity to storage environment. In seeds showing intermediate seed storage behavior, the trend toward increasing longevity the lower the seed storage moisture content (within the air-dry range) is reversed at a comparatively high moisture content, whereby at lower moisture contents longevity is reduced (Ellis and others 1990a, 1991a, 1991b, 1991c). But in such species it is also often (but not always) observed that seeds may be damaged immediately by desiccation to relatively low moisture contents, about 7 to 12 percent moisture content depending on species. The critical moisture contents of intermediate seeds below which more rapid loss in viability occurs during hermetic storage varies considerably with species, degree of maturity, and method of seed extraction and/or postcollection handling. In general, seeds extracted from fruits at maturity tolerate desiccation to moisture contents in equilibrium with about 40 to 50 percent r.h., i.e., about 10 percent moisture content for arabica coffee (*Coffea arabica*) (Ellis and others 1991a, Hong and Ellis 1992a) and 7 percent moisture content for *Citrus* spp. (Hong and Ellis 1995). A further feature of intermediate seeds of tropical origin is the fact that the longevity of dry seeds (7 to 10 percent moisture content) is reduced with reduction in storage temperature below about 10 °C (Ellis and others 1990a, 1991a, 1991b, 1991c; Hong and Ellis 1992a). In such cases, then, there is an optimum air-dry storage environment for the maintenance of seed viability. In arabica coffee this is about 10 °C with 10 to 11 percent moisture content (Hong and Ellis 1992a).

It is now known that seeds of neem (*Azadirachta indica* A. Juss) (Gaméné and others 1996; Hong and Ellis 1998; Sacandé and others 1997a, 1997b), *Cinnamomum subavenium* (Lin 1996), several *Citrus* species (Hong and Ellis 1995), *Coffea arabica* (Ellis and others 1990a, 1991a; Hong and Ellis 1992a), robusta coffee (*Coffea canephora*) (Hong and Ellis 1995), oil palm (*Elaeis guineensis*) (Ellis and others 1991c), *Khaya senegalensis* (Hong and Ellis 1998), *Lindera megaphylla* (Lin 1996), *Neolitsea parvigemma* (Lin 1996), and *Swietenia macrophylla* (Hong and Ellis 1998, Pukittayacamee and others 1995) show intermediate seed storage behavior. Among about 7,000 species examined in a recent survey, about 134 species are believed to show intermediate seed storage behavior (Hong and others 1996). Many of these species are trees of tropical origin, but a few are herbaceous (Hong and others 1996). For example, that survey suggests that the following important tree species may show intermediate seed storage behavior: *Acer macrophyllum*, *Afrocarpus gracilior*, *Agathis* *macrophylla*, *Araucaria columnaris*, *A. nemorosa*, *A. rulei*, *A. scopulorum*, *Bertholettia excelsa*, *Bixa orellana*, *Chrysophyllum cainito*, *Dacrycarpus dacrydioides*, *Dipterocarpus alatus* Roxb. & G. Don, *D. intricatus*, *D. tuberculatus*, *Dipteryx odorata*, *Rhapidophyllum hystrix*, and *Virola surinamensis* (Hong and others 1996).

From the point of view of optimum air-dry seed storage environments, it may be helpful to distinguish between species with intermediate seed storage behavior adapted to tropical environments and those adapted to temperate environments (including high altitudes in the Tropics). For example, intermediate seeds of tropical origin such as arabica coffee (Bendana 1962, Wellman and Toole 1960) and papaya (*Carica papaya*) (Bass 1975) can be stored at moisture contents in equilibrium with 50 percent r.h. (9 to 10 percent moisture content) and 10 °C for up to 5 and 6 years, respectively, without loss in viability. The viability of intermediate seeds of temperate origin is also maintained well at moisture contents in equilibrium with about 50 percent r.h., but at cooler temperatures of 5 °C to -10 °C. For example, seeds of *Corylus avellana* can be stored hermetically at -5 °C with 10 to 13 percent moisture content (Degeyter 1987). Species showing intermediate seed storage behavior can be stored successfully over the medium term, therefore, provided optimum environments have been defined and can be maintained.

Like orthodox seeds, intermediate seeds can also be stored moist at cool temperatures if germination is either prevented or delayed. For example, seeds of arabica coffee (Van der Vossen 1979) and tea (*Camellia sinensis*) (Amma and Watanabe 1983) were stored moist at 15 °C and 1 °C, respectively, for 2.5 and 6 years, respectively, with little loss in viability.

The situation regarding the cryopreservation of intermediate seeds is a little confused. Despite reports of the immediate death of (whole) seeds of species with intermediate seed storage behavior following cryopreservation in liquid nitrogen, e.g. arabica coffee (Becwar and others 1983), *Corylus avellana* (Normah and others 1994), *Corylus cornuta* (Stanwood and Bass 1981), oil palm (Grout and others 1983), and *Roystonea regia* (Kunth) O.F. Cook (Ellis and others 1991c), there have been several reports of the survival of dry seeds of species with intermediate seed storage behavior following immersion in liquid nitrogen, e.g. *Camellia sinensis* (Hu and others 1994), *Carica papaya* (Becwar and others 1983, Chin and Krisnapillay 1989), *Citrus aurantifolia*, and *Citrus halimii* (Normah and Serimala 1997). This implies that it may be possible to improve cryopreservation techniques to make them suitable for intermediate seeds.

Since the whole seeds of species with intermediate seed storage behavior tolerate desiccation to relatively low moisture content (7 to 10 percent), desiccated embryos may have a greater chance to survive cryopreservation in liquid nitrogen than is the case for recalcitrant seeds (see above). Reports of successful cryostorage of excised embryos of *Azadirachta indica* (Dumet and Berjak 1977), *Camellia sinensis* (Chandel and others 1995; Chaudhury and others 1990, 1991; Dumet and Berjak 1977; Wesley-Smith and others 1992), *Citrus aurantifolia* and *Citrus halimii* (Normah and Serimala 1997), *Coffea arabica* (Abdelnour and others 1992), *Corylus avellana* (González-Benito and Pérez 1994, Normah and others 1994, Pence 1990, Reed and others 1994), *Elaeis guineensis* (Engelmann and others 1995a, 1995b; Grout and others 1983), *Howea forsteriana* (Chin and Krishnapillay 1989, Chin and others 1988), *Poncirus trifoliata* (Radhamani and Chandel 1992), and *Veitchia merrilli* (Chin and Krishnapillay 1989, Chin and others 1988) have shown the potential for long-term seed storage under such conditions.

DESICCATION TOLERANCE AND POTENTIAL SEED LONGEVITY IN DEVELOPING AND MATURING SEEDS

Application of the seed viability equation (1) reveals that initial seed quality (K_i)–potential longevity–plays an important role in ensuring good seed survival in storage. The higher the value of K_i the longer the period of viability for a given storage environment. During seed development and maturation, the value of the seed lot constant K_i–so the potential longevity of orthodox seeds–increases markedly. There is now good evidence in a wide range of contrasting orthodox crop species that potential longevity continues to increase during the period after the end of seed filling, as seeds mature (Demir and Ellis 1992, 1993; Ellis and others 1993; Ellis and Pieta Filho 1992; Pieta Filho and Ellis 1991a, 1991b; Sanhewe and Ellis 1996; Zanakis and others 1994). There is also good evidence that the ability of orthodox seeds to tolerate desiccation to very low moisture contents also improves considerably during the late stages of seed maturation, and indeed one study has, therefore, shown a strong association in developing and maturing seeds between potential longevity (K_i) and the ability of seeds to tolerate desiccation to very low moisture contents (Ellis and Hong 1994).

The ability of seeds to tolerate enforced rapid desiccation has been reported to increase during seed development and maturation of tree seeds with orthodox (Hong and Ellis 1990, 1992b, 1997a), intermediate (Ellis and others 1991a, Hong and Ellis 1995), and recalcitrant seed storage behavior (Finch-Savage 1992a, Hong and Ellis 1990, Tompsett and Pritchard 1993). Orthodox seeds of several crop, wild herb, and tree species are not able to tolerate rapid enforced desiccation to low moisture contents (4 to 5 percent) until some time after mass maturity [defined as the end of the seed-filling phase (Ellis and Pieta Filho 1992)] once maturation drying has reduced seed moisture content on the mother plant substantially (Ellis and Hong 1994; Fischer and others 1988; Hay and Probert 1995; Hong and Ellis 1990, 1992b, 1997a; Wechsberg and others 1993). Maximum tolerance of rapid enforced desiccation occurs some time after mass maturity, probably at natural dehiscence (Hay and Probert 1995; Hong and Ellis 1990, 1992b, 1997a; Sanhewe and Ellis 1996; Wechsberg and others 1993). In addition, the slow desiccation of immature seeds harvested before mass maturity, and well before maturation drying occurs naturally, enables tolerance of rapid desiccation to be acquired (Dasgupta and others 1982, Hay and Probert 1995, Hong and Ellis 1997a, Kermode and Bewley 1985a, Sanhewe and Ellis 1996). For example, in Norway maple (*Acer platanoides*), rapid drying (by reducing moisture content from 57.3 percent to 9 percent within 1 day, and to 5 percent within 4 days) of the seeds harvested at mass maturity reduced viability from 100 percent to 61 percent and 38 percent at 8.1 percent and 4.7 percent moisture content, respectively, while for seeds that were first dried slowly for 32 days, during which period seed moisture content was reduced from 57.3 percent to 29.9 percent, subsequent rapid drying to 3.5 percent moisture content (within 4 days) resulted in 93 percent viability (Hong and Ellis 1997a). The slow drying of seeds or fruits harvested before or at mass maturity has also been reported to increase seed vigor and potential longevity in several crop and wild species (Hay and Probert 1995, Sanhewe and Ellis 1996, TeKrony and Egli 1997).

In addition, desiccation tolerance also decreases when seeds are initially subjected to conditions under which germination is initiated. For example, prechilling, moist storage, presoaking, fermentation treatment for seed extraction, and seed priming can reduce desiccation tolerance, and hence alter seed storage behavior (see Hong and others 1996). Similarly, seeds that are produced in unfavorable environments may show reduced desiccation tolerance to low moisture contents. For example, seeds of a japonica rice (*Oryza sativa* subsp. *japonica*) produced in a hot environment of 32/24 °C (day/night) showed consistently poorer desiccation tolerance at each stage of maturation drying than those produced in a cooler environment of 28/20 °C (Ellis and Hong 1994).

The above implies that considerable care should be taken when collecting, extracting, and subsequently handling tree seeds. It also implies that further improvements in the success of tree seed storage, particularly in the Tropics, can be expected if greater attention is paid to the timing of seed collection, and to postcollection practices.

PREDICTION OF SEED STORAGE BEHAVIOR

Methods, and indeed feasibility, of seed storage depend upon the seed storage behavior shown by a particular species. A simple, two-stage protocol to determine seed storage behavior has been suggested elsewhere (Hong and Ellis 1996). This task is, however, considerable given that there are about 250,000 species of flowering plants. Estimation of likely seed storage behavior would therefore be helpful, and several approaches to doing this have been developed (Hong and Ellis 1996, 1997b, 1998; Hong and others 1996). No single criterion can provide a satisfactory estimation of likely seed storage behavior, but combining information for at least four of the six factors described below may be worthwhile (Hong and Ellis 1996).

PLANT ECOLOGY

There appears to be an association between plant ecology and seed storage behavior (Roberts and King 1980; Tompsett 1987, 1992). From information on seed storage behavior collated for almost 7,000 species from 251 families (Hong and others 1996), it is evident that species that show recalcitrant seed storage behavior do not occur naturally in (i.e. originate from) arid habitats, that is desert and savanna. In such environments, the majority of plant species show orthodox seed storage behavior, while a few may show intermediate seed storage behavior. It is clear, however, that further generalizations are not possible. In particular, for example, at the other ecological extreme it is quite clear that not all species native to moist habitats, rain forests, flooded forests, or aquatic environments show recalcitrant seed storage behavior; all three categories of seed storage behavior can be found among the species native to such moist environments.

TAXONOMIC CLASSIFICATION

Orthodox seed storage behavior can be found in all species within a few families (e.g. Chenopodiaceae, Combretaceae, Compositae, Labiatae, Solanaceae, Pinaceae), or almost all species in others with only a few exceptional species (e.g. Leguminosae, Graminae, Cucurbitaceae, Cruciferae and Rosaceae). However, most families of flowering plants including tree species have species with two (e.g. Anacardiaceae, Dipterocarpaceae, Euphorbiaceae, etc.) or three different categories of seed storage behavior (e.g. Meliaceae). Furthermore, seed storage behavior can differ among species within a genus (Hong and Ellis 1995, Tompsett 1983), or even among sections (Hong and Ellis 1997b).

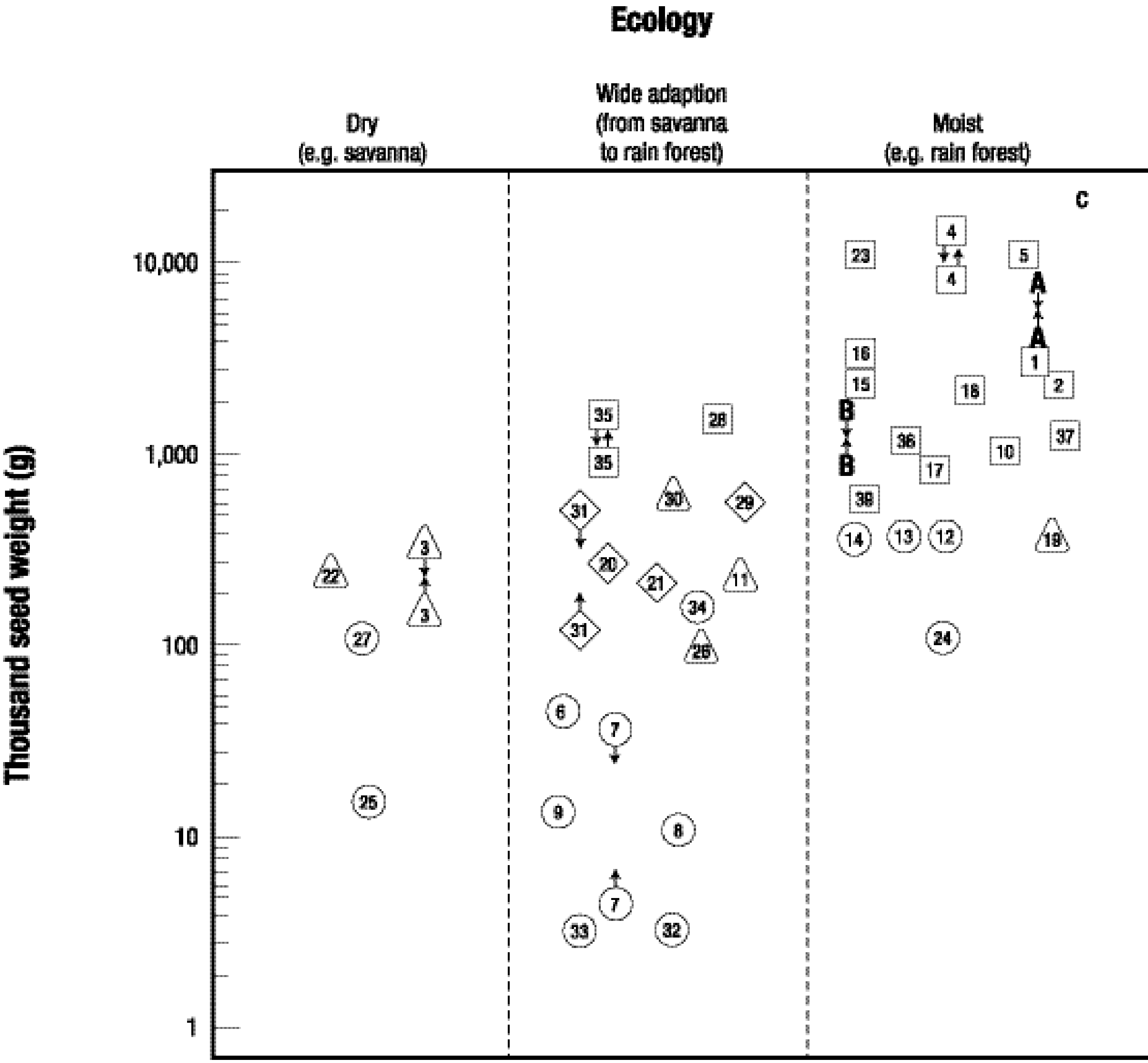
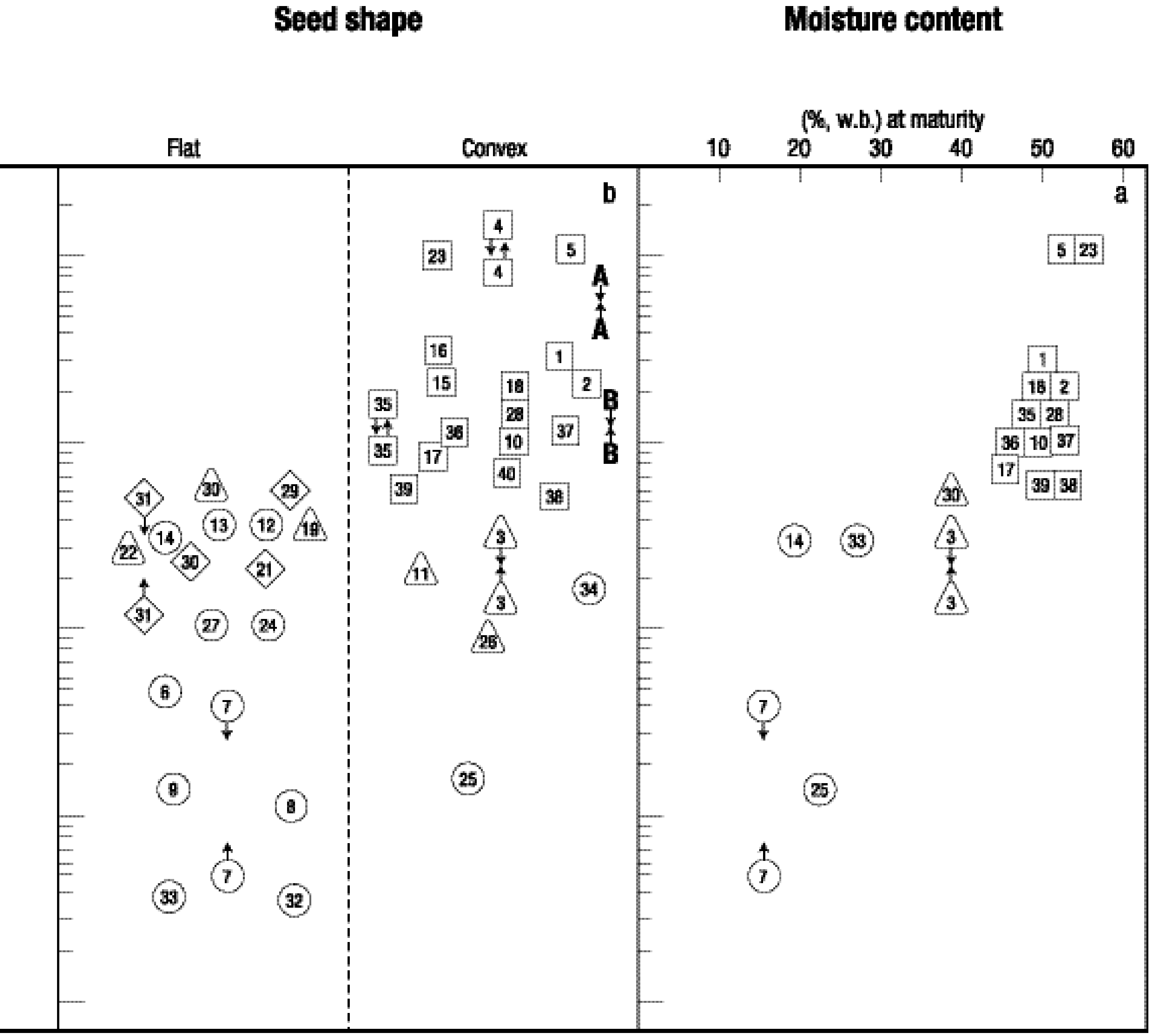


Fig. 1. Variation in thousand seed-weight (logarithmic scale) with that for seed moisture content at maturity or shedding (a), or seed shape (b), or ecology (c) and seed storage behaviour among 40 species of Meliaceae. Seed storage behaviour is classified as orthodox (s), intermediate or probably intermediate (n), recalcitrant or probably recalcitrant (u), or uncertain but not recalcitrant (e). The positions **A** and **B** for *Amoora wallichii* and *Turraeanthus africanus*, respectively, in both of which species seed storage behaviour is unknown, are included in order to test the prediction of seed storage behaviour (see text). Key to species: 1, *Aglaia clarkii*; 2, *Azadirachta excelsa*; 3, *Azadirachta indica*; 4, *Carapa guianensis*; 5, *Carapa procera*; 6, *Cedrela mexicana*; 7, *Cedrela odorata*; 8, *Cedrela toona*; 9, *Chukrasia tabularis*; 10, *Dysoxylum*



cauliflorum; 11, *Ekebergia senegalensis*; 12, *Entandrophragma angolense*; 13, *Entandrophragma candollei*; 14, *Entandrophragma utile*; 15, *Guarea cedrata*; 16, *Guarea sp.*; 17, *Guarea sp. nov (G. glabra)*; 18, *Guarea thompsonii*; 19, *Khaya anthothean*; 20, *Khaya grandiofoliola*; 21, *Khaya ivorensis*; 22, *Khaya senegalensis*; 23, *Lansium domesticum*; 24, *Lovoa trichiloides*; 25, *Melia azedarach*; 26, *Pseudobersama mossambicensis*; 27, *Pseudocedrela kotschyi*; 28, *Sandoricum koetjape*; 29, *Swietenia humilis*; 30, *Swietenia macrophylla*; 31, *Swietenia mahagoni*; 32, *Toona australis*; 33, *Toona ciliata*; 34, *Trichilia americana*; 35, *Trichilia emetica*; 36, *Trichilia martineaui*; 37, *Trichilia megalantha*; 38, *Trichilia monadelpha*; 39, *Trichilia prieuriana*; 40, *Trichilia tessmannii*. From Hong and Ellis 1998.

PLANT, FRUIT, OR SEED CHARACTERISTICS

Certain generalizations appear possible concerning associations between fruit and seed characteristics and seed storage behavior (Hong and others 1996). For example, orthodox seed storage behavior is shown by species that produce achenes, many-seeded berries, many-seeded dehiscent capsules, many dry-seeded pods (but not arillate), many dry-seeded follicles, schizocarps, and utricles (Hong and others 1996). Most species that produce siliques (one exception) and caryopses (three known exceptions) also produce orthodox seeds. On the other hand, however, all three categories of seed storage behavior can be found among species that produce one seed or a few large seeds (from 1 to 10 seeds) per fruit, or many arillate seeds per fruit, among drupes, pods, capsules, berries, and nuts.

SEED SIZE

Recalcitrant seeds often tend to be larger than intermediate seeds, which in turn tend (on average) to be larger than orthodox seeds. However, there is a very wide range of seed weights or sizes over which seeds of different species show all three types of seed storage behavior (Hong and Ellis 1996).

SEED MOISTURE CONTENT AT SHEDDING

A recent survey shows that seed moisture contents at maturity or shedding for species with recalcitrant, intermediate, and orthodox seed storage behavior overlap considerably. No generalization on seed storage behavior is possible for species in which seeds at maturity or shedding have between about 25 percent and 55 percent moisture content (Hong and Ellis 1996).

SEED SHAPE

There appears to be a weak association between seed shape and seed storage behavior. For example, recalcitrant seeds are often spherical or oval (Chin 1988). Seeds that are thin and flat, shapes that aid natural maturation drying, tend to show orthodox seed storage behavior (Tompsett 1994). Flat seeds tend to show orthodox seed storage behavior in *Acer* spp. (Hong and Ellis 1997b), but show both orthodox and intermediate seed storage behavior in Meliaceae (Hong and Ellis 1998). The spherical form is present in all three categories of seed storage behavior (Hong and Ellis 1997b, 1998).

All of the single criterion suggested above are not sufficiently reliable to be very useful as indicators of likely seed storage behavior. However, it may be possible to develop a multiple-criteria predictive framework in order to suggest likely seed storage behavior (Hong and Ellis 1996). For example, combining information on the four criteria–seed weight, seed moisture content at shedding, seed shape, and taxonomy (section)–can form the basis of a guide to likely seed storage behavior among *Acer* spp. (Hong and Ellis 1997b). Similarly, the four criteria of seed weight, seed moisture content at maturity, seed shape, and plant ecology may provide a guide to the estimation of seed storage behavior in Meliaceae (fig. 1). For example, from the two positions designated A in figure 1 for seed weight (TSW = 4300 to 7700 g), ecology (moist evergreen forest of India), seed shape (convex, fleshy aril) of *Amoora wallichii*–information from Luna (1996)–we suggest that this species is likely to show recalcitrant seed storage behavior. Similarly, from the two positions designated B for seed weight (TSW= 1000 to 1400 g [FAO 1975]), seed shape (convex, fleshy aril), and ecology (rain forest of East Africa) (Styles and White 1991) of *Turraeanthus africanus*, we suggest that recalcitrant seed storage behavior is likely in this species also. Furthermore, the information available on natural habitat of the two species can also be used to suggest likely suitable moist seed storage conditions. Given that the *Amoora wallichii* tree is described as “frost-hardy” (Luna 1996), and that *Turraeanthus africanus* grows at an altitude of 1525 m (Styles and White 1991), we suggest that chilling injury is unlikely if the seeds of both species are stored moist at about 10°C.

We propose that a collaborative investigation by seed scientists worldwide may realize larger, more accurate multiple-criteria keys to estimate seed storage behavior and so suitable environments for seed storage. We believe that the approach summarized here is particularly useful for tree seed storage, and that such a collaborative venture is urgently required.