Collecting and handling seeds in the field

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Introduction

If gene banks are to be successful, the seed samples placed into storage need to be of the highest initial quality. It has been known for a long time that initial seed quality determines the absolute longevity of seed collections, but few studies have been made of the effect of different seed collecting methods on quality. The seed collector is thus left to make important judgements about how to handle seeds in the field with little well-researched advice, when also facing a daunting array of decisions from plant identification and sampling strategies to navigation, team morale and menu planning. This chapter updates earlier attempts (Smith, 1984, 1985) to synthesize laboratory studies of the factors that influence the quality of seed samples and to relate such studies to the practical options available under field conditions.

Angiosperm seeds are borne in a wide variety of types of fruits (Heywood, 1978, Plate VI, pp. 18–19), each of which presents its own particular problems for the collector. Seeds borne in dry dehiscent fruits will only be available for collecting before they are dispersed. They can easily be detached to reduce their bulk for transport. The availability of seeds in dry indehiscent or schizocarpic fruits will depend on how and when the fruits themselves are dispersed from the parent plant. Here the separation of fruit parts and seeds for ease of transport during the collecting mission will be more difficult. However, the weight of fruit parts relative to seeds will still be small and in practice the fruit can be considered as part of the seeds. Major cereal and pulse crops are unique among angiosperms in having been selected against natural seed dispersal. The seeds of these species will remain within the fruit and the fruit will remain attached to the mother plant until harvested by farmers or collectors. The most problematic seeds for collectors are those borne

in fleshy fruits, whether they are derived from single flowers (as in berries, drupes, hesperidia or pseudocarps) or from an inflorescence (soroses, syconia and cocnocarpia). Here the ratio of the weight of flesh to seed can be in excess of 10 : 1, which can place the collector in the predicament of either cleaning the seeds in the field or overcoming the logistical problems of transporting large quantities of material.

Before answers to the practical problems of seed handling in the field can be attempted, we need to know more about seed storage behaviour. To date, three types of seeds have been identified, in terms of their storage characteristics:

- desiccation-intolerant (recalcitrant);
- partially desiccation-tolerant (intermediate);
- fully desiccation-tolerant (orthodox).

As the advice is different for each seed storage type, the first problem for collectors is to recognize which type they are dealing with.

Seed storage types

For well-known crop species, the problem of determining seed storage type can be solved by consulting published work. *Seed Abstracts* is a useful entry to the literature. This should quickly produce an answer, sensible though not necessarily correct. Unfortunately, the literature on the storage behaviour of seeds of wild plants such as forages or forest species is much less extensive. Collectors may then need to make some educated guesses. What information can they base such guesses on?

The use of evolutionary relationships to extrapolate from the known behaviour of species is of little help (Fig. 20.1). For example, all storage physiologies can be found in the genus *Araucaria*, with sympatric species exhibiting different behaviour (Tompsett, 1984). Aldridge and Probert (1993) have shown differences in storage physiologies between the closely related grass genera *Porteresia* and *Oryza*. Fruit type is an equally poor indicator, with both desiccation-tolerant and intolerant seed being known from schizocarpic samaras, caryopses, legumes and drupes.

Improved prediction can be achieved using a combination of seed weight, provenance and morphology. Figure 20.2 shows that, as seed weight rises from 0.001 g to 100 g, the probability of the seed being desiccation-intolerant rises from less than 1% to 100%. Similar correlations between storage behaviour and seed weight have been suggested for the families Dipterocarpaceae and Araucariaceae (Tompsett, 1984, 1988). While this correlation should be treated with caution due to the relatively small size of the sample (546 out of well over 250,000 plant species), a lack of caution can perhaps be justified by invoking the spirit of the *Convention on Biological Diversity*, which states that a 'lack of full scientific certainty cannot be used as a reason for postponing

measures to avoid or minimise a threat of significant reduction or loss of biological diversity'. Insufficient species with intermediate seed storage behaviour are known for any meaningful general conclusions to be drawn, other than perhaps to note that their seed weights are also intermediate.

The ability of seed weights to predict seed storage behaviour can be enhanced by also considering the habitat of species (Jurado *et al.*, 1991). The implication of this is straightforward: in wetter habitats seed is likely to be larger. Combining the two observations, seed from wetter habitats are also more likely to be desiccation intolerant. However, even in the driest habitat there are species that produce seeds of sufficient weight for desiccation intolerance to be as likely as tolerance. Similarly, there are species in the wettest habitats whose seeds fall in the (low) weight classes for which only desiccation tolerance is so far known.

Plant habit can also be added to the equation. In both mesic British (Waller, 1988) and arid Australian (Jurado *et al.*, 1991) vegetation, seed weights of herbaceous species are lower than those of trees, suggesting a higher frequency of desiccation-tolerant seeds in the former group. Among the trees of the rain forest reserve of Los Tuxtlas in Mexico, the pioneer species have lighter seeds, while the dominant species of the mature forest have heavier seeds (Ibarra-Manriquez and Oyama, 1992). Storing seeds of dominant species would appear to be more problematic than storing seeds of pioneer species. Another expected 'hot spot' of desiccation intolerance is among aquatic species, based on the early work of Muenscher (1936) and the subsequent confirmation of many of his findings by Probert and Longley (1989).

To determine the practical implications of seed storage behaviour for germplasm collecting, a brief résumé is necessary, for each seed storage category, of the relationship between seed viability and the relevant environmental variables, i.e. temperature, relative humidity, atmospheric composition and time (both physical and physiological).

Desiccation-intolerant (recalcitrant) seeds

As the name implies, desiccation-intolerant seeds cannot withstand drying. Species as varied as aquatic tropical grasses and dominant temperate trees have seeds that lose viability when dried to water potentials in the range from -5 to -3 MPa (Probert and Longley, 1989; Pritchard, 1991). Over the range of normal biological temperatures, these water potentials would be in equilibrium with a relative humidity of $>95\%$. Such high ambient relative humidities are rarely found in nature. Consequently, recalcitrant seeds will dry to lethal water potentials even if they are held under the ambient conditions in which the species occurs in the wild. Not surprisingly, the seed-coats of some of these species are adapted to restrict dangerous moisture loss, as in *Acer pseudoplatanus* (Dickie *et al.*, 1991) and *Araucaria hunsteinii* (Tompsett, 1982). However, other desiccation-intolerant seeds, such as those of *Porteresia*, have no such adaptation, and drying

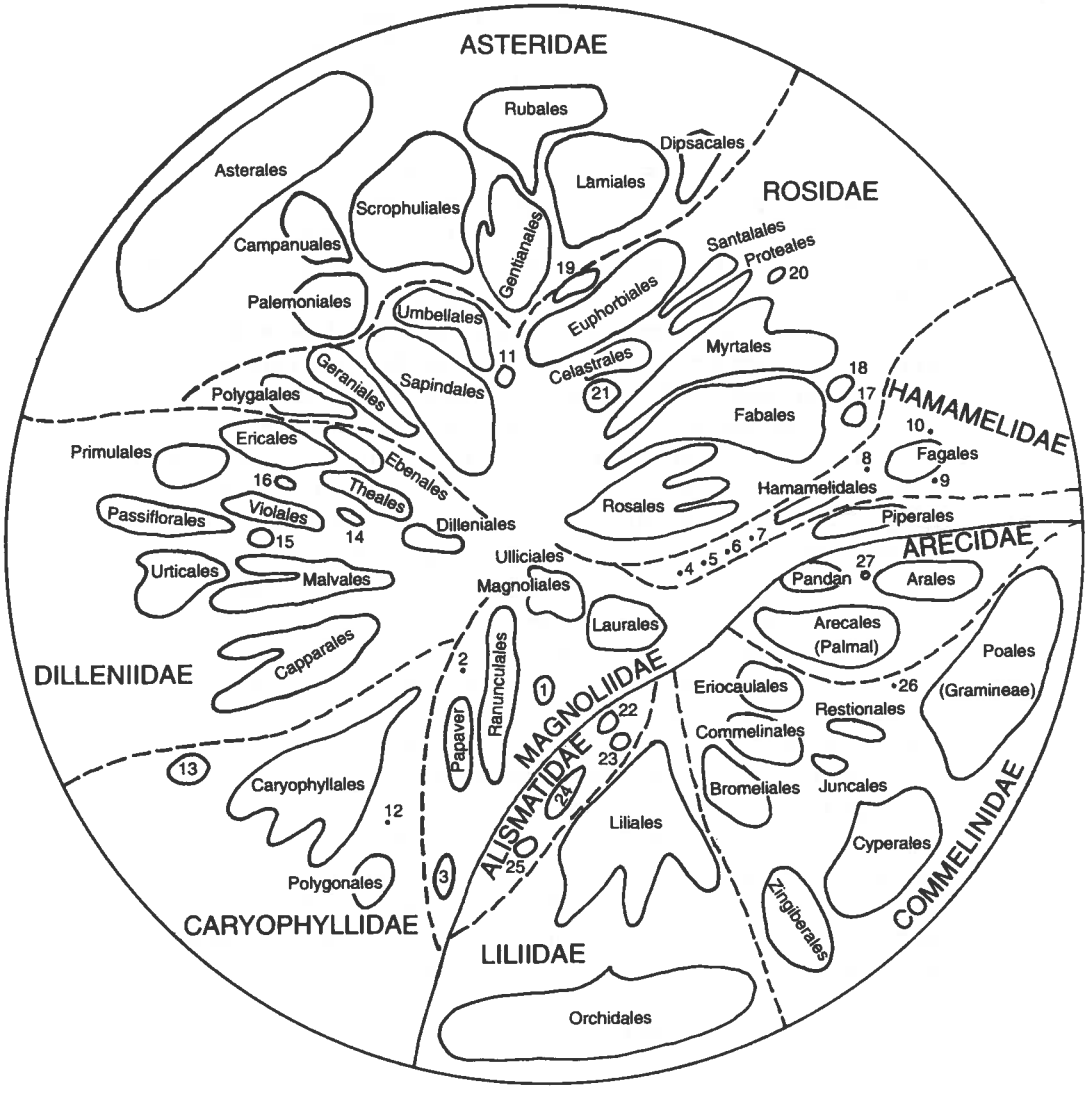


Fig. 20.1.(a) Evolutionary diagram showing the relative degree of specialization of the orders of Angiosperms (after Heywood, 1978). Smaller orders are indicated by numbers as follows: (Dicotyledons) **(1)** Nymphaeales; **(2)** Sarraceniales; **(3)** Aristolochiales; **(4)** Trochodendrales; **(5)** Cercidiphyllales; **(6)** Didymeleales; **(7)** Eupteleales; **(8)** Eucommiales; **(9)** Casuarinales; **(10)** Leitneriales; **(11)** Juglandales; **(12)** Batales; **(13)** Plumbaginales; **(14)** Lecythidales; **(15)** Salicales; **(16)** Diapensiales; **(17)** Podostemales; **(18)** Haloragales; **(19)** Cornales; **(20)** Rafflesiales; **(21)** Rhamnales; (Monocotyledons) **(22)** Alismatales; **(23)** Triuridales; **(24)** Najadales; **(25)** Hydrocharitales; **(26)** Typhales; **(27)** Cyclanthales.

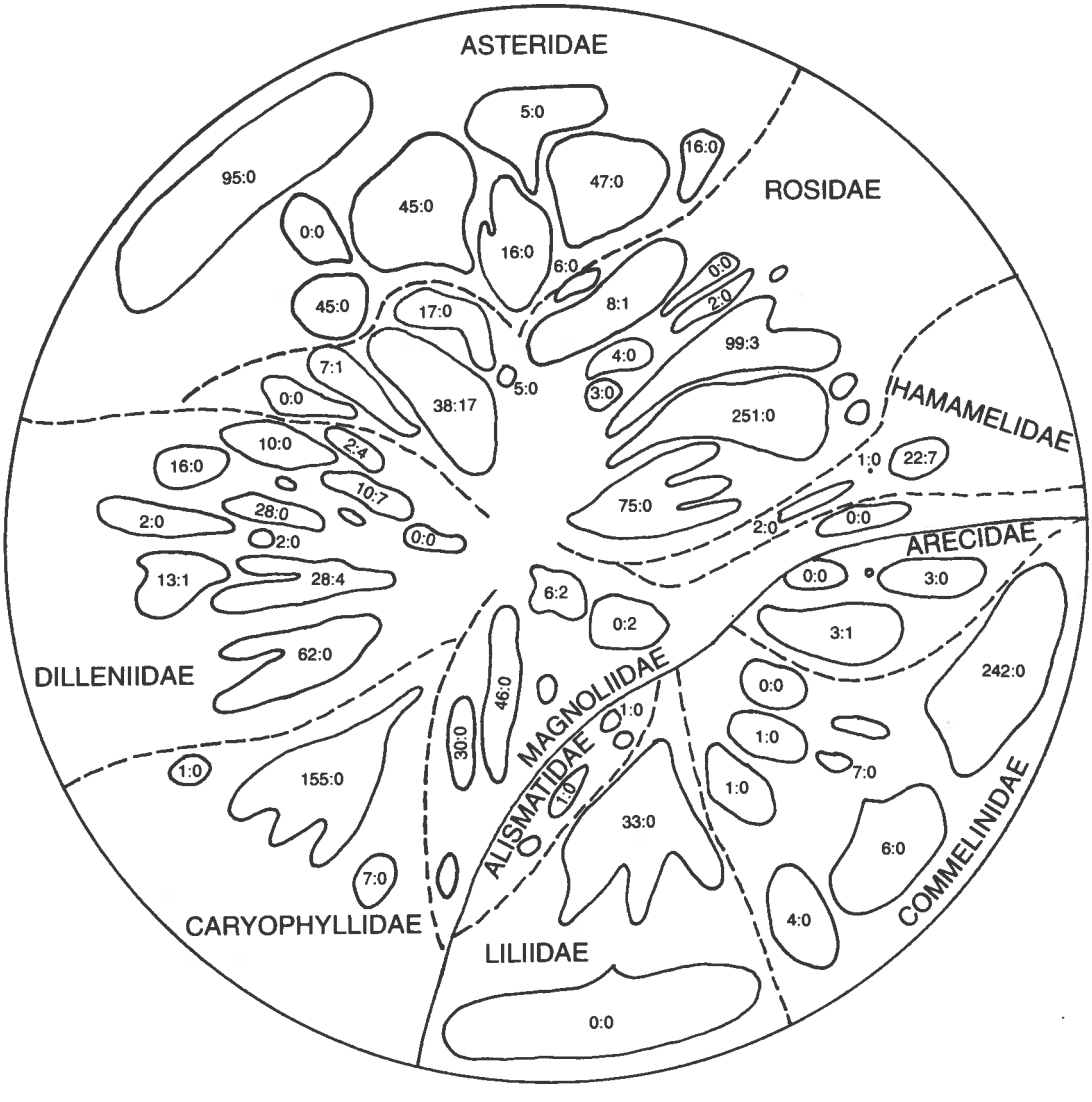


Fig. 20.1.(b) The same diagram as Fig. 20.1(a), showing the numbers of fully desiccation tolerant (first number) and desiccation intolerant (second number) species known to occur in each order from the literature. Where no numbers are shown for the smaller orders, the data are 0:0.

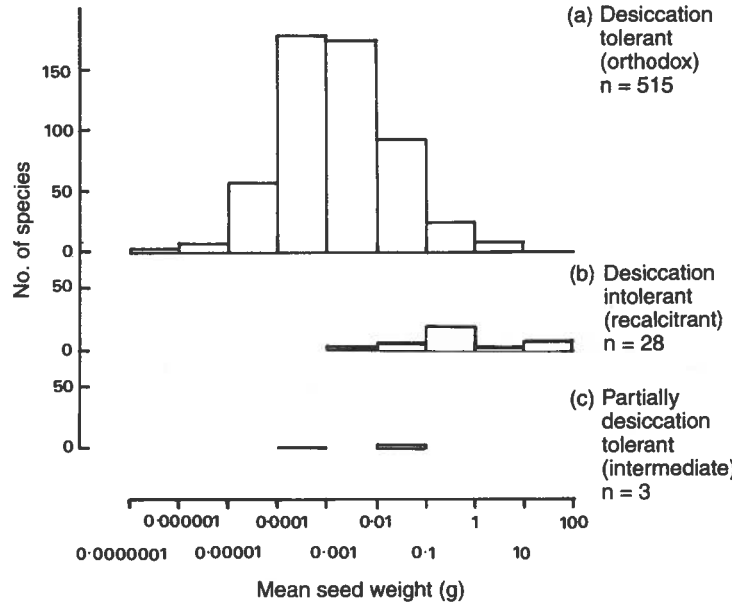


Fig. 20.2. The distribution of individual seed weight among species, showing the three kinds of seed storage behaviour. Data from Cromarty *et al.* (1985).

to lethal water potentials on the plant has been observed (R.J. Probert, pers. comm.).

If the seed is in a fleshy fruit, such as in mango or mangosteen, the flesh will act as a reservoir of moisture, effectively 'buffering' the seed against drying. The problem of maintaining high seed moisture can be overcome most easily for recalcitrant seeds in fleshy fruits by keeping them in their fruits and paying the logistical, physical and financial price of moving the seeds together with their highly adapted buffers.

One way collectors can minimize loss of moisture from seeds is by holding them in impermeable containers such as plastic bags. However, this raises the problem of controlling the gaseous atmosphere inside the bags. At the water potentials at which the seeds retain their viability, they are metabolically active. They are therefore consuming oxygen and releasing carbon dioxide and water. In desiccation-intolerant species, the rate of loss of seed viability increases as the oxygen concentration falls in the surrounding atmosphere. In practice, this depletion of oxygen can be avoided by keeping the seeds in relatively light-gauge polythene bags and regularly reoxygenating the internal atmosphere by deflating and reinflating the bags on a weekly basis (Tompsett, 1983). Even so, under such a regime the seed moisture content gradually increases, presumably due to uptake of the water

respired into the atmosphere by the seeds themselves. This reduces a restraint on germination.

Little is known of the effect of temperature on the longevity of desiccation-intolerant seed, as experimental studies have often confounded this with the effects of temperature on the rates of drying and of germination. For temperate species of oak, reducing the temperature as low as −1.5 °C increases seed storage life, but further reduction to −5 °C is lethal (Suszka and Tylkowski, 1980). However, even modest reductions in temperature risk rapidly killing the seeds of tropical species through so-called chilling injury. Despite its name, this can occur in *Inga*, *Shorea* and other tropical genera between 21 °C and 16 °C. While chilling injury is unlikely at the ambient conditions where these species generally occur, injurious conditions could well be encountered in excessively air-conditioned rooms and during air freight shipment, for example in light aeroplanes and during transfers from one plane to another at intermediate airports during indirect intercontinental flights. Labelling seed consignments as 'perishable' will risk their refrigeration by customs officials while they await import clearances.

Holding desiccation-intolerant seeds at higher temperatures, well above those at which chilling injury can occur, can cause problems of its own. Most such tropical seeds are non-dormant and germination will be hastened under these conditions. The more the radicle has protruded in *Avicennia marina* seeds, the less tolerant of desiccation they become and so the more demanding in their storage requirements (Farrant *et al.*, 1986). Efforts to discover a phase of greater desiccation tolerance have proved unsuccessful in aquatic grasses (Probert and Brierley, 1989). Work on seeds of *Aesculus hippocastanum* (Tompsett and Pritchard, 1993) and of oak (Finch-Savage, 1992) showed that their desiccation tolerance increased as they developed and was at its maximum during peak seed fall. So harvesting desiccation-intolerant seeds just before natural dispersal is better than collecting fallen fruits, because in the latter case the seed lot contains both individual seeds close to maximum desiccation tolerance and also older seeds already advanced in their germination and so losing desiccation tolerance. Where tree climbing and hand picking is impractical, and limb felling unacceptable, this collecting problem can be overcome by laying sheeting beneath the tree and collecting the seeds/fruits that fall over a short time period.

In practice, when dealing with desiccation-intolerant seeds the most easily manipulated variable is time. Working quickly between harvest and dispatch will not only reduce the practical difficulties of keeping the seeds ventilated and alive but will also ensure that the material is received still ungerminated, which will maximize the options for conservation. The evidence suggests that 30 days is the longest period which should be allowed between harvesting and receipt by the seed bank.

Partially desiccation-intolerant (intermediate) seeds

Variants of both major seed storage types exist. *Zizania palustris* seeds, for example, share many of the characteristics of desiccation-intolerant seeds and yet under highly specific conditions can be dried to low moisture content and subsequently germinated (Kovach and Bradford, 1992). However, under the less controlled conditions likely to be available to collectors, these seeds may behave as recalcitrant. They should always be treated as such. On the other hand, there are species that can be partially dried with ease but where full desiccation is lethal. They include coffee (Bacchi, 1956; Vossen, 1979), *Araucaria columnaris* (Tompsett, 1984) and oil-palm (Grout *et al.*, 1983). Recently, they have been categorized as of 'intermediate' storage behaviour (Ellis *et al.*, 1990). Under field conditions their behaviour is expected to be that of desiccation-tolerant species. Only active seed drying (i.e. in the sun or using silica gel) needs to be treated with caution.

Few such variants have been identified. Their existence demands that their storage characteristics be carefully specified and raises the possibility of a continuum of storage types. However, the imperative to act despite our uncertainties means that the advice offered here will assume that deviation in seed storage behaviour from the two major types is rare and that these two will describe most species.

Desiccation-tolerant (orthodox) seeds

Seed development

Considerably more is known about orthodox seeds than about the recalcitrant or intermediate types. The accepted general model for development is shown in Fig. 20.3a. It should be treated with due caution. The literature is dominated by studies of annual crops, but the duration of seed development can range from a few days in ephemeral species to over two years in conifers. Also, the model is derived from studies of seed populations, with mean values used to model the behaviour of individual seeds.

Soon after fertilization, which can be delayed by up to 14 months after pollination in *Pinus*, the weight of water in the seed reaches a constant, maximum value. This is followed by a period in which embryo development and the deposition of storage products result in seed dry weight increasing sigmoidally, until it too reaches a maximum. As dry matter increases while the amount of water in the seed remains constant, so percentage seed moisture content necessarily declines. At maximum dry weight, the moisture content of seeds from dry fruits falls rapidly, due to the net loss of water that follows the severing of the vascular connection between seed and mother plant. The seed is now hygroscopic, its moisture content coming into equilibrium with the ambient atmosphere. Inside fleshy fruits, seed moisture content remains close to its

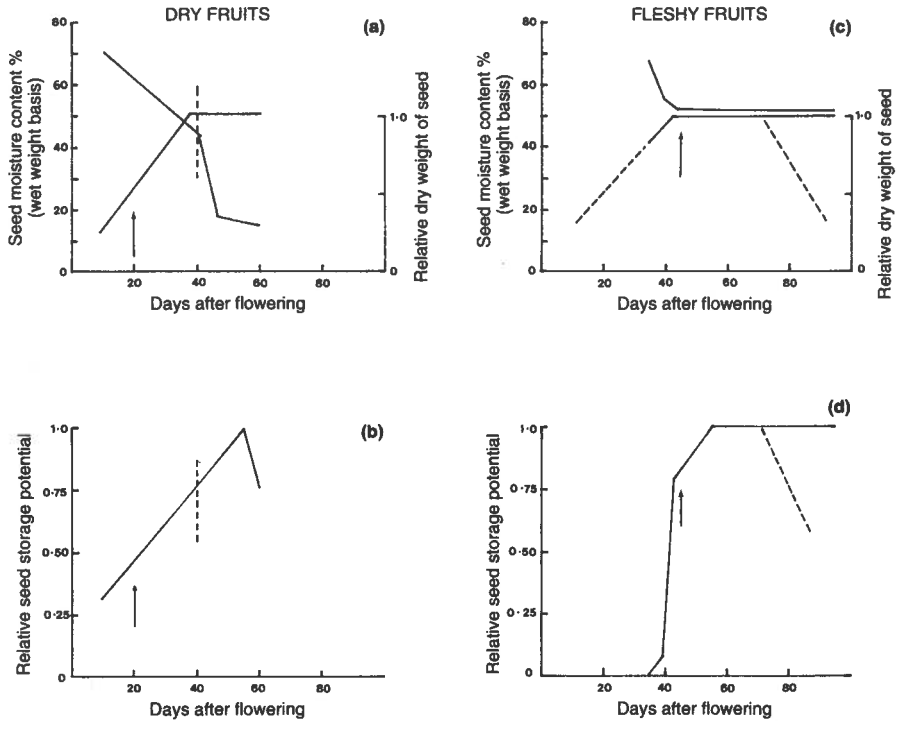


Fig. 20.3. Diagrammatic representation of seed development and seed storage potential in fully desiccation-tolerant species, with seeds borne in dry fruits (**a** and **b**) and fleshy fruits (**c** and **d**). The arrows indicate the acquisition of desiccation tolerance, the vertical dashed line the start of water loss from the seed. The dashed lines in (**c**) and (**d**) indicate the likely course of events if fruits begin to dry.

value at maximum dry matter and seeds only behave hygroscopically if taken from the fruit.

There are deviations from this general pattern. In the drupe fruits, the development pattern has a quiescent period of dry matter accumulation interpolated in the sigmoidal growth pattern. It is during this period that the embryo grows (Tukey, 1934). In the wetland herb *Ranunculus sceleratus*, even though maximum seed dry weight is achieved, no phase of rapid drying takes place before the seeds are shed (Wechsberg *et al.*, 1993).

Where known, seed moisture content at the time of maximum dry weight ranges from about 30% on a wet weight (WW) basis for dry-fruited cereals up to 65% for the fleshy-fruited coffee berry. Across all species and fruit types, the mean value is close to 45%. However, for dry dehiscent, indehiscent and schizocarpic fruits, these moisture contents are unlikely to be those at which collecting will be done. The moisture content of seeds of domesticated cereals and pulses will oscillate in equilibrium with ambient conditions until harvested and go up as the

seeds take up moisture from any rain falling on them (Yaklich and Cregan, 1987). Among naturally dispersed British species, seeds of *Lathyrus sphaericus* have moisture contents in the pod close to 15% at the point of explosive dispersal, while the grasses *Melica uniflora* and *Bromus sterilis* shed their seeds at 38% and 54% moisture contents respectively. In the wetland herb *R. sceleratus*, seed dispersal takes place at 56% moisture content. Even higher values (67%) have been recorded for the seeds of the tropical timber tree *Agathis macrophylla* as the cone shatters to release them (Smith, 1985). The range of seed moisture contents at dispersal is even wider than at maximum dry weight. This variation provides collectors with a considerable challenge to ensure the appropriate handling of each species following collecting.

Furthermore, the seed's desiccation tolerance and its storage potential vary through its development. Seeds that are desiccation-tolerant when mature are intolerant of drying at earlier stages of development. This is shown in a generalized way in Fig. 20.3b. Initially, developing seeds are desiccation-intolerant. Then, at least in the case of *Ricinus communis*, about halfway through seed development they become tolerant of slow desiccation. Finally, close to maximum dry weight, they become tolerant of rapid desiccation, such as that imposed by silica gel drying (Kermode and Bewley, 1985). This increasing tolerance of seeds to desiccation with maturation also manifests itself in *Acer* and coffee as a progressive lowering of the moisture content to which the seed population can be dried without loss of viability (Ellis *et al.*, 1991; Hong and Ellis, 1992). While this may not directly affect the survival of seeds under collecting conditions, it will certainly affect their subsequent prospects for long-term storage.

In other studies, not using different drying rates, tolerance to drying in dry-fruited cereals, pulses and crucifers is achieved about halfway through seed development. In cereals, seed storage potential continues to rise until well after maximum seed weight is reached and seed moisture has fallen to equilibrium with the surrounding air (Pieta Filho and Ellis, 1991). Seed storage potential is subsequently lost as the seed ages in the field. The dry-fruited achene of the wild wetland herb *Ranunculus sceleratus* differs from this pattern in two respects. First, the rapid drying phase does not occur. Secondly, the storage potential of seeds is still rising on the mother plant until they are shed (Wechsberg *et al.*, 1993). In contrast, in the fleshy-fruited tomato, tolerance to rapid desiccation does not occur until just before or just after maximum seed weight has been reached. Seed storage potential then continues to increase for the next ten days and remains constant for a further 40 days (Demir and Ellis, 1992).

There has been too little research to take these patterns as settled. Different authors working on the same species have produced different results and even the same author working on the same species has on different occasions come to different conclusions. None the less, collec-

tors would do well to bear in mind that in dry-fruited species the early period of desiccation intolerance is followed by a period of drying sensitivity before maximum storage potential is achieved. After the seed has dried to equilibrium with ambient conditions, it then begins to age. This initial period of drying sensitivity may last longer in seeds borne in fleshy fruits, but, as long as the seeds remain in the fruits, the storage potential is maintained.

Morphological markers of seed maturity

The colour changes that are associated with fruit and seed ripening do not appear to correlate sufficiently well with the achievement of maximum storage potential to be of much use to collectors in the field. For example, the skin colour of tomato (*Lycopersicon esculentum*) fruits varies from between 20 and 25% red to completely green at the same level of seed maturity, namely attainment of maximum seed weight. In barley, the colour of the palea and lemma is not correlated with subsequent seed longevity (Ellis and Roberts, 1981). Developmental studies of grass species suggest that endosperm characteristics would also be of little value as markers of seed maturity (Komatsu *et al.*, 1979). In the drupe fruits, only final fruit size correlates with the completion of seed development. More obvious markers, such as pit (pericarp) hardening, take place just as the embryo begins its development. Dehiscence or dispersal remains uninvestigated as a natural diagnostic of maximum seed storage potential.

Rapid gravimetric techniques for measuring seed moisture content have been developed for field use, for example using hot exhaust gases from motor vehicles (Klein and Harmond, 1971). However, the relatively constant moisture content of tomato seeds over the period during which they achieved first desiccation tolerance and then maximum seed storage potential suggests that single moisture content measurements would be of little use when dealing with fleshy fruits.

Where seeds are being collected from regeneration plots, say, seed development can be monitored and harvest timed to coincide with the attainment of maximum storage potential. However, collectors working in the field cannot afford to remain for sufficient periods at any one site in this way to monitor the rate of change in the various indicators. Consequently, the knowledge that seed storage potential can rise or fall dramatically over a few days (so that bank storage lives will first double and then halve over the same period) will remain of little value to collectors until a simple, reliable and rapid field diagnostic is developed.

Seed longevity at high moisture content

Earlier discussions of the problems faced by seed collectors in keeping their material viable in the field (Smith, 1984) were hampered by the lack of comprehensive data on the rate of loss of seed viability over the range of moisture contents, temperatures and gaseous environments which seed

samples could expect to experience during a collecting trip. Such data sets are now available for commercial seed lots of lettuce (Roberts and Ellis, 1989). Figure 20.4 summarizes these relationships schematically.

The whole response can be divided into two phases, determined by seed moisture content. In both phases, the effect of temperature appears to be very similar, higher rates of loss occurring at higher temperatures. The effect of the gaseous environment is dramatic in the first phase and insignificant in the second. Under aerobic conditions, as seed moisture content falls over the first phase from full imbibition to about 15% moisture content, the rate of loss of viability increases. Further drying below this value decreases the rate of loss of viability until it is considerably lower than that at full imbibition. The rates of loss of viability are approximately equal at 40% and 8% moisture content, or in equilibrium with 99% and 65% relative humidity respectively.

In the first phase, the rate of loss of viability is 50 times higher under anaerobic conditions than under aerobic conditions at the same moisture content of 40% (close to full imbibition). At 25°C, seed viability could be expected to fall from 84% to 16% in seven days under anaerobic conditions, compared with 300 days under aerobic conditions. As the seeds dry further under anaerobic conditions during the first phase, the rate of viability loss decreases only slightly, until the seeds have dried to around 22% moisture content. The rate of viability loss then decreases sharply until at 15% moisture content it is the same as in seeds held aerobically.

This comprehensive study on the dry indehiscent fruit of lettuce is

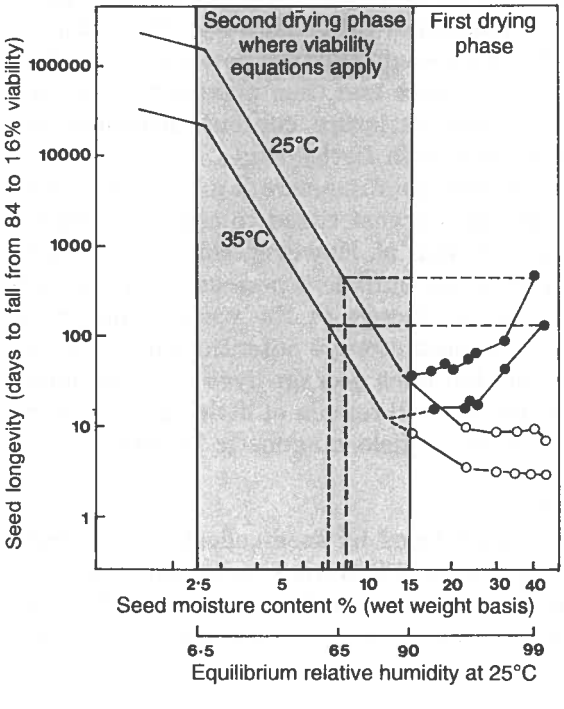


Fig. 20.4. Diagrammatic representation of the viability behaviour of lettuce seed at 25°C and 35°C over the range from 40 to 2.5% moisture content, under aerobic (filled circles) and anaerobic (open circles) conditions. The equivalent equilibrium relative humidity values are shown for four moisture content levels, i.e. where maximum longevity under hydrated condition (99%) occurs, where minimum longevity occurs (90%), where longevity under air dry storage is the same as maximum longevity under hydrated conditions (65%), and where the benefit of further drying decreases (6.5%).

matched very well by the much less extensive data for coffee presented by Smith (1984). The equilibrium relative humidity value for the switch between the two phases is acceptably close in both species to 90% relative humidity. A slightly higher value of close to 93% relative humidity has been reported for durum wheat (Petruzelli, 1986), while *Phleum* exhibits an intermediate value of about 91% (Smith, 1984).

Three important practical conclusions can be drawn from these limited data on viability loss in desiccation-tolerant seeds at high moisture contents:

- Great care must be taken to keep seeds aerated when they are held at high moisture contents, for example those at which many non-domesticated species are shed. Holding the seeds in sealed conditions, such as in a plastic bag, or packing the seeds tightly into limited space will result in an anaerobic environment rapidly developing around the seeds, due to their respiration. This will dramatically increase the rate of loss of viability.
- The switch to air-dry storage behaviour appears to occur at relative humidities close to 90%.
- The benefits of drying will only be realized if ambient relative humidities are $<65\%$.

Relative humidity values as high as 90% are rare in nature. Consequently, if samples of seeds or dry indehiscent fruits are kept aerated, their hygroscopic nature will soon result in the seeds shifting quickly to the second, air-dry storage phase. However, seeds from fleshy-fruited species will either shift much more slowly or not at all if the fruit itself does not dry.

The behaviour of air-dry seeds has been much studied, with the viability constants determined for tens of species. The responses of interest to seed collectors are summarized below.

The behaviour of air-dry seeds

The effect of temperature on the storage of air-dry seeds

First, the effects of temperature and seed moisture content on seed longevity are independent. The effect of a given change in temperature is the same at any seed moisture content. Over the range $-13°C$ to $80°C$ the effect of temperature is the same for seeds from eight widely different species, including monocotyledons and dicotyledons, herbs and trees, annuals and perennials, and temperate and tropical species, as well as grains, leafy vegetables and timber crops (Dickie *et al.*, 1990). The relationship between temperature and the rate of loss of viability is quadratic. At the ambient temperatures likely to be encountered while collecting, warming will cause greater increases in the rate of seed viability loss than cooling by the same amount will reduce it. In practical terms, this means that if cooling is possible (e.g. in air-conditioned rooms), it should be taken advantage of. However, the bigger concern should be to prevent seeds being heated by the sun, either directly or

indirectly, as in a parked vehicle. Vehicles fitted with reflective false sun roofs are less prone to such heating. A roof rack full of luggage will serve much the same purpose.

The effect of moisture content on the storage of air-dry seeds

In contrast, the effect of moisture content on the rate of viability loss in air-dry seeds is much more variable among species. However, where a wide range of air-dry moisture content has been studied, the relationship between seed moisture content and the rate of loss of viability has been shown to be logarithmic (Ellis *et al.*, 1986; Tompsett, 1986; Kraak and Vos, 1987; Dickie *et al.*, 1991). This means that for each progressive similar reduction of seed moisture content, say 1%, an ever-increasing reduction in the rate of loss of viability is achieved. In practice, this makes lowering moisture content a potent tool in managing seed viability loss during collecting.

What controls seed moisture content is thus important to collectors. Desiccation-tolerant seeds are hygroscopic and so give out or take up moisture until they are in equilibrium with the surrounding air. Consequently, seed moisture content is, in part, determined by the temperature and relative humidity of that air. Species from some plant families, such as the Leguminosae and Malvaceae, produce 'hard' seeds, which are adapted only to desorb, i.e. give out moisture when the ambient air is drier than the equilibrium relative humidity of the seed and prevent moisture uptake when the air is wetter. This characteristic accounts for the extreme longevity of hard seeds and makes collecting such species relatively straightforward.

For fully hygroscopic seeds, oil content is the other factor that determines the final equilibrium moisture content. The role of oil is passive, acting as a constant but hydrophobic element of the seed weight. Consequently, seeds with high oil contents equilibrate to lower moisture contents under the same conditions than seeds with low oil contents.

The relationship between seed moisture content, oil content, temperature and relative humidity has been described in an empirical equation (Cromarty *et al.*, 1985), which can be combined with the viability equation of Ellis and Roberts (1981) to predict the rate of seed viability loss under ambient atmospheric conditions. For many species, this reveals that, at a constant temperature, similar changes in the equilibrium relative humidity produce the same relative changes in the rate of seed viability loss. While this allows the construction of a simple tool to predict relative seed viability losses for a majority of species under ambient collecting conditions (Fig. 20.5), it is not absolutely applicable for all species. *Ulmus carpinifolia* is one species for which it is inappropriate and one that also differs in the relative humidity at which the minimum rate of loss of viability is achieved (Smith, 1992). Figure 20.5 presents a standard psychrometric chart, on which the combinations of temperature and relative humidity calculated to bring about the same monthly viability losses have been identified. (See also Box 20.1.) When planning collecting

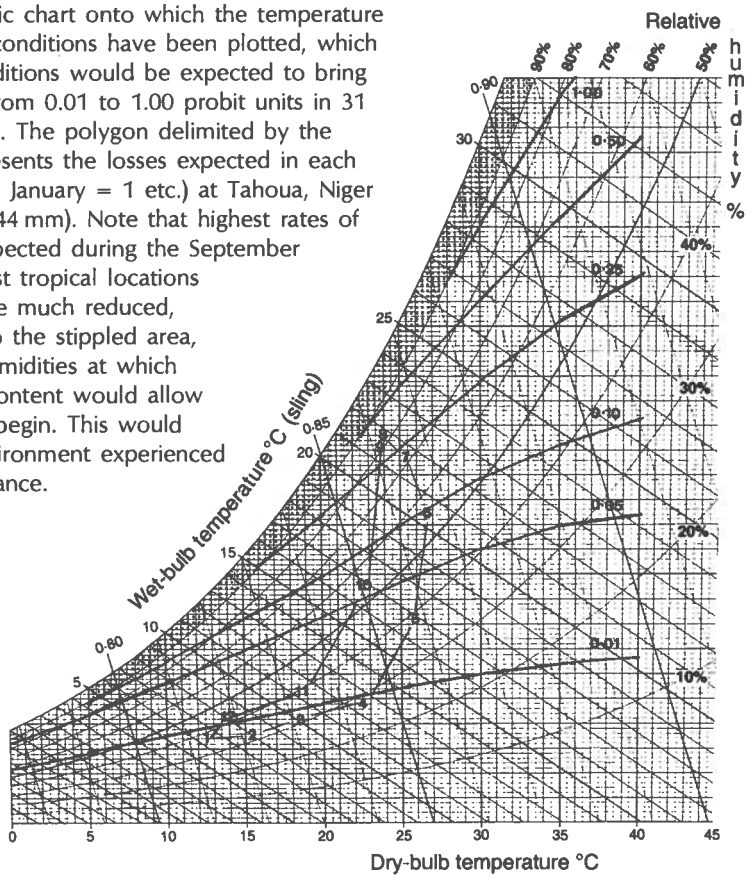


Fig. 20.5. Psychrometric chart onto which the temperature and relative humidity conditions have been plotted, which under equilibrium conditions would be expected to bring about viability losses from 0.01 to 1.00 probit units in 31 days in chickpea seeds. The polygon delimited by the numbers 1 to 12 represents the losses expected in each month of the year (i.e. January = 1 etc.) at Tahoua, Niger (annual precipitation 444 mm). Note that highest rates of loss of viability are expected during the September harvest period. In moist tropical locations monthly differences are much reduced, but values are close to the stippled area, which indicates the humidities at which equilibrium moisture content would allow aerobic respiration to begin. This would make the gaseous environment experienced by the seed of importance.

trips, plotting mean monthly temperature and relative humidity values, taken from meteorological tables, on to such a chart allows collectors to quickly identify the relative rates of loss of seed viability likely to be encountered in the field.

This method overcomes many of the drawbacks associated with the earlier approach of Smith (1984). However, its purpose is much the same, i.e. to help collectors calculate whether the expected monthly rates of loss of viability are sufficiently large (i.e. in excess of 0.1 probit/month[1]) for either: (i) active field drying of seeds to moisture levels below the likely equilibrium value to be necessary; or (ii) the time spent in the field before a base is reached where seed drying facilities exist to have to be reduced. Indeed, once the timing of the trip has been set, maps of the collecting area can be produced showing contours of equal levels of

[1] At this stage, the 0.1 probit/month is a somewhat arbitrary value which is discussed further below. However, note that this rate of loss of seed viability is 20 times greater than would be expected under recommended seed drying-room conditions. The meaning of 'probit' is discussed in Box 20.1.

Box 20.1
Percentages, probits and seed viability

As long as the germination test conditions adopted overcome any dormancy, seeds in viability monitoring tests can be classified as either viable (if they germinate) or dead (if they do not germinate). When the results of a time series of monitoring tests are plotted as percentage viability values, the graph takes the form of a reversed S-shaped or negative sigmoidal curve (opposite).

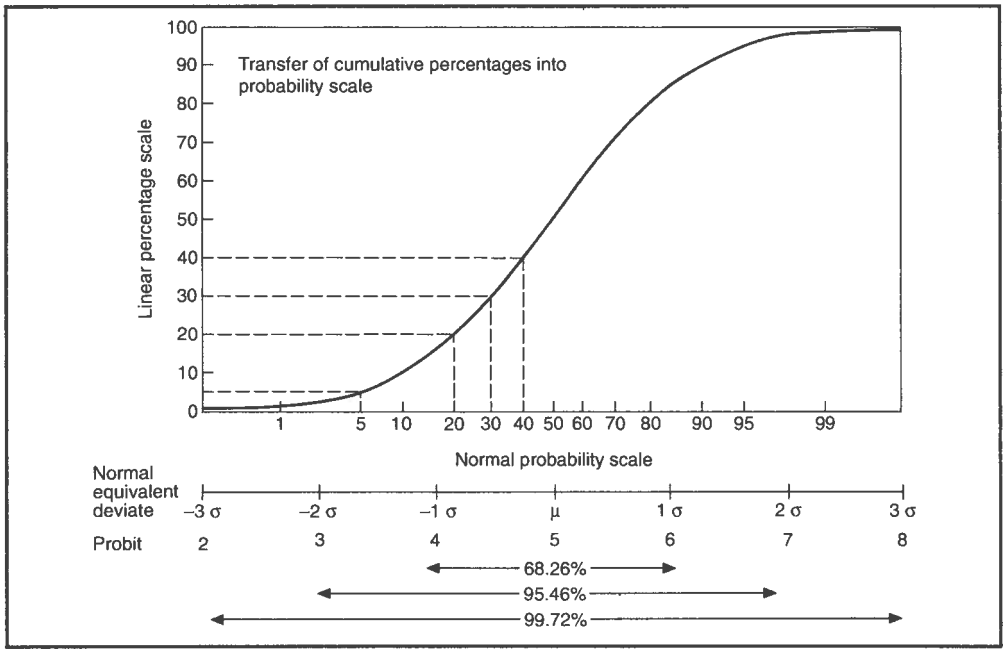
Given that the classes used were 'all' (germinating and therefore viable) or 'nothing' (non-germinating and therefore dead), the negative sigmoidal graph approximates a negative cumulative normal distribution of individual life spans within the seed lot (Smith, 1984).

A negative cumulative normal distribution is cumbersome to manipulate as a curve, but it can be converted into a straight line by transforming the raw percentage values to probit or normal equivalent deviate values, in the same way as transformation to logarithms is used in some applications. Variation in individual attributes (in this case life spans of seeds) about the population mean may be expressed in terms of standard deviations. The proportion (% age) of the population which lies within each standard deviation unit is fixed (see figure). Using values related to standard deviations rather than raw percentages thus gives a linear scale of viability loss with time. Tables, not unlike log tables, can be produced to convert percentages to probits (see table).

The value of this transformation is that it makes clear that, though the same damage is caused in any single seed lot by similar exposure to identical adverse storage conditions, at high viabilities this damage will be much more difficult to detect than at lower viabilities. For example, if we assume that the damage is sufficient to reduce viability by 1 probit, the % viabilities will be from 99.9 to >97.7, >84 and >50 as the probit values fall from 8 to >7, >6 and >5. The fall from 99.9% will be statistically undetectable in a standard germination test using 400 seeds, but the fall from 84% will be very significant.

Table 20.A The probit transformation.

Response rate	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.00	–	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
0.10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
0.20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
0.30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
0.40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
0.50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
0.60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
0.70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
0.80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
0.90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
Response rate	0.000	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009
0.97	6.88	6.90	6.91	6.93	6.94	6.96	6.98	7.00	7.01	7.03
0.98	7.05	7.07	7.10	7.12	7.14	7.17	7.20	7.23	7.26	7.29
0.99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09



viability loss. This will be important in itinerary planning. For example, Fig. 20.6, from Prendergast *et al.* (1992), shows that in Mali in October, the normal harvest month, there is a ten- to 40-fold difference in the expected rate of viability loss from the west to the east. Thus, all other things (e.g. seed availability) being equal, collecting from west to east will be a good idea, as the seed will dry during collecting. If collecting is from east to west, the seed will absorb moisture during the mission, and the quality of samples therefore deteriorate.

Drying seeds in the field

Where the ambient conditions are such that the expected viability losses are acceptable, collectors will only need to make sure that seeds from dry dehiscent or indehiscent fruits are stored in permeable containers such as cotton or paper bags. These should be held in such a way that air circulates freely between and through them. It could be argued that seeds are adapted to such conditions and damage should be minimal. However, extrapolation of the research findings presented earlier suggests a more cautious approach. The risk of damage caused by rapid drying of immature seed must be balanced against that caused by mature seed drying slowly between high moisture contents, where viability loss is slow, and much lower moisture contents, where viability loss is again slow. In lettuce, this involves drying seed from 40% to 8% moisture content. This problem remains little researched, and advice given is therefore uncertain, though less so for dry-fruited than

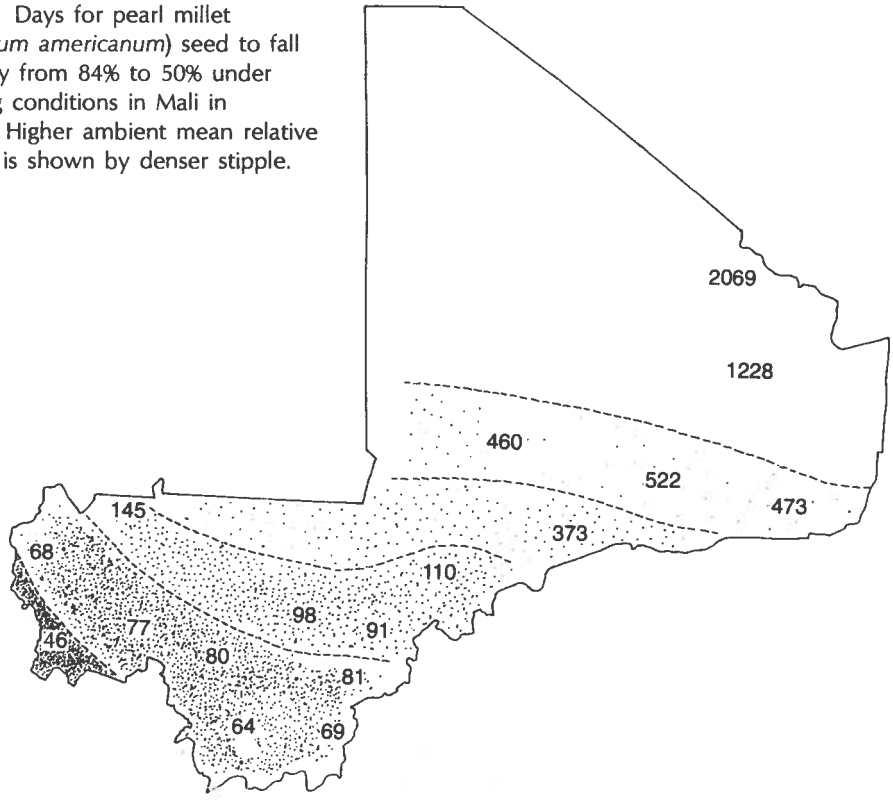


Fig. 20.6. Days for pearl millet (*Pennisetum americanum*) seed to fall in viability from 84% to 50% under prevailing conditions in Mali in October. Higher ambient mean relative humidity is shown by denser stipple.

for fleshy-fruited species. First, tolerance to rapid desiccation appears to occur sooner in seed development, so seed maturity at the time of collecting may exert a smaller influence, and secondly, provided they have achieved their maximum dry weight, the seeds of dry-fruited species may already have undergone drying to ambient equilibrium moisture content and so will be in the air-dry phase. Further drying will probably be less stressful. Indeed, where rapid drying was found to induce desiccation stress, this took place in seeds of high initial moisture content and so was likely to be in equilibrium with relative humidities above the transition zone.

Therefore, dry-fruited seed lots can probably be dried to below ambient moisture content safely, the only risks being to immature seed. To overcome this final risk, holding the fruits loosely packed, and thus aerated, under ambient conditions for a further three to seven days after collecting would cause only tolerable damage in the mature seed while providing an opportunity for the immature seed to mature. Following this period of air-drying, the seed could then be dried further.

For small, many-seeded fleshy fruits, if the logistics of the trip do not prevent holding the seeds inside the fruits, this is the most practical and most successful option. Apple seeds held in the fruits survived much

better than those extracted from the fruit and held at the same moderate temperature in a collecting bag (Dickie, 1988). Seed longevity in the fruit was also reduced if the fruits were held at higher temperatures or under semisealed conditions where the oxygen supply was limited. The advice appears to be: keep the fruit 'sound' and the seed will also keep sound. What is not clear, and would be helpful for collectors to know, is whether seed development can continue within detached fruits and if so under what conditions this process is most successfully completed.

Successfully dealing with large, many-seeded fruits or relatively small, single-seeded fruits, when the logistics prevent keeping the seeds in their fruits, provides the most difficult challenge. In fleshy fruits, desiccation tolerance occurs late in seed development, useful morphological markers of seed development do not appear to be displayed by the fruit and the seeds themselves will be at high moisture contents on extraction from the fruits. All that can be recommended is to extract the seeds carefully by hand, spread them into a thin layer to maximize aeration and allow them to dry under ambient conditions in the shade until they have achieved equilibrium. However, as seed drying theory predicts that over the initial drying phase the rate of moisture loss under most ambient conditions will be close to that expected over silica gel, the risks are high. Yet transporting constant humidity solutions into the field to slow seed drying rates to lower levels of damage in the same way as in laboratory studies is probably more logistically demanding than holding the seeds in the fruits.

When drying seed to below equilibrium moisture content, whether from fleshy or dry fruits, one of the least demanding methods is direct sun-drying. However, despite its common use in traditional agriculture, the advantages and disadvantages of the technique remain unquantified. Therefore, sun-drying cannot be recommended, despite the expectation that evaporative cooling should probably counteract the effects of increased temperature.

Another, more logistically complicated, possibility involves the use of desiccants such as silica gel. This raises the questions of how much to dry each seed lot and how much silica gel is required in total on the trip. Figure 20.7 allows the estimation of the weight of silica gel needed to reduce the moisture content of a sample to 5% from its fresh weight, provided seed oil and moisture contents are known. Fresh weight can be measured in the field using a spring balance, but the latter are more difficult. However, Earle and Jones (1962) analysed the seed oil content of 900 species in 501 genera in 113 families representing 35 angiosperm orders and two gymnosperm orders. The average seed oil content for this wide sample is about 25%. If 90% relative humidity is taken as the upper limit for the air-dry storage phase and 20°C as a reasonable global average temperature, an approximation of the worst likely equilibrium moisture content can be calculated. The value is 15% moisture content (WW basis). From Fig. 20.7, the weight of silica gel required to dry 1 kg of wet seeds to 5% moisture content is about 0.7 kg. This value rises to

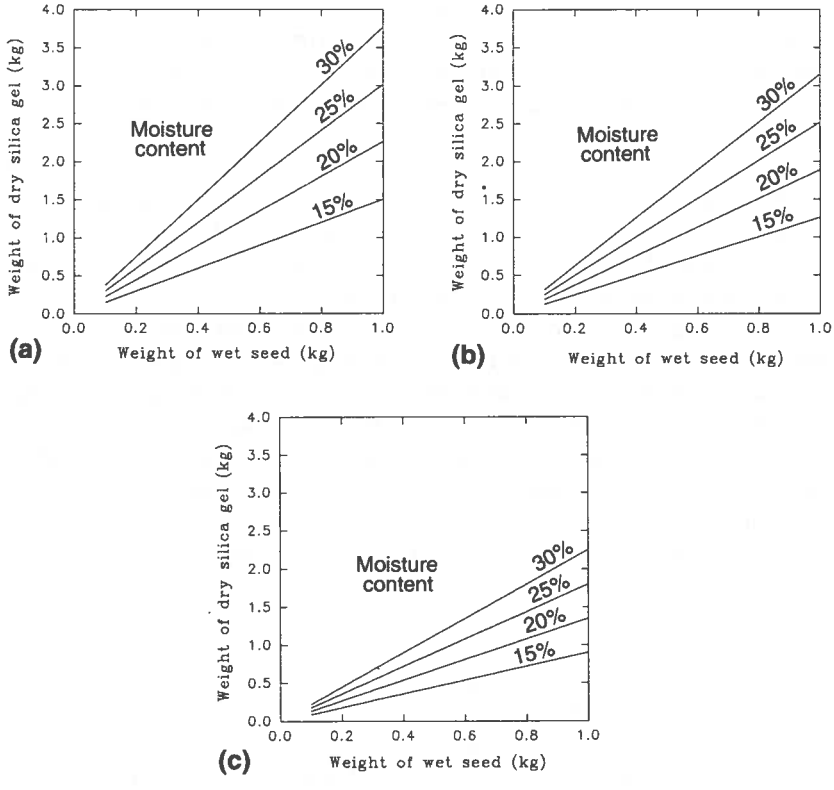


Fig. 20.7. Calibration charts to allow rapid estimation of the weight of silica gel required to dry seed lots of known weight and moisture content to 5% moisture content at 20°C. **(a)** 1.0% oil content; **(b)** 10.0% oil content; **(c)** 25.0% oil content. Adapted from Cromarty *et al.*, 1985.

1.4 kg as the seed oil content falls to 1%, as in the major grains. In practice, three parts seeds to two parts silica gel seems to be an acceptable compromise.

Seed moisture content will be reduced most quickly if the seeds and silica gel are sealed in a container that minimizes the air volumes enclosed and maximizes contact between the two. Alternate layers of packeted silica gel and packeted seeds, all enclosed in a sealed plastic container, may be an option. Rapid drying will result in the container becoming warm to the touch as the absorption of the water by the silica gel releases latent heat. This heating will be offset to some extent by evaporative cooling of the seeds as they dry.

If the transport of the necessary weights of desiccant is a problem and the logistics of collecting and transporting fruit overwhelming, then a more complex drying protocol can be used. This involves the regular reactivation of smaller weights of silica gel so that the seed moisture content is 'stepped down' to its final low value. This requires blue silica

gel, which can be reactivated when it has turned pink by heating to 175°C for 6 hours or to 125°C for 16 hours. When reactivated, the silica gel returns to its original colour and can be used again. Only when the silica gel stays blue for several days will the seed have been dried to sufficiently low levels. The slower drying rate of this technique will mean that greater levels of damage are accumulated. When the seeds are dry, they can be packed tightly into a sealed heavy-duty polythene bag. If condensation is seen inside the bag, the seeds must be re-dried.

Seed cleaning in the field

If seed lots must be cleaned during a collecting mission for logistical or quarantine reasons, only manual methods that do not reduce subsequent longevity should be employed. Mechanical methods, even traditional ones such as threshing with flails (Saini *et al.*, 1982), reduce subsequent longevity by twice as much as is proposed as acceptable under ambient collecting conditions. Some mechanical methods are described in Chapter 23, but they are not recommended for seeds destined for long-term conservation. Flotation methods are sometimes used to separate viable seeds from unwanted material. The effect of this on viability has not been explicitly studied. However, there is evidence that most, if not all, orthodox seeds suffer imbibition damage when dried below 15% moisture content and then imbibing through contact with liquid water (rather than water vapour). There is also the need to redry the seeds back to their original or lower moisture contents for storage. The recommendation must again be to avoid such methods when collecting for long-term conservation.

Quarantine and transport effects

If seeds that have been collected through international collaboration are to be transferred from the country of origin, quarantine treatments and conditions during transport could reduce their viability further. While there are many studies on the immediate effects of fungicides and other quarantine seed treatments on germinability, most do not investigate their effects on subsequent storage. The literature is also ambivalent, some studies reporting that such treatments result in higher germination, others lower. Therefore, a strategy must be adopted that reduces the risk of damage to seed storage potential without increasing the risk of unwitting disease transfer.

An option is for the recipient organization to obtain permission from its own national authorities to carry out post-entry quarantine. Getting such agreement should be possible, as the seed lots will usually be small and not intended for general distribution and will enter the country in a sealed package destined for a single location, where they will remain

banked and so isolated from the normal processes of disease transmission. Should such an arrangement be acceptable, collectors will need to have the necessary paperwork showing that post-entry quarantine has been agreed before the host country plant health officials can be expected to waive any treatment. The importance of quarantine inspection of germplasm should not be underestimated, as the conditions that prolong the longevity of desiccation-tolerant seeds also prolong the longevity of the desiccation-tolerant fungal and bacterial spores that are borne upon them (Lenné and Wood, 1991).

Seed samples should be transported to the seed bank for further drying as quickly as possible. Airmail is uncertain, with neither collector nor recipient able to trace the package from the moment of posting to delivery at its destination. It should only be considered as a last resort. Experience shows that air freight is not as certain or as quick a means of transporting seeds between countries as some agents might contend. Collectors need to take some defensive measures on behalf of their seeds if the vicissitudes of the air transport system are to be avoided. Ideally, the collector should accompany the package to its destination, so that delays between collecting and storage are kept to a minimum and loss of viability is minimized. On the surface, this may appear extreme. However, when agents' fees and air freight charges are added together, the comparison between excess baggage and air freight costs is acceptable. When the staff time of the recipient institute spent in monitoring the progress of the consignment towards its destination is added to the cost, the comparison is even more favourable. If air freight is the only practical way forward, the minimum defence is to pack the seed so that subsequent moisture uptake, either from being left standing in the rain or under conditions of high relative humidity, is unlikely. Collectors must inform the recipient institute by Telefax, telex or telephone of the airway bill number of their consignment(s), given to them by the freight agent. This will allow the progress of the consignment to be monitored through the international air freight computer system and the seed 'pulled' towards its destination and safety. Seed lots are forgotten and left to stand in airports or in agents' warehouses more often than might be hoped.

Why should the collector heed this advice?

Why is it important to minimize losses of seed viability between collecting and storage, as the advice given here attempts to do? The traditional argument is still valid and by itself is enough to justify the call for high viability seed lots. This points to declining seed lot viability as a reflection of the increased frequency of mutations in the surviving seeds. These mutations, though reduced by diplontic selection as the resulting seedlings develop into plants, do pass to the next generation (Dourado and Roberts, 1984). Thus, the surviving seed lot will gradually diverge

from the population from which it was collected. Those responsible for the germination, establishment and subsequent cultivation of aged seed lots would also point to increased intolerance of stress, reduced growth rates and reduced seed yield of the surviving plants from such material. Those responsible for the funding of seed banks may also question why seed collected in the field was not directly storable and why an additional costly step of rejuvenation was required at all.

More disconcertingly for collectors, it is becoming apparent that the loss of seed viability can exert directed selection pressure. However, the selection pressures are not for 'seed bankability', as some have proposed. The arguments against this ill-considered view are straightforward. First, environmental conditions worldwide are such that desiccation-tolerant seed, when severed from the parental vascular supply and not in contact with soil water through capillarity, will soon air-dry to below the critical relative humidity and fall within the range of water potentials at which the viability equation applies. Here temperature (ambient or bank) and seed moisture content (ambient or bank) control the rate of loss of viability. There is no evidence of discontinuities in these relationships for temperatures over the range −13°C to 80°C or for moisture contents in equilibrium over the range of 10% to 90% relative humidity. Therefore, the rate of seed viability loss can be deduced to be under the same control in all these conditions, being slowed and accelerated by changes in these parameters but not changed qualitatively. At a single pair of temperature and moisture content values throughout this wide range of conditions, seed survival curves can be described by a negative normal distribution of individual life spans. Some seeds will be more short-lived and others more long-lived than the average, but there is no evidence for systematic changes in the shape of the survival curve with storage conditions. This suggests that the relative longevity of individual seeds within these populations remains unchanged irrespective of the storage conditions under which they are kept. Hence the shortest-lived individuals under a single pair of storage conditions (ambient) will remain the shortest-lived individuals if moved to any other storage conditions (in the bank, say).

The selection pressure exerted during seed storage can be deduced from the seed viability equation to be for those genotypes which have the highest storage potential at the moment of collecting. The magnitude of this selection pressure is likely to be determined by: (i) whether the quantity of seed taken from each mother plant was similar or whether it reflected the seed available from each mother plant on the day of collecting; (ii) how great the differences were between the storage potentials of the seed lots taken from each mother plant; (iii) how high the storage potentials were of these seed lots; and (iv) how far seed viability is allowed to fall before rejuvenation is undertaken.

As has been mentioned earlier, barley and tomato seed storage potential varies with developmental age, and yet the rate of loss of viability between samples of different developmental age is the same

when stored under similar conditions. A model can therefore be developed which reflects collecting practice by proposing that on the day of harvest each mother plant is different in development and so produces a seed lot which has different storage potential. The seed lot from each mother plant is then combined through normal seed collecting protocol into a single seed lot. Table 20.1 shows the expectation from theory for a seed lot drawn from individual plants producing seed of low storage potentials which were collected in equal amounts and then bulked together. Table 20.1 also shows the same model for seed lots taken at higher storage potentials, again in equal amounts. For all seed lots, the variation in storage potential of the sublots around the mean is the same, storage conditions are identical (so that the rates of loss of viability are the same) and the duration of storage is the same, as would be seed bank practice. In the low-storage potential lots the relative frequencies of surviving individuals from each mother plant are substantially different after storage from those which pertain at the time of collecting. However, this is not the case for the high potential lots.

Such a model seems well founded. Roos (1984) was able to show that observed experimental behaviour fitted the predictive model well when a synthetic population was constructed from beans of different initial viabilities (each with a different seed-coat colour). Through the use of the model, it is possible not only to predict the effects of higher and lower mean seed lot storage potentials on selection during ageing, but also to examine the effects of tolerating larger or smaller degrees of seed ageing following collecting on the selective deletion of genotypes, as well as the effects of collecting unequal seed amounts from each mother plant.

From Table 20.1, higher mean seed storage potentials will reduce to practically zero any deletion of genotypes with lower than average viability. However, at lower mean storage potentials, the same level of ageing will change the relative frequency of the surviving genotypes dramatically. At low mean levels of viability, this change in frequencies can be kept to acceptable limits by reducing the field ageing that is tolerated during expeditions. Clearly, the practical advice is to make every effort to collect seed lots at the highest possible initial mean storage potential.

The necessity of this advice in comparison with current practice would be easier to appreciate if the seed storage potentials of collections arriving at seed banks for storage were known. Regrettably, no values from actual collections are known. Experimental determinations have been made for very few species and vary greatly, from low values for seeds of *Malus domestica* taken directly from the fruits to moderate values for the dry dehiscent fruits of barley and wheat and high values for the desiccated berries of *Nicandra pysalodes*. Given so few data and such a large variation, there is no alternative but to base advice on the assumption that the relatively low storage potential (about 97.7%) found in a wide variety of apples by Dickie (1988) will be common and so only low levels of ageing acceptable.

Collecting unequal seed numbers from each mother plant can either increase or decrease the selective deletion of genotypes, depending on the initial viability of the seeds that are over-represented. If seeds with higher than average storage potential are over-represented, the relative deletion of the genotypes contributing the lower than average viable seed will be greater. If it is seed with lower than average viability which is overcollected, the effect will be less.

The genotypes that are vulnerable to deletion are clearly those that either directly or indirectly result in seed lots being contributed to the sample that are of lower than average viability. From the patterns of seed storage potential associated with maturation in the dry-fruited cereal barley, these would be those genotypes that matured much earlier or later than on the day of collecting. The more extreme their earliness or lateness, the more susceptible to deletion they would be. For the fleshy-fruited berry tomato, only the later-maturing genotypes would be at risk as within the fruit there is no subsequent fall in seed storage potential. This latter case is also more likely to be found in dry dehiscent and indehiscent-fruited species, where the seeds or fruits are dispersed as they mature and so are no longer available to be collected.

The preceding discussion suggests that genetic selection linked to seed maturity ought to be well known and commonplace in seed storage. Yet, when Roos and Rincker (1982) investigated selection among the four parental clones that make up the synthetic variety of *Dactylis glomerata* cv. Pennlate, no genetic shifts could be found following seed ageing and regeneration. This suggests that the four parental clones produce seed of substantially similar storage potentials. Two very different processes could produce such a result. The first involves all the clones sharing similar phenologies of seed maturation and fruit shedding. The second demands that consideration be given to the structure of inflorescences and the seed maturity patterns produced within them (Fig. 20.8). Clearly, any inflorescence reflects the temporal differences in the seeds' development in their spatial separation. For example, within a wheat spike seven weeks after anthesis, seed moisture contents differ from 30% in the apical grains to 60% in the basal grains, reflecting differences in stage of maturation (Wellington, 1956). Selective harvesting of individual seeds from different positions within inflorescences of different age could none the less result in collecting seeds of similar maturities (and hence storage potentials). Indeed, such selective harvesting will inadvertently result when mature seeds are shed from the plant and desiccation tolerance is achieved only late during seed maturation, as only seed with a more limited variation in storage potential will be available for collecting. In naturally occurring populations the variation in flowering time among the many inflorescences that can be borne on single plants may further 'indemnify' collectors who take random samples of the seed that is available on a single day from putting together samples where seed storage potential and maturation genotype are so linked that genetic selection occurs in subsequent storage.

Table 20.1. Genetic selection following seed collection, assuming differences in storage potential.

		Mother plant A	Mother plant B	Mother plant C[1]	Mother plant D	Mother plant E
Very high viability, high loss						
On collection	Probit viability	4.0	4.5	5.0	5.5	6.0
	% viability	99.99+	99.99+	99.99+	99.99+	99.99+
	Relative frequency in survivors	1.0	1.0	1.0	1.0	1.0
After loss of viability (1 probit) in the field	Probit viability	3.0	3.5	4.0	4.5	5.0
	% viability	99.86	99.98	99.99+	99.99+	99.99+
	Relative frequency in survivors	0.998	0.999	1.0	1.0	1.0
High viability, high loss						
On collection	Probit viability	3.0	3.5	4.0	4.5	5.0
	% viability	99.86	99.98	99.99+	99.99+	99.99+
	Relative frequency in survivors	0.998	0.999	1.0	1.0	1.0
After loss of viability (1 probit) in the field	Probit viability	2.0	2.5	3.0	3.5	4.0
	% viability	97.7	99.38	99.86	99.98	99.99+
	Relative frequency in survivors	0.98	0.99	1.0	1.0	1.0

Medium viability, high loss						
On collection	Probit viability	2.0	2.5	3.0	3.5	4.0
	% viability	97.7	99.38	99.86	99.98	99.99+
	Relative frequency in survivors	0.98	0.99	1.0	1.0	1.0
After loss of viability (1 probit) in the field	Probit viability	1.0	1.5	2.0	2.5	3.0
	% viability	84.1	93.3	97.7	99.38	99.86
	Relative frequency in survivors	0.86	0.95	1.0	1.02	1.02
Low viability, high loss						
On collection	Probit viability	1.0	1.5	2.0	2.5	3.0
	% viability	84.1	93.3	97.7	99.38	99.86
	Relative frequency in survivors	0.86	0.95	1.0	1.02	1.02
After loss of viability (1 probit) in the field	Probit viability	0.0	0.5	1.0	1.5	2.0
	% viability	50.0	69.2	84.1	93.3	97.7
	Relative frequency in survivors	0.59	0.82	1.0	1.11	1.16
Low viability, low loss						
On collection	Probit viability	1.0	1.5	2.0	2.5	3.0
	% viability	84.1	93.3	97.7	99.38	99.86
	Relative frequency in survivors	0.86	0.95	1.0	1.02	1.02
After loss of viability (0.1 probit) in the field	Probit viability	0.9	1.4	1.9	2.4	2.9
	% viability	81.6	91.9	97.1	99.18	99.81
	Relative frequency in survivors	0.84	0.95	1.0	1.02	1.02

[1]Assuming equal seed numbers are taken from each plant, those values will also represent the values for the seed collection as a whole.

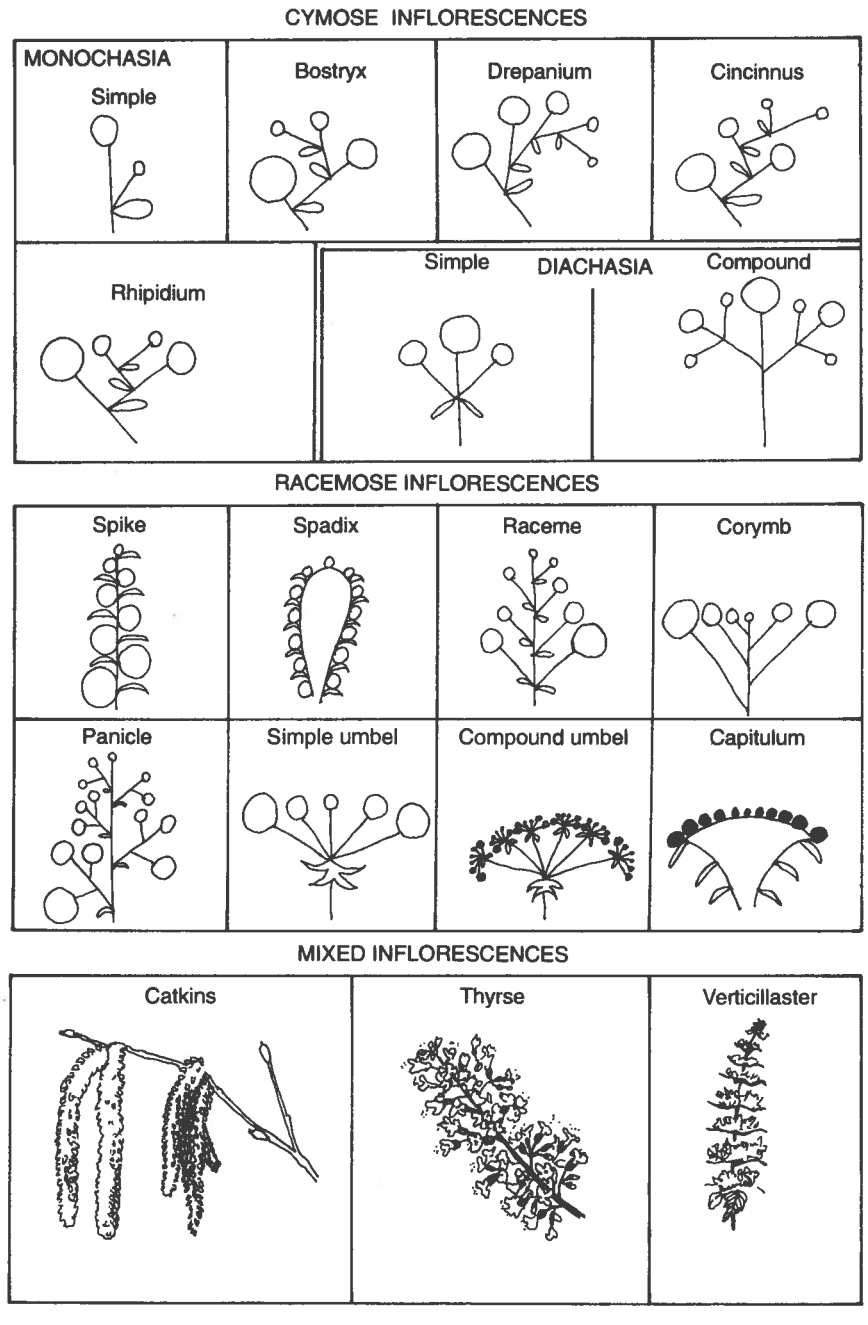


Fig. 20.8. Types of inflorescence structures found among the Angiosperms and variation in seed maturity within each. The relative maturity is indicated by the size of the circle representing the flower (after Heywood, 1978).

In single plants of *Pastinaca sativa*, primary umbels are mature 10–14 days earlier than secondary umbels, which in turn mature 10–14 days earlier than tertiary umbels. For the whole population, seeds are dispersed naturally over a protracted period from August to October (Hendrix, 1984). For the weed *Echinochloa oryzicola*, the inflorescences, in this case panicles, borne on one plant varied by 11 days in their flowering dates and their glumous flower number varied from 27 to 493 per panicle. Within one of the earlier panicles to flower, seed ripened between 17 and 46 days after the first flowering of that panicle was observed. However, in the panicle that first flowered six days later, a similar delay was observed in the ripening of the earliest seeds compared with the earlier-flowering panicle. The latest seeds to ripen on the later panicle did so at much the same time as the latest seeds of the earlier panicle (Toshioka *et al.*, 1985).

The practical situations encountered by seed collectors in the field may therefore not be accurately reflected by the experimental situation reported in barley, where genetically homogeneous cultivars were used, and would be expected to flower and the seeds to mature over a very restricted period, while the plants themselves are grown at such densities that very few inflorescences are produced by each. In the same way, inflorescences possessing a more varied spread of flowering time than that of the barley spike may also reduce the peak in seed storage potential.

Overlying all these possibilities are the observations that pollination with pollen from different donors can result in the differential maturation of the fruits produced, while within individual plants ovules fertilized sooner are more likely to develop into seeds than those fertilized later, which could link male genotypes to seed maturation (Lee, 1988).

What is clear from this discussion is that the likelihood of genetic selection during storage will be unique for each seed lot, being an interaction between the phenology of the species in question, the maturity and hence storage potential of the seed populations on the day of collecting, the methods used by the collectors to assemble the sample and its subsequent storage. To minimize the risk of selective deletion of maturity types, collecting at least early and late during the period of seed dispersal may be necessary.

However, seed maturity need not be the only factor affecting seed storage potential. Different subspecies of rice have recently been reported to possess different seed storage characteristics (Ellis *et al.*, 1992), resulting in significantly different rates of loss of viability occurring under the same storage conditions. As all three subspecies were derived from a common ancestral stock, it is reasonable to assume that the differences in their seed storage behaviour also existed in the common ancestral stock and the selection for this character was passive. The trebling of seed oil content in *Zea mays* that can be achieved through 24 cycles of selection (Misevic and Alexander, 1989) is of similar concern. Here, either the differences in seed moisture contents which must result

at the same equilibrium relative humidity will make a single moisture content term for the viability equation unacceptable or the relationship between equilibrium moisture content, oil content and relative humidity is not properly understood. Together, they draw into question the current assertion that, as all seed lots from the same species so far investigated experimentally respond in the same way to temperature and moisture content, the values of such viability constants apply to all genotypes within species. Clearly, this assertion now needs to be re-investigated over a wider range of the naturally occurring variation within species, both wild and cultivated.

The differences in storage behaviour of the rice subspecies under the same conditions have been modelled for seed lots of different storage potential as shown in Table 20.2. This model can also be used to illustrate the more general situation where seed storage characteristics vary between individuals in the population. As Table 20.2 shows, the practical solution of this problem is the same as for the variation in storage potential. The higher initial seed viability is at harvest, the less the separation will be of the different seed storage variants in the survivors for the same loss of viability. At lower initial viabilities the only technique that will reduce such a separation is to hold the loss of viability to relatively low levels. A similar model will apply to differing rates of viability loss resulting from the variation in individual seed moisture contents found within equilibrated rice (and so presumably all species) samples (Siebenmorgen *et al.*, 1990). Whether this will exert a directed or random selection pressure remains unclear.

Accessions of genotypes of *Zea mays* have been ranked for their subsequent storability under constant conditions (Moreno Martinez *et al.*, 1988). While different accessions of the same genotypes could be found in a variety of storage classes, suggesting environmental and harvesting effects, there were genotypes that regularly achieved higher and lower rankings. This work was followed up by Lozano *et al.* (1989), who showed these differences to be heritable and chose to seek direct differences in the seed's behaviour but found only slight supporting evidence. Reanalysis of their storage data shows that these differences can be just as validly explained by differences in storage potential. Genetically determined factors that affected subsequent seed drying rate, such as the tightness of the husks covering the cob and the thickness of the pericarp and testa could consistently affect the accumulation of damage within the seeds before they have dried to equilibrium. In turn, this would consistently affect seed storage potential. All such variants have been recorded among maize genotypes (Kang *et al.*, 1986; Baker *et al.*, 1991). Again, this would lead to selection pressures of the type described in Table 20.1.

Clearly, selection of some kind (though not for 'seed bankability') can occur as viability is lost. How frequent this is in practice remains unquantified. Consideration of the work of Scott (1981), who actively sought to select maize for improved response to accelerated ageing, sug-

gests it may be a bigger problem in theory than in practice, though one that needs to be taken seriously none the less. Therefore, the earlier recommendation that viability losses should be restricted to 0.1 probit/month in the field appears to be a reasonable practical balance. To allow more will undo much of the purpose of seed collecting.

Before leaving the problems of genetic selection, whether brought about by differences in seed maturity or genetically controlled differences in either seed storage behaviour or storage potential, one final point must be made. The advice offered here is of equal use to those who prefer to keep plant genetic resources *in situ* as plants, and collect seeds on demand as convenient disseminules, as it is to those who collect seed as convenient disseminules with which to build *ex situ* collections of germplasm resources, to both meet the demand and underwrite the continued existence of the population in the field. In both systems, the largest single annual loss of seed viability will occur between collecting the seeds and their receipt by either the user of an *in situ* resource or the *ex situ* collection. This will also be the period in which the greatest genetic selection is most likely. It is peculiar that genetic selection is thought of as an exclusive problem of *ex situ* conservation and that research is focused on the locations where annualized rates of selection should be lowest (i.e. in gene banks) and explanations sought based on inherent seed characteristics over which collectors can have no control.

Conclusion

In summary, what practical advice can be given to collectors? Not perhaps as much as one would have hoped. It is clear that the longevity of samples in the seed bank is determined by their quality at the moment of banking. However, there are very few direct investigations into the effects and interactions of seed development, seed maturation, subsequent storage in transit and cleaning under field conditions on subsequent long-term storage. Most of what is presented here would easily fit Albert von Szent-Györgi's conclusion that 'the basic texture of research consists of dreams into which the threads of measurement and reasoning are woven'. Only here seed collectors find themselves a little short of measurement and a little overdependent on dreams and reasoning. Yet again invoking the UN Conference on Environment and Development (UNCED) imperative to act rather than anguish, the advice is as follows.

All seeds

For all seeds:

- Attempt to collect equal numbers of seeds from each plant sampled, and at the same maturity, ideally when seed storage potential/desiccation tolerance is highest. Avoid damaged seeds (mechanical damage, pest attack).

Table 20.2. Genetic selection in *Oryza sativa* subspecies following seed collection, assuming differences in storage characteristics.

		ssp. *indica*	ssp. *japonica*	ssp. *javanica*	Collection
Very high viability, high loss					
On collection	Probit viability	5.0	5.0	5.0	5.0
	% viability	99.99+	99.99+	99.99+	
	Relative frequency in survivors	1.0	1.0	1.0	
After loss of viability (1 probit) in the field	Probit viability	4.37	3.41	4.27	4.0
	% viability	99.99+	99.99+	99.99+	
	Relative frequency in survivors	1.0	1.0	1.0	
High viability, high loss					
On collection	Probit viability	4.0	4.0	4.0	4.0
	% viability	99.99+	99.99+	99.99+	
	Relative frequency in survivors	1.0	0.99	1.0	
After loss of viability (1 probit) in the field	Probit viability	3.37	2.41	3.27	3.0
	% viability	99.96	99.2	99.95	
	Relative frequency in survivors	1.0	0.99	1.0	
Medium viability, high loss					
On collection	Probit viability	4.0	4.0	4.0	4.0
	% viability	99.99+	99.99+	99.99+	
	Relative frequency in survivors	1.0	1.0	1.0	
After loss of viability (1 probit) in the field	Probit viability	3.37	2.41	3.27	3.0
	% viability	99.96	99.2	99.95	
	Relative frequency in survivors	1.0	0.99	1.0	

Medium viability, low loss					
On collection	Probit viability	3.0	3.0	3.0	3.0
	% viability	99.86	99.86	99.86	
	Relative frequency in survivors	1.0	1.0	1.0	
After loss of viability (1 probit) in the field	Probit viability	2.37	1.41	2.27	2.0
	% viability	99.91	92.1	98.84	
	Relative frequency in survivors	1.0	0.92	0.99	
Low viability, high loss					
On collection	Probit viability	2.0	2.0	2.0	2.0
	% viability	97.7	97.7	97.7	
	Relative frequency in survivors	1.0	1.0	1.0	
After loss of viability (1 probit) in the field	Probit viability	1.37	0.41	1.27	1.0
	% viability	91.5	65.9	89.8	
	Relative frequency in survivors	1.0	0.72	0.98	
Low viability, low loss					
On collection	Probit viability	2.0	2.0	2.0	2.0
	% viability	97.7	97.7	97.7	
	Relative frequency in survivors	1.0	1.0	1.0	
After loss of viability (0.1 probit) in the field	Probit viability	1.94	1.84	1.93	1.9
	% viability	97.4	96.7	97.3	
	Relative frequency in survivors	1.0	0.99	1.0	

- If seeds must be cleaned during the trip, do so by hand to minimize the chance of mechanical damage.
- If it is possible to avoid quarantine seed treatments without breaking quarantine regulations, for example through post-entry quarantine, do so.
- Personally ensure that seed arrives at the seed bank without undue delay.

Desiccation-intolerant seeds

For desiccation-intolerant seeds, which are more likely to be large seeds from dominant trees growing under wet conditions:

- Collect close to fruit fall. Do not collect from the ground unless you can be sure seeds are only recently dispersed.
- Keep seeds aerated and moist in inflated polythene bags, changing the air at least weekly by deflation and reinflation.
- Do not allow such seeds collected in the tropics to either cool below 20°C or heat up above ambient shade temperatures in the field or during transport.
- Plan your activities so that no more than one month elapses between collecting and reception by the seed bank.

Desiccation-tolerant seeds or their fruits

For desiccation-tolerant seeds or their fruits, which are more likely to be small seeds from herbs growing under dry conditions:

- If meteorological data suggest that more than 0.1 probit/month will be lost during the collecting trip, either modify your itinerary or prepare to dry actively with silica gel.
- For fleshy-fruited species, if logistically possible keep the seeds in the fruits and the fruits aerated and at ambient temperatures.
- If the above is not logistically possible, and for fruits that are dry dehiscent or indehiscent, air-dry the hand-cleaned seeds in a thin layer (to ensure aeration) under shade for three days or more (larger seeds need longer) to reduce the seed moisture content towards equilibrium with ambient relative humidities before packing, to use space more efficiently.

References

Aldridge, C.D. and R.J. Probert (1993) Seed development, the accumulation of abscisic acid and desiccation tolerance with aquatic grasses *Porteresia coarctata* (Roxb.) Tateoka and *Oryza sativa L. Seed Science Research* 3:97–103.

Bacchi, O. (1956) Novos ensaios sôbre a seca da semente de café ao sol. *Bragantia* 15:83–91.

Baker, K.D., M.R. Paulsen and J. van Zweden (1991) Hybrid and drying rate effects on seed corn viability. *Transactions of the American Society of Agricultural Engineers* 34:499–506.

Cromarty, A.S., R.H. Ellis and E.H. Roberts (1985) *Design of Seed Storage Facilities for Genetic Conservation.* IBPGR, Rome.

Demir, I. and R.H. Ellis (1992) Changes in seed quality during seed development and maturation in tomato. *Seed Science Research* 2:81–87.

Dickie, J.B. (1988) Prospects for the long-term storage of apple seeds. *Veröffentlichungen Landwirtschaftlich-chemische Bundesanstalt Linz* 19:47–63.

Dickie, J.B., R.H. Ellis, H.L. Kraak, K. Ryder and P.B. Tompsett (1990) Temperature and seed storage longevity. *Annals of Botany* 65:197–204.

Dickie, J.B., K. May, S.V.A. Morris and S.E. Titley (1991) The effects of desiccation on seed survival in *Acer platanoides* L. and *Acer pseudoplatanus* L. *Seed Science Research* 1:149–162.

Dourado, A.M. and E.H. Roberts (1984) Phenotypic mutations induced during storage in barley and pea seeds. *Annals of Botany* 54:781–790.

Earle, F.R. and Q. Jones (1962) Analyses of seed samples from 113 plant families. *Economic Botany* 16:221–250.

Ellis, R.H. and E.H. Roberts (1981) An investigation into the possible effects of ripeness and repeated threshing on barley seed longevity under six different storage environments. *Annals of Botany* 48:93–96.

Ellis, R.H., T.D. Hong and E.H. Roberts (1986) Logarithmic relationship between moisture content and longevity in sesame seeds. *Annals of Botany* 57:499–503.

Ellis, R.H., T.D. Hong and E.H. Roberts (1990). An intermediate category of seed storage behaviour? 1. Coffee. *Journal of Experimental Botany* 41:1167–1174.

Ellis, R.H., T.D. Hong and E.H. Roberts (1991) An intermediate category of seed storage behaviour? 2. Effects of provenance, immaturity and imbibition on desiccation tolerance in coffee. *Journal of Experimental Botany* 42:653–657.

Ellis, R.H., T.D. Hong and E.H. Roberts (1992) The low-moisture-content limit to the negative logarithmic relation between seed longevity and moisture content in three sub-species of rice. *Annals of Botany* 69:53–58.

Farrant, J.M., N.W. Pammenter and P. Berjak (1986) The increasing desiccation sensitivity of recalcitrant *Avicennia marina* seeds with storage time. *Physiologia Plantarum* 67:291–298.

Finch-Savage, W.E. (1992) Seed development in the recalcitrant species *Quercus robur* L.: germinability and desiccation tolerance. *Seed Science Research* 2:17–22.

Grout, B.W.W., K. Shelton and H.W. Pritchard (1983) Orthodox behaviour of oil palm seed and cryopreservation of the excised embryo for genetic conservation. *Annals of Botany* 52:381–384.

Hendrix, S.D. (1984) Variation in seed weight and its effects on germination in *Pastinaca sativa* L. (Umbelliferae). *American Journal of Botany* 71:795–802.

Heywood, V.H. (1978) *Flowering Plants of the World.* Oxford University Press, Oxford.

Hong, T.D. and R.H. Ellis (1992) Development of desiccation tolerance in Norway maple (*Acer platanoides* L.) seeds during maturation drying. *Seed Science Research* 2:169–172.

Ibarra-Manriquez, G. and K. Oyama (1992) Ecological correlates of reproductive traits of Mexican rainforest trees. *American Journal of Botany* 79:383–394.

Jurado, E., M. Westoby and D. Nelson (1991) Diaspore weight, dispersal, growth form and perenniality of central Australian plants. *Journal of Ecology* 79:811–830.

Kang, M.S., M.S. Zuber, T.R. Colbert and R.D. Horrocks (1986) Effects of certain agronomic traits on and relationship between rates of grain moisture reduction and grainfill during the filling period in maize. *Field Crops Research* 14:339–347.

Kermode, A.R. and J.D. Bewley (1985) The role of maturation drying in the transition from seed development to germination. 1. Acquisition of desiccation tolerance

and germinability during development of *Ricinus communis* L. seeds. *Journal of Experimental Botany* 36:1906–1915.

Klein, L.A. and J.E. Harmond (1971) Seed moisture – a harvest timing index for maximum yields. *Transactions of the American Society of Agricultural Engineers* 14: 124–126.

Komatsu, T., N. Shimizu and S. Susuki (1979) [Studies on development and ripening of seeds in temperate grasses. 1. Seed development and ripening of Italian rye grass, and changes in germination and seeding vigour during seed development and ripening.] *Bulletin of the National Grassland Research Institute* 15:59–69.

Kovach, D.A. and K.J. Bradford (1992) Imbibitional damage and desiccation tolerance in wild rice (*Zizania palustris*) seeds. *Journal of Experimental Botany* 43:747–758.

Kraak, H.L. and J. Vos (1987) Seed viability constants for lettuce. *Annals of Botany* 59:353–359.

Lee, T.D. (1988) Patterns of fruit and seed production. In: Doust, J.L. and L.L. Doust (eds) *Plant Reproductive Ecology: Patterns and Strategies.* pp. 179–202. Oxford University Press, Oxford.

Lenné, J.M. and D. Wood (1991) Plant diseases and the use of wild germplasm. *Annual Review of Phytopathology* 29:35–63.

Lozano, J.L., S.H. Wettlaufer and A.C. Leopold (1989) Polyamine content related to seed storage performance in *Zea mays. Journal of Experimental Botany* 40:1337–1340.

Misevic, D. and D.E. Alexander (1989) Twenty-four cycles of phenotypic recurrent selection for percent oil in maize. 1. *Per se* and test cross performance. *Crop Science* 29:320–324.

Moreno Martinez, E., J. Ramirez Gonzalez, M. Mendoza Ramirez and G. Valencia Ramirez (1988) Comparison of Mexican maize races stored under adverse humidity and temperature. In: *Recent Advances in the Conservation and Utilization of Genetic Resources.* Proceedings of the Global Maize Germplasm Workshop. pp. 94–98. CIMMYT, Mexico City.

Muenscher, W.C. (1936) Storage and germination of seeds of aquatic plants. *Cornell University Agricultural Experimental Station Bulletin* 652:1–17.

Petruzelli, L. (1986) Wheat viability at high moisture content under hermetic and aerobic storage conditions. *Annals of Botany* 58:259–265.

Pieta Filho, C. and R.H. Ellis (1991) The development of seed quality in spring barley in four environments. I. Germination and longevity. *Seed Science Research* 1:163–177.

Prendergast, H.D.V., S.H. Linington and R.D. Smith (1992) The Kew Seed Bank and the collection, storage and utilisation of arid and semi arid zone grasses. In: Chapman, G.P. (ed.) *Desertified Grasslands: Their Biology and Management.* Academic Press, London.

Pritchard, H.W. (1991) Water potential and embryonic axis viability in recalcitrant seeds of *Quercus rubra. Annals of Botany* 67:43–49.

Probert, R.J. and E.R. Brierley (1989) Desiccation intolerance in seeds of *Zizania palustris* is not related to the developmental age or the duration of post harvest storage. *Annals of Botany* 64:669–674.

Probert, R.J. and P.L. Longley (1989) Recalcitrant seed storage physiology in three aquatic grasses (*Zizania palustris, Spartina anglica* and *Porteresia coarctata*). *Annals of Botany* 63:53–63.

Roberts, E.H. and R.H. Ellis (1989) Water and seed survival. *Annals of Botany* 63:39–52.

Roos, E.E. (1984) Genetic shifts in mixed bean populations. I. Storage effects. *Crop Science* 24:240–244.

Roos, E.E. and C.M. Rincker (1982) Genetic stability in 'Pennlate' orchard-grass seed following artificial aging. *Crop Science* 22:611–613.

Saini, S.K., J.N. Singh and P.C. Gupta (1982) Effect of threshing method on seed quality of soybean. *Seed Research* 10:133–138.

Scott, G.E. (1981) Improvement for accelerated aging response of seed in maize populations. *Crop Science* 21:41–43.

Siebenmorgen, T.J., M.M. Banaszek and M.F. Kocher (1990) Kernel moisture content variation in equilibrated rice samples. *Transactions of the American Society of Agricultural Engineers* 33:1979–1983.

Smith, R.D. (1984) The influence of collecting, harvesting and processing on the viability of seed. In: Dickie, J.B., S.H. Linington and J.T. Williams (eds) *Seed Management Techniques for Genebanks.* pp. 42–87. IBPGR, Rome.

Smith, R.D. (1985) Maintaining viability during collecting expeditions. In: IBPGR Advisory Committee on Seed Storage (ed.) *Report of Third Meeting.* pp. 13–20. IBPGR, Rome.

Smith, R.D. (1992) Seed storage temperature and relative humidity. *Seed Science Research* 2:113–116.

Suszka, B. and T. Tylkowski (1980) Storage of acorns of the English oak (*Quercus robur* L.) over 1–5 winters. *Arboretum Kornickie* 25:199–229.

Tompsett, P.B. (1982) The effect of desiccation on the longevity of seeds of *Araucaria hunsteinii* and *A. cunninghamii. Annals of Botany* 50:693–704.

Tompsett, P.B. (1983) The influence of gaseous environment of the storage life of *Araucaria hunsteinii seed. Annals of Botany* 52:229–237.

Tompsett, P.B. (1984) Desiccation studies in relation to the storage of *Araucaria. Annals of Applied Biology* 105:551–586.

Tompsett, P.B. (1986) The effect of temperature and moisture content on the longevity of seed of *Ulmus carpinifolia* and *Terminalia brassii. Annals of Botany* 57:875–883.

Tompsett, P.B. (1988) A review of the literature on storage of dipterocarp seeds. In: *Proceedings of the IUFRO Symposium on Forest Seed Problems in Africa.* pp. 348–365. Swedish University of Agricultural Sciences, Umea.

Tompsett, P.B. and H.W. Pritchard (1993) Water status changes during development in relation to the germination and desiccation tolerance of *Aesculus hippocastanum* L. seeds. *Annals of Botany* 71:107–116.

Toshioka, T., Y. Yamasue and K. Ueki (1985) [Seed ecological studies in relation to the asynchronous emergence of *Echinoctilea oryzicola* Vasing. 1. Variations in weight and ripening date of seeds among setting positions within one plant.] *Weed Research Japan* 30:58–64.

Tukey, H.B. (1934) Growth of the embryo, seed and pericarp of the sour cherry (*Prunus cerasus*) in relation to season of fruit ripening. *Proceedings of the American Society for Horticultural Science* 31:125–144.

Vossen, H.A.M. van der (1979) Methods of preserving the viability of coffee seed in storage. *Seed Science and Technology* 1:65–74.

Waller, D.M. (1988) Plant morphology and reproduction. In: Doust, J.L. and L.L. Doust (eds) *Plant Reproduction Ecology: Patterns and Strategies.* pp. 203–227. Oxford University Press, Oxford.

Wechsberg, G.E., C.M. Bray and R.J. Probert (1993) The development of longevity and response to priming of *Ranunculus sceleratus* seeds In: Côme, D. and F. Corbineau (eds) *4th International Workshop on Seeds: Basic and Applied Aspects of Seed Biology.* Vol. 3, pp. 845–850. ASFIS, Paris.

Wellington, P.S. (1956) Studies on the germination of wheat grains in the ear during development, ripening and after-ripening. *Annals of Botany* 20:105–120.

Yaklich, R.W. and P.B. Cregan (1987) A field study of moisture content of soybean pods and seeds after harvest maturity. *Journal of Seed Technology* 11:62–68.

Appendix 20.1
Collecting fern spores

L. Guarino

Roos and Verduyn (1989) discuss how to collect fern spores. The optimal time for collecting spores is when the sporangia are ripe or nearly ripe. The frond from which spores are to be collected should be placed in a paper pouch or envelope. If kept under dry conditions, most of the spores will have been shed after a couple of days, and the frond may be discarded. Most fern species have spores that are yellow or dark. These remain viable for considerable periods if kept dry and cool. In contrast, green spores (e.g. *Osmunda, Grammitis*) maintain their viability for only a few hours to a few days, and must therefore be sown as soon as possible after collecting.

References

Roos, M.C. and G.P. Verduyn (1989) Collecting and germinating fern spores. In: Campbell, D.G. and H.D. Hammond (eds) *Floristic Inventory of Tropical Countries*. pp. 467–468. New York Botanical Garden, New York.