

Chapter

6

Seed Maturity:

*when to collect seeds from wild
plants*



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Summary

Seed collectors should aim to ensure that seeds are collected at peak quality such that their longevity in the seed bank is optimal. Seed maturity is therefore an important consideration; harvest too early and losses may be incurred because the seeds have not yet acquired desiccation tolerance and/or because the seeds lose viability more rapidly in storage due to impaired longevity. Ideally the seeds should be collected when they are on the point of natural dispersal when seed equilibrium relative humidity (eRH) is first falling and equilibrating with the air immediately around the seeds.

Crude indicators used to identify increasing seed maturity include fruit or seed colour. Signs that the fruits are dehiscent (e.g., splitting capsules) are better. Measuring the eRH of the seeds (and comparing this with ambient RH) will be a useful and precise tool for identifying this point. It is equally important to check that the seeds are well filled. In the case of those borne in fleshy fruits, which will not be able to equilibrate with the air, no better indicators are currently available.

Many plant populations will naturally have fruits of varying maturity at any point in time. This is particularly true for wild plant species. As a consequence, it is highly likely that a collection will include seeds with a wide variation in maturity. A slow-drying treatment may allow the continuation of late developmental events which will improve the overall quality of such collections.

Introduction

In theory, seed collection is a simple process: go out in the field and collect. In practice, since we are interested in wild plant conservation and, as conservationists, we do not wish to devastate a population from which a collection is made, a seed collector will have to decide which fruits or seeds should be harvested from a fruiting population. A major factor that a collector is likely to automatically take into account is the ripeness or maturity of the seed or fruit. Efforts will be made to ensure that each seed in the collection is at 'peak' maturity, using indicators such as size, colour, and hardness as markers of maturity. Of course there are good physiological and genetic reasons why seed maturity should be considered when collecting seeds for long-term storage. Those reasons will be presented in this chapter. For those already experienced in seed collecting, hopefully this framework is in accordance with the intuitive decisions already made in the field regarding the best time to harvest seeds to ensure that they will remain viable for as long as possible in the seed bank. For people new to collecting, it is hoped that this guide will save the disappointment of repeating the earlier mistakes of others.

Why is the Timing of Seed Collection Important?

Seeds start to develop after fertilisation has occurred, going through a number of morphological and physiological changes until they reach the point of dispersal. From a seed conservation point of view, the first important physiological change that must have taken place is that the seeds have acquired the ability to germinate. Furthermore, since we are concerned with orthodox (desiccation-tolerant) seeds and dry storage, they must be able to germinate after drying. By this, we usually mean rapid enforced drying to low moisture contents (*c.* 5% fresh weight), perhaps in a dry-room or in desiccators (with a drying agent such as silica gel) depending on the facilities available.

Foxglove (*Digitalis purpurea* L.), a native British woodland plant that has racemes of 20–80 sequentially developing flowers, has been the subject of a detailed study of seed development carried out over a number of years (Hay, 1997). Foxglove was chosen as an experimental species, in order to investigate some of the problems facing seed collectors working with populations of wild species. The timing of pollination is simply dated by tagging open flowers. Each dehiscent capsule which subsequently forms is easily harvested and has plenty of seeds (up to *c.* 1,000) for experimental purposes.

Over the three years in which the progression of seed development was followed, there were some differences in the timing of different developmental phases from year to year, however the timing of those phases relative to each other did not change. In 1993, the onset of the development of ‘germinability’ (germination of fresh seeds immediately after harvest) occurred at around 24 d after flowering (DAF) and approximately 8 d before the seeds started to acquire desiccation tolerance (Figure 6.1). Total desiccation tolerance in the seeds was not attained until 44 DAF. Collecting seeds before they are fully desiccation tolerant would result in the loss of a proportion of the individual seeds in the collection during the seed bank drying process. This is something that should be avoided, especially when collecting seeds of a wild plant species where there is likely to be considerable genetic heterogeneity across the fruiting population and each seed is likely to have a unique genetic makeup.

This pattern of germinability preceding the acquisition of desiccation tolerance is typical of a number of orthodox seeds. However it is not a fixed pattern for all orthodox seeds (Figure 6.2). In some species, there is a pattern of concurrent development of germinability and desiccation tolerance. In cereals such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rice (*Oryza sativa* L.) the seeds appear to acquire desiccation tolerance before they are able to germinate upon removal from the parent plant (Rasyad *et al.*, 1990; Pieta Filho and Ellis, 1991; Aldridge and Probert, 1993). This latter observation may be

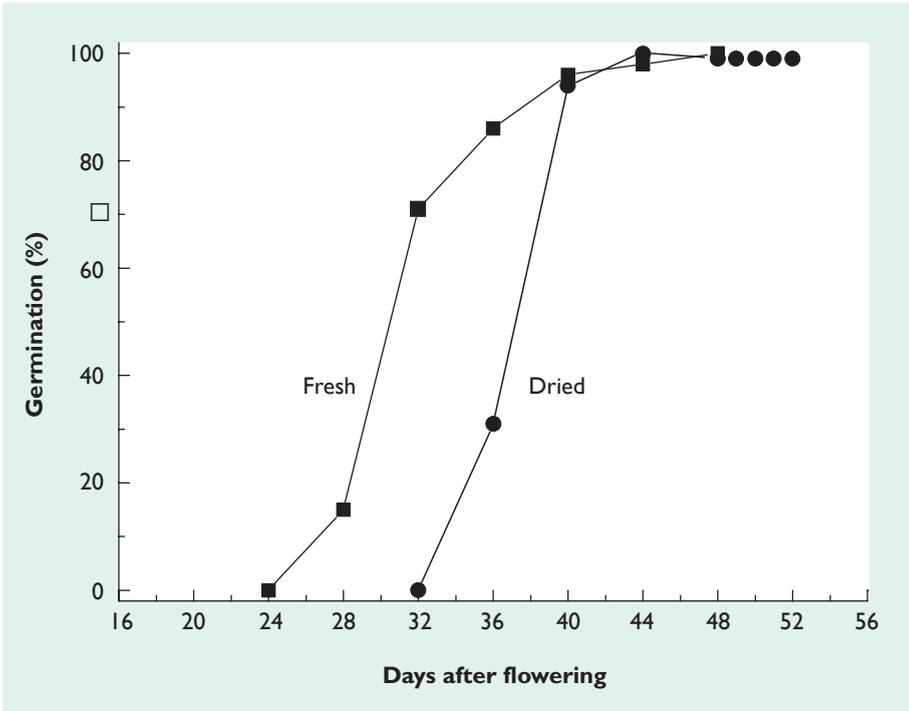


Figure 6.1 The development of germinability and the acquisition of desiccation tolerance in seeds of foxglove (*Digitalis purpurea*) harvested in 1993 (adapted from Hay and Probert, 1995).

related to dormancy rather than desiccation tolerance *per se*. For orthodox species in general, the failsafe position for collectors is to assume that desiccation tolerance is acquired after germinability has been achieved.

Other physiological changes that occur after the acquisition of desiccation tolerance contribute to the overall quality of the seeds. Improvements are observed in seed quality traits such as the ability of the whole population of seeds to germinate and establish in the soil, and the ability to germinate under stress conditions, *i.e.*, conditions where temperature and/or water availability are sub-optimal. Most importantly, from a seed conservation perspective, the longevity of the seeds increases. These increases in seed longevity are clearly apparent when seed storage experiments are carried out on seeds harvested at different stages of development.

Storage experiments involve ageing seeds under controlled conditions (moisture content and temperature) and taking regular samples for germination testing in order to monitor the rate of viability loss. In a population of seeds, seed death follows a normal distribution; a few seeds are

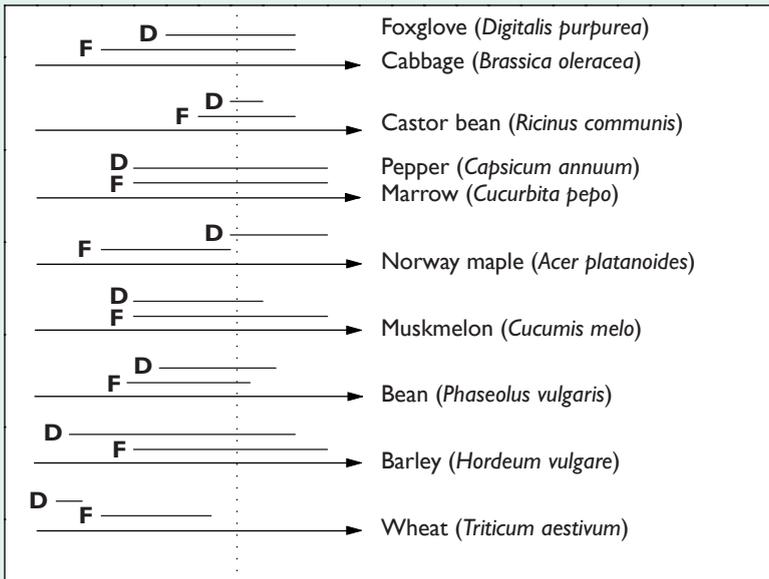


Figure 6.2 Schematic showing the relative timing of the development of germinability (F) and the acquisition of desiccation tolerance (D) during seed development in a range of orthodox species. The vertical line indicates the approximate timing of mass maturity (see later text). Information incorporated from Hay and Probert (1995), Gray *et al.* (1985), Kermode and Bewley (1985), Demir and Ellis (1992a, 1993), Hong and Ellis (1990), Welbaum and Bradford (1989), Sanhewe and Ellis (1996), Pieta Filho and Ellis (1991), and Rasyad *et al.* (1990). Adapted from Hay (1997).

very short-lived, a few are very long-lived, most die around the mean time of death. As a result, when the viability data for a seed storage experiment is plotted against time, a negative sigmoidal survival curve is usually seen. If the viability scale is converted from percentages to probits or normal equivalent deviates (NEDs), a linear relationship is apparent. This linear relationship is described by the intercept on the vertical axis, K_i and a slope parameter, σ [see Pritchard and Dickie (2003) – Chapter 35 for further explanation of K_i and σ]. Alternatively, rather than fitting this linear relationship to transformed survival curves, an index such as time taken for viability to fall to 50% (P_{50}) or the mean time to death (MTD) during storage is used as a measure of seed longevity.

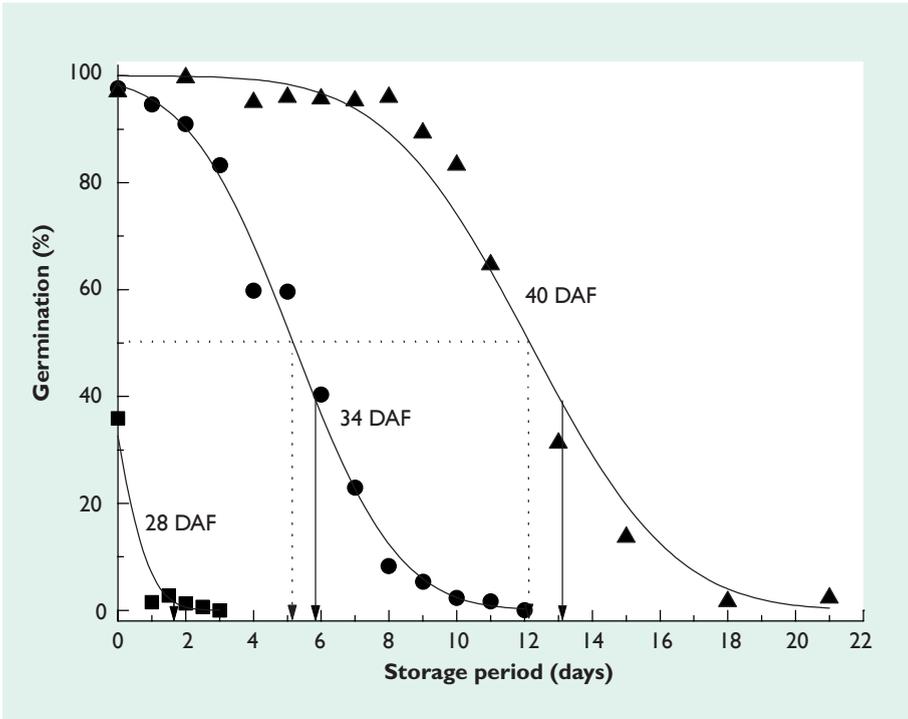


Figure 6.3 Survival curves for seeds of foxglove (*Digitalis purpurea*) harvested at 28, 34, and 40 days after flowering (DAF) in 1995. The dotted arrows show the estimation of the time for viability to fall to 50% (P_{50}) which may be used as an index of seed longevity. Alternatively, since in some cases it is not possible to determine P_{50} , the mean time to death (MTD) may be calculated (solid arrows).

$$MTD = \frac{\sum_{i=1}^n (T_i \times d_i)}{n} \text{ where } \sum_{i=1}^n \text{ indicates the sum (of whatever follows) for every}$$

sample point i , T_i is the time in storage for sample i , d_i is the number of seeds dying between samples $i-1$ and i , and n is the total number of seeds that die during the storage experiment.

In foxglove, there are increases in desiccation tolerance (K_i) and in the slope (σ) of the survival curves during seed development (Figure 6.3). These increases in K_i and σ are reflected in the increases in P_{50} and MTD (Figure 6.4). Interestingly, although the timing of the increases in longevity varied in the different years, the rate at which P_{50} or MTD increased was constant. Most importantly from a seed conservation point of view, seed longevity continues to increase after all the developing seeds have seemingly acquired desiccation tolerance.

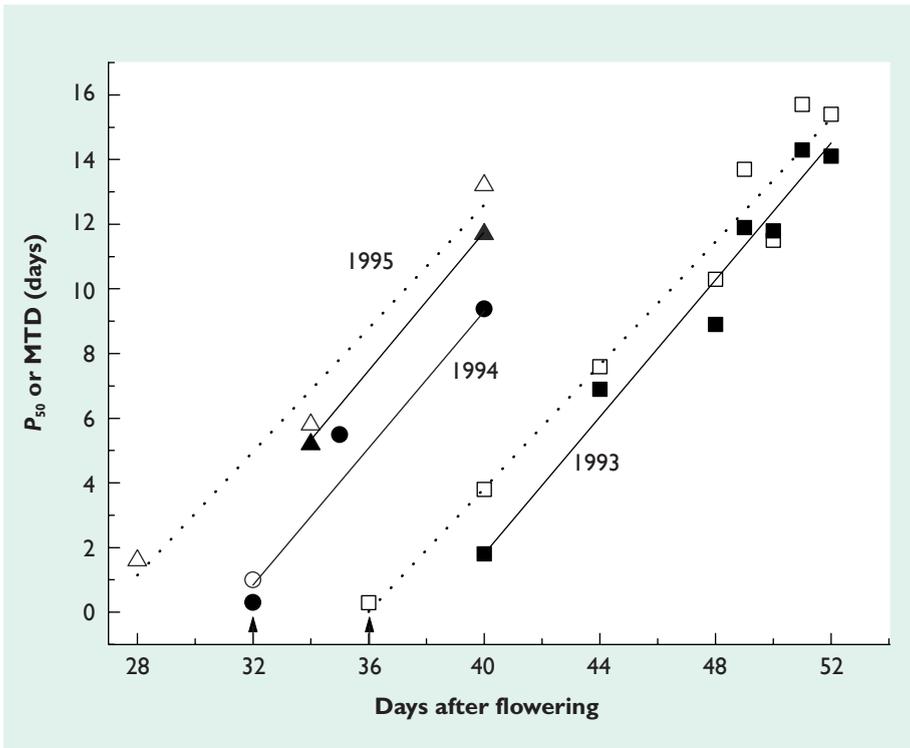


Figure 6.4 Increases in the time for viability to fall to 50% (P_{50}) (solid symbols and lines) or in the mean time to death (MTD) (hollow symbols, dashed lines) for seeds of foxglove (*Digitalis purpurea*) harvested at different times in three years. The arrows indicate the timing of mass maturity in 1993 (at 36 days after flowering) and 1994 (at 32 days after flowering). Adapted from Hay (1997).

Foxglove is an example of a species in which pollination of each flower gives rise to a many-seeded, dry dehiscent fruit. A similar pattern of seed behaviour during seed development is seen in other species resulting from the pollination of a single flower. In developing seeds of rapid cycling Brassica (*Brassica rapa* L. cited as *B. campestris* [*rapa*] L.), K_i increased from -0.5 NED at 24 days after pollination (DAP) to +3.8 NED at 44 DAP (Sinniah *et al.*, 1998). However, by 55 DAP, K_i had dropped to +3.3 NED (Figure 6.5). So there can be reductions in longevity if harvesting is delayed.

In annual crop species, where the plants have been selected and grown to give uniform growth and development, similar patterns of behaviour can be observed in the whole seed crop, when measured against the mean flowering

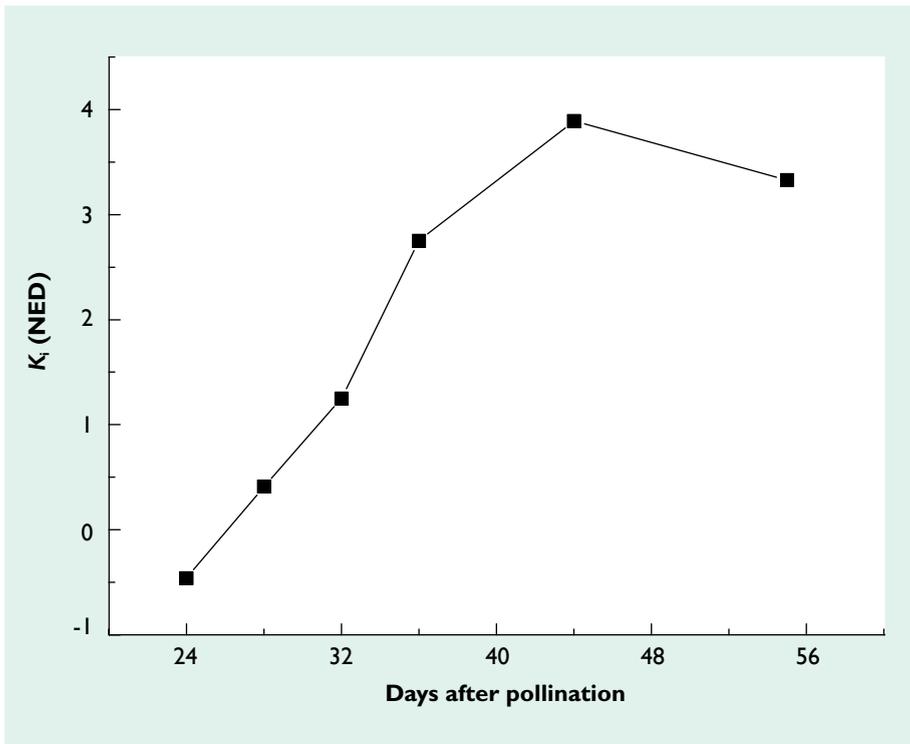


Figure 6.5 Changes in K_i during development (days after pollination) in seeds of *Brassica rapa*. Data extracted from Sinniah et al., 1998.

time. Thus, for example, the K_i of seeds of bean (*Phaseolus vulgaris* L.) continued to increase with developmental time up to 56 d after 50% first flowering (Sanhewe and Ellis, 1996) (Figure 6.6) and there was a positive linear relationship between K_i and time from anthesis in developing seeds of marrow (*Cucurbita pepo* L.) (Demir and Ellis, 1993).

Taken together, these findings suggest that the fruit type does not change the pattern of development, with respect to the acquisition of seed longevity and that seed longevity continues to increase late in seed development. Ideally seeds should be harvested when these three attributes – germinability, desiccation tolerance, and longevity – are optimal. So, how do we know that the seeds have reached that point?

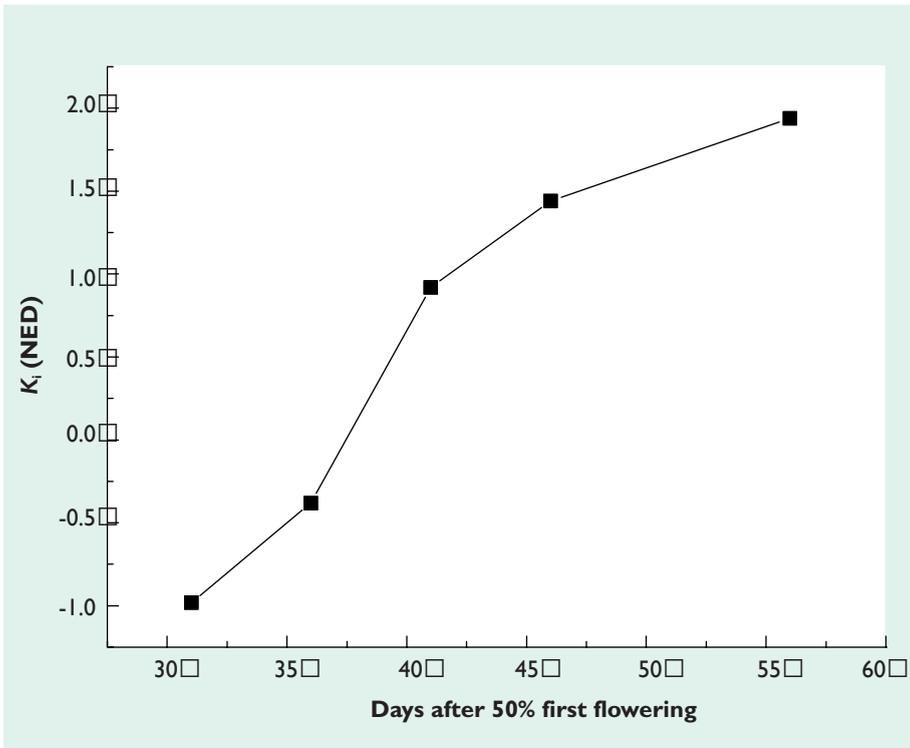


Figure 6.6 Increases in K_i during development (time from 50% first flowering) in seeds of *Phaseolus vulgaris*. Data extracted from Sanhewe and Ellis, 1996.

Knowing When to Collect

1. Experimental Monitoring

One way of trying to identify when seeds are likely to have reached the optimal point, is to track the progress of seed development by monitoring parameters such as fresh or dry mass, or the amount of water in the seeds or their moisture content. These measures are inter-related and are therefore determined simultaneously when a moisture content determination is carried out (Box 6.1).

In the early stages of development immediately following fertilisation, there is a period of rapid cell division and differentiation into the different tissues which make up the seed; the fresh or dry mass of the seed is low and the

Box 6.1 Determining seed moisture content

Seed moisture content is determined by measuring the fresh mass of a sample of seeds, drying the seeds in an oven to drive-off all the water, and then, after allowing the seeds to cool to room temperature usually in a desiccator or over silica gel, measuring the dry mass. The International Seed Testing Association (1985) recommends drying seeds at 103°C for 17±1 h or, in the case of non-oily seeds, at 130°C for 1 h. Other seed research laboratories use a lower oven temperature and dry the seeds for a longer period of time or until there is no further loss of water (no further change in mass). For example the National Seed Storage Laboratory, Colorado, USA routinely dry seeds at 95°C for 5 d (e.g., Walters and Hill, 1998).

Seed 'moisture' or 'water' content may be expressed on a fresh or dry weight basis, as either a proportion or a percentage. Although these two terms are interchangeable, usually seed 'moisture' content is expressed as a percentage of the fresh weight and is

thus calculated as $MC = \frac{f-d}{f} \times 100$ where f and d are the fresh and dry weights, respectively.

Seed 'water' content is most often expressed as a proportion of the dry-weight:

$WC = \frac{f-d}{d}$. This measure has the advantage that the dry-weight is constant and

therefore the denominator will never change for a given sample of seeds. For reference purposes, it is useful to know the formulae for translating the two measures:

$$WC = \frac{MC}{100 - MC} \qquad MC = \frac{100 \times WC}{1 + WC}$$

By counting the number of seeds used in the determination, the mean seed fresh or dry weight may be calculated by dividing the total fresh or dry mass of the sample by the number of seeds in the sample. The mean mass of water in the seeds is calculated by subtracting the mean seed dry weight from the mean seed fresh weight and dividing by the number of seeds in the sample.

moisture content high. This is followed by a maturation phase – a period of dry-mass accumulation as storage reserves (proteins, lipids, and carbohydrates) are laid down; during this period the moisture content of the seeds may start to decline gradually as the dry mass increases, whilst the weight of water remains constant. The attainment of maximum dry weight at 'mass maturity' (see for example, Ellis and Pieta Filho, 1992) is thought to coincide with the formation of an abscission layer that closes the vascular connection between the seed and the parent plant. At this point, the seeds become hygroscopic and, for seeds borne in non-fleshy fruits, there will be a (normally rapid) loss of water. This pattern is seen, for example in developing seeds of foxglove (Figure 6.7).

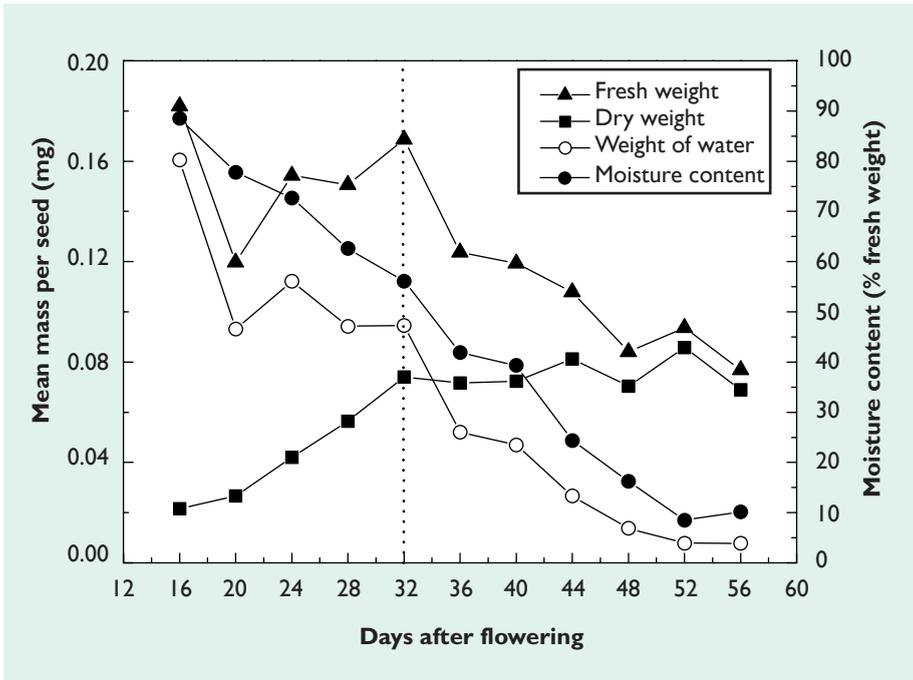


Figure 6.7 Changes in the mean seed dry weight, fresh weight, weight of water, and moisture content (% fresh weight) during seed development in foxglove (*Digitalis purpurea*). The dashed vertical line indicates when mass maturity is attained. Data taken from Hay *et al.* (1997).

In the case of foxglove seeds harvested in 1994, mass maturity was attained at 32 DAF, in the middle of the period when desiccation tolerance was being acquired; total desiccation tolerance in the seed population as a whole was not observed until four days later. Many orthodox species acquire desiccation tolerance around the time when mass maturity is reached. However, the timing of mass maturity relative to the acquisition of germinability and desiccation tolerance varies between species (Figure 6.2). Furthermore, significant increases in longevity occur after mass maturity has been attained. For example, for foxglove seeds harvested in 1993, the mean time to death increased from 0.14 d at 36 DAF when mass maturity was attained, to 15.28 d at 52 DAF (Figure 6.4). Similarly in bean, the large increases in seed longevity through K_i are observed after the attainment of mass maturity at 33 d from 50% first flowering (Figure 6.6) (Sanhewe and Ellis, 1996). Mass maturity can therefore be used as an indicator that seeds are tolerably mature. However, in general, seeds must have progressed beyond mass maturity before they are ideal for seed banking.

Alternatively, seed moisture content or equilibrium relative humidity (eRH) could potentially be used as a more precise indicator of maturity. From the point of mass maturity, with the closure of the vascular connection to the parent plant, water will be lost from the seeds. There will be no further inflow of water from the plant and, provided the relative humidity (RH) of the air around the seed is lower than the eRH of the seeds, the seeds will lose water to the air until they are in equilibrium. The rate at which the water is lost to the air will depend on the RH gradient between the seed and the air around the seed. This may be influenced by the structure of the fruit, the inflorescence, the plant, and/or the surrounding vegetation as well as the ambient humidity of the air. For example, for seeds borne in capsules (such as foxglove), it is probable that there will be a micro-environment around the seed which means the seed eRH remains much higher than the general, ambient air RH and so the seeds remain relatively wet until they are dispersed. If the seeds do become more exposed, their eRH is more likely to be approach ambient RH. Even if there is a relatively moist micro-environment around the seed, seed eRH and moisture content are likely to fluctuate, at least to some extent (which may be very little if there is little air flow through the micro-environments) with fluctuations in ambient RH, until they are dispersed from the parent plant. As long as the eRH of the seeds is still high ($\geq 85\%$), metabolic turnover and repair will maintain the quality of the seeds; when the eRH falls below *c.* 85%, the seeds are thought to become incapable of metabolic synthesis, and ageing will occur at its most rapid rate. Only when the seeds have reached *c.* 60% eRH do they die more slowly than would be expected for fully hydrated seeds.

Measuring seed eRH and the ambient RH will therefore indicate whether the seeds have reached an appropriate stage of maturity for making a collection, and, if a collection is made, how the seeds should be treated afterwards (Probert, 2003 – Chapter 19). The lower the seed eRH, and the closer it is to ambient RH (assuming ambient RH has not been inflated due to early morning dew or rainfall), the more likely the seeds are close to being dispersed (or should have been dispersed already in cases where there is an impediment *e.g.*, a capsule which has not fully opened). This observation that seed eRH is likely to be higher than ambient RH at the time when the seeds are at or near optimal quality is corroborated by a small survey of seeds from wild plants in the UK and in Niger, West Africa. The seeds, collected when they appeared to be on the point of natural dispersal, generally had an eRH that was less than 100% but still typically 25–40% higher than ambient RH (Table 6.1). Even though the vascular connection with the parent plant had been closed and the seeds had started to dry, they had not yet equilibrated to ambient RH. This is further illustrated by a small series of pictures and eRH measurements made of the yellow flag iris (*Iris pseudacorus* L.) in the final stages prior to natural dispersal (Figure 6.8). Even after the pods had fully opened, seed eRH was more than 90% (J. Adams, pers. comm.).

Table 6.1 Survey of ambient relative humidity (RH) and temperature (t°C) and the equilibrium relative humidity (eRH) and moisture content [MC (% fresh weight)] of seeds collected from a range of wild plant species in the UK and in Niger, West Africa

Species	Family	Ambient		Seed	
		RH%	t°C	eRH%	MC
UK survey					
<i>Hyacinthoides non-scripta</i>	Liliaceae	49.4	28.5	74.9	14.4
<i>Ranunculus acris</i>	Ranunculaceae	49.4	28.5	65.7	10.9
<i>Anthriscus</i> sp.	Umbelliferae	49.4	28.5	72.5	14.5
<i>Carex pendula</i>	Cyperaceae	59.5	27.2	97.9	37.6
<i>Stachys sylvatica</i>	Labiatae	59.5	27.2	98.3	25.4
<i>Silene dioica</i>	Caryophyllaceae	35.0	30.9	74.7	16.0
<i>Impatiens glandulifera</i>	Balsaminaceae	44.8	29.1	98.8	35.9
<i>Rumex</i> sp.	Polygonaceae	40.2	32.5	55.2	12.1
<i>Carex sylvatica</i>	Cyperaceae	43.5	29.7	36.4	12.4
<i>Scrophularia auriculata</i>	Scrophulariaceae	43.5	29.7	66.3	14.8
<i>Lathyrus pratensis</i>	Leguminosae	-	-	68.6	14.4
<i>Lotus corniculatus</i>	Leguminosae	-	-	72.3	8.3
<i>Ranunculus repens</i>	Ranunculaceae	-	-	81.9	14.1
<i>Geum urbanum</i>	Rosaceae	-	-	68.4	12.3
<i>Prunella vulgaris</i>	Labiatae	-	-	70.3	11.2
Niger survey					
<i>Triumfetta pentandra</i>	Tiliaceae	53.2	35.2	82.9	13.0
<i>Cassia mimosoides</i>	Leguminosae	61.7	32.8	70.3	13.1
<i>Sida</i> sp.	Malvaceae	61.7	32.8	90.2	12.9
<i>Merremia pinnata</i>	Convolvulaceae	61.7	32.8	95.6	29.3
<i>Sesbania pachycarpa</i>	Leguminosae	73.3	30.7	99.2	50.0
<i>Brachiaria xantholeuca</i>	Poaceae	45.6	38.4	96.2*	26.7
<i>Digitaria gayana</i>	Poaceae	67.5	30.6	97.2*	32.1
<i>Ceratotheca sesamoides</i>	Pedaliaceae	67.5	30.6	85.1	12.7
<i>Fimbristylis</i> sp.	Cyperaceae	67.5	30.6	83.3	13.1
<i>Cleome gynandra</i>	Capparaceae	48.0	34.3	94.9	19.5
<i>Jacquemontia tamnifolia</i>	Convolvulaceae	45.0	35.0	38.7	6.8
<i>Amaranthus graecizans</i>	Amaranthaceae	35.6	36.5	70.9	12.9
<i>Monechma ciliatum</i>	Acanthaceae	30.0	39.0	37.9	6.0
<i>Borreria scabra</i>	Rubiaceae	34.4	36.8	39.7	8.8
<i>Mollugo nudicaulis</i>	Molluginaceae	40.6	35.0	81.6	17.6

*Indicates that fruit rather than seed eRH was measured.

**Closed pod**

Pod eRH: 100%
Seed eRH: 100%
Seed MC: 46%

**Half-open pod**

Pod eRH: 100%
Seed eRH: 99%
Seed MC: 45%

**Open pod**

Pod eRH: 88%
Seed eRH: 93%
Seed MC: 40%

Figure 6.8

Seed pods of *Iris pseudacorus*, just prior to seed dispersal in October 2002. Measurements of pod and seed equilibrium relative humidity (eRH) carried out at 20°C; ambient conditions were 70% RH and 14°C at the time of collection. Photos taken by J.B. Dickie; measurements made by J. Adams.

N.B. Since the eRH measurements were made at a slightly higher temperature than ambient temperature, the values may be slightly higher than they would have been *in situ*.

From a seed conservation point of view, a collection should not be made until, ideally seed eRH first approaches 85–90% (Box 6.2). Delaying the harvest beyond this point may lead to losses either due to dispersal and/or due to impairment of seed quality (including seed longevity) as the moisture content of the seeds fluctuates with ambient RH. Of course, ageing in the field will depend on local climatic conditions and is more of an issue for domesticated plants that have been selected and bred to retain their seeds until mass harvesting.

For wild plant species, making a measurement of seed eRH in the field can help to determine whether or not the seed population is likely to be close to that point when seed quality is likely to be optimal. If any seeds are left on the plant whilst most other have been dispersed (for example, lodged in a fruit which has not been blown about as much as other fruits), it might be advisable not to collect those seeds – they may have started to age and therefore be of poor quality.

Of course, equilibration to ambient RH after mass maturity only applies for dry fruits. In fleshy fruits such as tomato (*Lycopersicon esculentum* Mill.) (Berry and Bewley, 1991; Demir and Ellis, 1992b) and muskmelon (*Cucumis melo* L.) (Welbaum and Bradford, 1989), the reduction in moisture content after mass maturity is relatively small. For fleshy fruits, the dry mass of the seeds is a more useful measure to follow through development than the moisture content or mass of water in the seeds.

2. Field Markers of Seed Maturity

Of course the equipment to check seed or fruit eRH may not be available in the field, although there are some small RH meters that can be easily carried and used (Probert *et al.*, 2003 – Chapter 20). Also, it is highly unlikely that seed weights or moisture contents can be monitored, except on a very few occasions, for research purposes. Thus, seed collectors will often have to use other markers of seed maturity, perhaps using a single marker such as fruit colour, or a combination of a number of field markers.

Naturally, the potential markers that might be used will differ from species to species. At a more general level, potential markers might vary according to fruit type *i.e.*, fleshy *vs.* dry fruits. This separation has already been alluded to earlier in this chapter, in the description of seed development. A collector should be knowledgeable about dealing with either fruit type. To illustrate this requirement, fleshy and dry fruits occur with a 52% and 47% frequency amongst the 1,340 species of indigenous angiosperm trees of Southern Africa, (Knight and Siegfried, 1983).

2.1. Changes in fruit colour

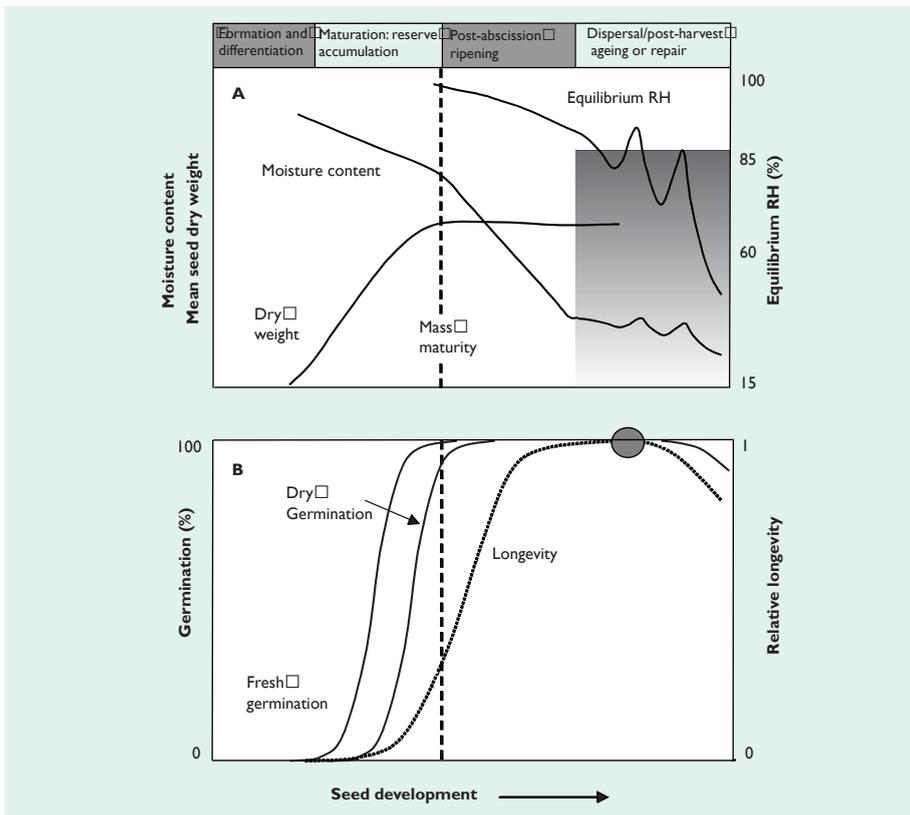
In the case of fleshy fruits, fruit size is a risky diagnostic of seed development. Amongst the fleshy drupes of the genus *Prunus* L. in the *Rosaceae*, the fruit can

Box 6.2 Schematic of orthodox seed development

A) Following fertilisation, there is an increase in the dry weight of the seeds up to the point of mass maturity after which the mean seed dry weight remains constant. At mass maturity there is an increase in the rate at which seed moisture content decreases and the equilibrium relative humidity (eRH) of the seeds starts to fall. Seed eRH is likely to remain high ($\geq 85\%$) until the seeds become exposed to ambient air at which point seed eRH and moisture content will fluctuate with changes in ambient RH.

B) The relative timing of changes in germinability (fresh germination), desiccation tolerance (dry germination), and longevity during seed development [this pattern can vary between different orthodox species (Figure 6.2)]. The circle indicates the point when we should aim to collect seeds as seed quality traits are likely to be optimal. This is when the seeds are first equilibrating to the RH of the surrounding air. With further exposure of the seeds and fluctuations in seed eRH and moisture content with ambient conditions, there may be ageing. If ambient RH is low and seed eRH less than c. 30%, ageing is likely to be at a low rate (light shading). If seed eRH is between 30 and 85% there will be a significant level of ageing (increasing shading; see Probert, 2003 – Chapter 19; Pritchard and Dickie, 2003 – Chapter 35) and seed quality, including longevity will fall. If seed eRH goes above 85%, repair processes may occur instead of ageing.

Adapted from Probert and Hay (2000).



achieve full size before the embryo has begun to develop (Hawker and Buttrose, 1980; Finch-Savage *et al.*, 2002). In complete contrast, in other fruits such as the berries of *Opuntia ficus-indica* (L.) Mill., seeds have achieved a maximum dry weight whilst the fruits continue to increase in size (Barbera *et al.*, 1992). Change in fruit colour may therefore be a more reliable indicator of seed maturity.

In tomato, although the change of fruit colour from pink to red does not correlate well with the development of seed dry weight, the red colour identified a 10 d period either side of peak seed longevity during which seed quality remained within 85% of the optimal value achieved. Earlier, pink and later, over-ripened fruits yielded seeds of poorer quality (less than 80% of the maximal longevity value achieved) (Demir and Samit, 2001). In another solanaceous berry, *Capsicum annuum* L., maximum seed dry weight was achieved 50 d after anthesis when fruit colour was changing from green to green with red stripes. Greater seed vigour was reported to occur 55 to 60 d after anthesis, at the time when the fruit colour had changed further to red with green flecks and then intense red (Mantovani *et al.*, 1980). This would fit well with the observations of Demir and Ellis, (1992a) that maximum longevity was achieved some 10 to 12 d after seed maximum dry weight. In *Solanum dulcamara* L. seeds harvested at the later red stage of fruit maturity showed a large (c. 50%) increase in longevity compared with the earlier orange stage. Seeds from both stages were of similar weight suggesting mass maturity had been reached (Hay, 1997).

Similarly, 'ripe fruits' of *Cucurbita moschata* (Duchesne) with their colour changing from green to yellow-brown and with dry peduncles, produce seeds of significantly higher quality than either "unripe", green fruits harvested 23 d earlier when the peduncles are still turgid, or over-ripe, yellow-brown fruits harvested 38 d later (Goldbach, 1978). The quality of the seed within this relatively broad window of ripeness was not further discriminated. In the drupes of *Rhus aromatica* Ait. and *R. glabra* L., colour changes appear to be associated with the embryo achieving full size and developing the ability to germinate (Li *et al.*, 1999).

These reports suggest colour changes in the maternal tissues of fleshy fruits can be used as indicators that seed has achieved maximum dry weight and hence germinability, but not necessarily optimal seed storability. They are at best a coarse tool, which can be used to make preliminary assessments.

Fruit size is an equally unreliable diagnostic of seed development in dry fruits (Muthoka *et al.*, 2003 – Chapter 7). Fortunately colour changes still occur as the fruits mature, usually, due to loss of chlorophyll from the maternal tissues, from green to yellow or brown. In *Brassica oleracea* L., silique colour changes from green to yellow. However, this change in fruit colour varies in relation to the achievement of maximum dry weight between years. In one year, 50% of siliques had turned yellow; the following year 100% of the siliques were yellow at the same stage. Seed moisture contents at this stage were 47% and 37% respectively (Gray *et al.*, 1985).

The legumes (pods) of many *Fabaceae* similarly change colour. In *Millettia leucantha* Vatke the legume changes from green through yellow green, sulphur yellow to brown-grey. The pod colour at mass maturity varied between 2 years observations. In the second year, seed viability and longevity was also monitored. The pod colour remained the same between mass maturity and a harvest taken 26 days later. In this period, seed longevity increased by 85% (Muthoka *et al.*, 2003 – Chapter 7). In *Lotus pedunculatus* Cav., the legumes change from dark purple on top, with green underneath, through purple to light brown on top and yellowish green underneath to light brown all over and finally dark brown all over. Maximum seed weight was achieved at the purple to light brown on top, yellowish green underneath stage, when seed moisture contents were about 60%. The light brown all over the pod was achieved when the seeds were at 20% moisture content or lower (Hare and Lucas, 1984). In *Glycine max* (L.) Merr., pods also pass through different colour phases as they mature: from green to green-yellow to yellow to yellow-brown to brown. However, when the seeds of a field grown population achieve maximum dry weight, only yellow, yellow-brown, or brown pods could be found. At this time, the seed moisture content is close to 60% (TeKrony *et al.*, 1979).

In the samaras of *Ailanthus excelsa* Roxb. the pericarp again passes through a sequence of colours from green to green-yellow to yellow to yellow-brown to brown to dark brown. At the time the population of fruits achieved maximum dry weight, the pericarp was brown and the fruit moisture content had fallen to 18% (Ramakrishnan *et al.*, 1990). In *Atriplex cordobensis* Gand. and Stuck. dispersal units start to change colour from green to yellow *c.* 10 d before maximum dry weight is achieved and the seed moisture content is 30%. When green and yellow dispersal units are found with an equal frequency, maximum dry weight has been achieved and the seed moisture content (16% fresh weight) is in equilibrium with conditions inside the fruit (Aiazzi *et al.*, 1998). In both *Ceratotheca sesamoides* (Endl.) and *Alysicarpus ovalifolius* (Schumach. and Thonn.) J. Léonard seeds from fruits which had changed colour from green to brown showed increased longevity of 300% and 500% respectively. For both species, significant increases in seed weight were also recorded for the later harvests. This suggests seeds from the earlier harvest had not yet reached mass maturity (Hay, 1997).

As for fleshy fruits, fruit colour in dry fruits can be used as a coarse tool to make preliminary assessments. However, it appears unlikely that it can be used to indicate when seeds have achieved maximal longevity.

2.2. Changes in seed coat colour

As with fruits, changes in seed coat colour have also been correlated with seed maturation characteristics. However, seed coat colour changes associated with maturation appear to occur less frequently than in fruits. Inside the fleshy *Carica papaya* L. berry, seed coat colour changes from white to brown to black during development. The final colour change occurs as the seed population achieves its maximal dry weight and seed moisture content is *c.* 80% fresh weight (Zhou and Paull, 2001).

In the dry fruits of *Milletia leucantha*, seed colour changes from leaf green, through herbage-green and yellow-green to milk-coffee. In the two years of study, mass maturity was achieved at herbage green- and milk-coffee in different years. In the 1998 season when seed longevity was also studied, seed from the last two harvests (26 days apart) had the same colour. However, seeds from the later harvest showed significant increases in initial viability and longevity (85% increase in mean longevity; Muthoka *et al.*, 2003 – Chapter 7). In a number of cultivars of *Glycine max*, seeds pass from green, through green-yellow and yellow-green, until the seed becomes completely yellow. At this final colour stage, radiocarbon studies indicate this is the point that the movement of photosynthate into the seed has ceased. Similarly, respiration studies showed that the rapid reduction in respiration rate that occurred at 60% seed moisture content was coincident with the seed coat colour change from yellow-green to yellow (TeKrony *et al.*, 1979). In another legume, *Sesbania bispinosa* (Jacq.) W. Wight (cited as *S. aculeata* Poir.) the maximum dry weight of the seed population can be calculated to occur when the seed coat colour changes from yellow to dark-green and the moisture content is around 40%. However, maximum seed vigour (quality) occurs when a further colour change, from dark green to olive green has taken place; seed moisture content is *c.* 14% at this time. The final change from olive green to greenish brown occurs shortly afterwards at much the same moisture content (Selvaraj and Ramaswamy, 1984). In two other legumes, *Trifolium pratense* (L.) and *T. repens* (L.), seed coat colour started to change when the seed population was 80% of its maximum dry weight and the moisture content was above 60% (Hyde *et al.*, 1959).

In the cultivated cabbage (*Brassica oleracea*), seed coat colour changes do not appear to be correlated with the achievement of maximum dry weight of the seed population. Indeed, the same seed coat colour changes occur at different moisture contents in different years (Gray *et al.*, 1985). In soft red winter wheats, *Triticum aestivum* L., the red colouring of the seed before the development of darkening of the pigment strand was approximately coincident with dry matter accumulation in the seed (Housley *et al.*, 1982). In crested dogstail grass (*Cynosurus cristatus* L.) seed colour was no indicator of quality (Clark, 1982).

Whilst for many species, coat colour changes as the seed develops, the available evidence suggests that these are at best coincident with the achievement of maximum dry weight, but not with maximum longevity.

2.3. Changes in the hardness and consistency of seed reserves

In a small number of dry-fruited species, the relationship between the consistency/hardness of the seed reserves and development have been investigated. This approach mirrors the traditional agricultural practice of squeezing seeds between teeth to determine whether or not the seeds should be harvested. In *Brassica oleracea*, the same change in consistency, from the 'hard cheese' to the 'flint hard' stage, was achieved at different stages of development in different years. In one year it coincided with maximum seed

dry weight of the population, when the moisture content was around 40% fresh weight. In the next, there was no correlation with maximum population seed dry weight and the moisture content had fallen to 20% (Gray *et al.*, 1985). In *Lolium multiflorum* Lam. a similar picture of variation in the timing of the endosperm change from the 'doughy' to the 'solid' condition between years and between diploid and tetraploid cultivars was found (Komatsu *et al.*, 1979).

An overall summary of the value of fruit and seed changes as field diagnostics of optimum seed longevity is a balance of positives and negatives. On the positive side, they indicate increasing seed maturity and with it increased seed longevity, reducing the likelihood of harvesting seeds which may not have achieved the maximum dry weight, germinability, and/or desiccation tolerance. On the negative side, the colour changes are often subjective. If the full range of states is not represented in the population at the time of collection, the collectors may have difficulty in interpreting the significance of what they find. The colour changes themselves are not necessarily coincident with changes in desiccation tolerance or longevity. Hence, they cannot on their own, confirm that seed populations have achieved these states.

2.4. Fruit or seed dispersal

Viewed from the perspective of seed collecting logistics, seed dispersal appears to be a good practical marker of seed maturity. It is the last natural stage in seed development before the seed becomes detached from an identifiable parent. However, there is little evidence in the literature which illuminates the relationship between fruit/seed dehiscence or dispersal and seed longevity; any guidance that can be offered is provisional. The literature does provide however, some further evidence of the importance of RH (both air and seed eRH) in relation to the timing of fruit or seed dispersal.

In considering other markers of seed maturity, the findings were analysed against the background of fruit type (fleshy *vs.* dry). When considering dispersal, this approach appears less valuable; fruits of the same type can be dispersed by a variety of mechanisms. Care is also required in the interpretation of observations at the population/crop level with regard to the behaviour of a single fruit in that population. This is often easier for the collector who has the visual evidence in front of them in the field, than for the reader of an article.

For example, in a number of dry-fruited agricultural crops, seed dispersal has not been eliminated through human selection. In such crops, seed dispersal is often reported to start before the maximum seed weight of the crop has been achieved. It is likely that the fruit/seeds being dispersed are those resulting from the earliest fertilised ovules, are at maximum dry weight and have undergone maturation drying. The literature provides no evidence to support the alternative interpretation that within a population, seeds are naturally shed over a range of maturities.

The first signs of dispersal in the population should therefore be interpreted as showing that some, but not all of the remaining fruits will be close to dispersal and appropriate for collection. Other fruits will still be developing and be less suitable for collection for long-term conservation. In *Lotus corniculatus* L., 33% and 38% of umbels contained immature pods when seed dispersal (shattering) began in successive years (McGraw and Beuselinck, 1983). In *Allium tuberosum* Rottler ex Spreng., some capsules from both the inner and outer florets of the umbel begin to split when the seed crop moisture content remains around 50% (*c.* 99% eRH) and maximum seed weight has not been achieved (Reddy *et al.*, 1998).

In foxglove (*Digitalis purpurea*), the relationship between seed longevity, capsule dehiscence and seed dispersal has been closely observed (Hay and Probert, 1995). Capsule dehiscence began 48 DAF when the seed eRH was 92.3%. Incipient dispersal of seeds from the capsule was reported to occur at 52 DAF when seed eRH had fallen to 84.2%. Seed longevity increased by *c.* 50% over this 4 d period. In *Atriplex cordobensis*, dehiscence began when *c.* 40% of dispersal units had changed colour from green to yellow. At this time, the seed crop moisture content has reached equilibrium with conditions inside the dispersal unit (Aiazzi *et al.*, 1998), with an estimated moisture content of 16% and an estimated eRH of *c.* 80%.

Fruit dehiscence in *Lotus corniculatus* is reported to be controlled by the RH of the surrounding air; dehiscence only took place at less than 40% RH (Metcalf *et al.*, 1957). The increase in pod temperature in full sunlight was considered sufficient to lower the RH of the surrounding air and the moisture content of the pod to levels that brought about dehiscence. Interestingly, within the genus *Lotus*, there are both dehiscent and indehiscent species. A suggestion is also reported that one species (*L. micranthus* Benth.) produces both dehiscent and indehiscent legumes (Grant, 1996). Combining these two sets of observations, conjecture about the extent to which dehiscence is genetically or environmentally controlled becomes of interest.

A change in dispersal behaviour, perhaps due to a change in climate, has also been observed in *Eschscholzia californica* Cham. Despite being reported in the botanical literature as having explosively-dispersed capsules, when it was introduced as a crop in New Zealand, the dry fruit was found to be indehiscent. Instead the intact capsules are shed, at a time when the seeds had dried to *c.* 8% moisture content (*c.* 55% eRH) and appeared to be in equilibrium with the surrounding air (Reddy *et al.*, 1994).

In drylands, a significant number of wild species have evolved to delay the dispersal of some or all of the seed population, a mechanism termed “serotiny”. Release of seeds from these aerial seed banks is often coupled with environmental factors. Serotiny can be found in annuals, shrubs (Gunster, 1994) and trees (Lamont *et al.*, 1991). Varying degrees of serotiny (*i.e.*, the

proportion of seeds being shed with time) have been reported. Some species shed all of their seeds over two years or so, others over 12 or so years. Seed viability declines during the period that the seeds are held on the plant (Lamont and Enright, 2000). Whilst seeds collected during the earliest part of this serotinous period would have some value for seed conservation, seeds collected from the current year's crop will always be more valuable for long-term storage. Significant ageing-related damage will have already accumulated in older seeds, making them shorter lived in subsequent storage.

Difficulties exist in the wider interpretation of data from the stone fruit crops developed from fleshy simple drupes of the genus *Prunus* L. Here selection and breeding by humans has produced early maturing varieties in which the fleshy maternal pericarp develops at a faster rate than the seed. Thus, when the pericarp of the fruit softens and is ready for human consumption, the embryo of the seed is still immature. As the softening of the pericarp also makes the fruits attractive to other animals, the possibility exists that dispersal could take place before the seeds have completed their development. Whether this is restricted to the crop situation is unclear. No evidence is available for seed dispersal in the wild types of these species within their natural range and involving their co-evolved dispersal agents.

Fruit softening prior to dispersal is known for a variety of cactus species and occurs shortly before birds begin to remove the fruit and disperse the seed (Abendroth, 1969; Horobin, 1981). However, no observations were made on the developmental stage of the seeds at dispersal. In the musk melon, a cultivated form of *Cucumis melo* from the *Reticulatus* group, selection has taken place for the delayed softening of the fleshy pepo fruit to improve marketability. Losses in seed viability occur inside the fruit in the later stages of crop growth before harvesting. In the Armenian cucumber from the *Flexuosus* group of the same species (*C. melo*) such selection has not taken place. Here, seeds are dispersed following the natural breakdown of the fruit. No loss of viability is observed (Welbaum, 1993).

Within the fleshy berry of tomato, delayed harvesting of the commercial crop also results in losses in seed viability (Demir and Samit, 2001). Delayed harvest has also been shown to lead to losses of seed viability in dry-fruited/dry-seeded crops where dispersal has been eliminated by selection or breeding (*e.g.*, Coste *et al.*, 2001; Sinniah *et al.*, 1998). Extrapolation of the behaviour of crop plants across to wild species needs care. Particular attention should be paid to changes in fruit development or seed dispersal mechanisms that occurred in their transformation into crops.

Variable Maturity Within a Collection

1. Sources of Variation

The best collections for long term conservation in seed banks will be those where the seeds are of uniform maturity and at maximal longevity. Amongst annual, highly bred crops this can be achieved by good husbandry. In perennial crops or wild species, this will or will not be achieved depending on the variation in seed maturity that is selected by the collectors as they make their collections. This could be due to variation within fruits, within inflorescences, between inflorescences, and/or between individual plants in the same population. A short review follows of the spread of flowering, fertilisation and seed maturation periods in naturally occurring populations. Its purpose is to quantify this variation and so assist collectors by illustrating the problems that may face them in the field. Six levels of variation within a single species will be considered:

1. Between populations
2. Between individuals of the same population
3. Between inflorescences or solitary flowers on an individual plant
4. Between flowers within an individual inflorescence
5. Between individual ovules within a fruit
6. Between different years.

Variations at levels (1), (2), (3) and (4) will be those that confront collectors on all occasions. Variation at level (1) will need to be accommodated within any planning of an extended collecting trip. The same will be true for level (6), with backup plans put in place for early and late seasons. Collectors will be unable to do anything about the variation at level (5). As will be shown, this variation appears small in comparison with that found at other levels. It is included for completeness and as a possible way to check that the collecting strategy adopted [based on interpretation of levels (2), (3) and (4)] is working out.

The duration of seed development from flowering to mass maturity also varies greatly between species. In annual angiosperm species, it can take as little as 10 d; in long-lived perennials it can take more than 200 d. In gymnosperms, seed development can extend over more than 2 years. Across the 50 species of angiosperms for which this parameter has been defined, there appears to be a bimodal distribution in terms of seed developmental periods with peaks at 30 and 100 d. Such wide variation in developmental periods may be a confounding factor when trying to collect seeds of uniform maturity. For the most rapidly maturing species, the seed collecting process will cover a significant proportion of the development period. Precision in collecting will, therefore, be paramount.

1.1. Variation between populations

Since flowering is partly controlled by climatic conditions such as temperature and photoperiod, intuitively we might expect there to be at least some variation between populations in the time at which seeds are at peak maturity, depending on how widely the species is distributed and how much variation there is across its distribution in terms of climatic conditions. For example, in the stunted cruciferous shrub *Hormathophylla spinosa* (L.) Kupfer, which may be found in abundance in the high mountains of Mediterranean Spain, peak flowering times recorded over four years varied considerably between populations at different altitudes. The flowering peaks of the populations growing at 2,250 m and 3,130 m were, respectively, 15 and 38 d later than the flowering peak for the population growing at 2,130m. In distance, they were only separated by eight km (Gomez, 1993). Clearly the variation in peak flowering between populations is likely to be carried through to variation in timing of peak seed maturity.

Further examples of species in which variation in flowering time between populations has been recorded include the short-lived dryland grass *Sporobolus flexuosus* (Thurb. ex Vasey) Rydb. Two populations, 17 km apart in New Mexico, USA were found to have differences in peak flowering dates of 20, 18, and 7 d over three years of study (Gibbens, 1991). The duration of flowering at the sites also differed, ranging from 34 to 73 d. Similarly, in the mesic herb *Arum maculatum* L., a difference of 7 d in the maximum flowering time was recorded between two populations occurring within a short distance of each other on the Exeter University campus, UK (Ollerton and Diaz, 1999).

There is also evidence that there may be some genetic component determining peak flowering time in some species. Seeds of the mesic dicot herb, *Silene nutans* L. were collected from eleven populations over a relative short range (30–40 km) in Denmark and grown in an experimental plot in order to remove the effects of environment. The extreme differences in onset of flowering observed in the field were maintained in the controlled experiment. Nine of the populations started within overlapping periods, but two flowered much later; one of them, at least one month later than the earlier populations (Hauser and Weidema, 2000).

If, like the Millennium Seed Bank Project, the initial aim is to conserve genetic diversity at a species level rather than to sample from multiple populations, this source of variation in maturity may be less of an issue to seed collectors than other sources. When multiple populations are sampled, variation in the timing of peak maturity may be advantageous, enabling the collection of seeds from a number of populations in one year. Whichever approach is being taken, it is unlikely that the populations will be mixed and so this source of variation in maturity is less relevant than the other sources of variation which are likely to result in a mixed maturity collection.

1.2. Variation in flowering times between individuals in the same population

This source of variation is another that we would expect to encounter when collecting seeds from wild plant species. However the degree of variation across a population will obviously vary from species to species (and of course from population to population). In *Hormathophylla spinosa*, more than 70% of the plants studied over a four year period had a flowering synchrony value of 0.75 or more (Gomez, 1993). In contrast, in a population of the perennial herb, *Lotus corniculatus* growing in Wytham Woods, UK, the dates of peak flowering of individual plants varied by 70 d. Furthermore, the duration of flowering times of individual plants varied between 1–9 d and 100–109 d (Ollerton and Lack, 1998).

McIntosh (2002) studied the flowering phenologies of two sister species of perennial xerophytes, *Ferocactus cylindraceus* (Engelm.) Orcutt and *F. wislizeni* (Engelm.) Britton and Rose, looking at all the reproducing plants occurring within 1.5 (*F. cylindraceus*) and 3.0 (*F. wislizeni*) hectare plots over two consecutive years. Both plots were within 45 km of Tucson, Arizona, USA. The flowering of the *F. cylindraceus* population was bimodal, moderately well synchronised and had an average duration of 151 d. Individual plants were in flower for between 1% and 94% of this time. The flowering of the *F. wislizeni* population was unimodal, well synchronised and had an average duration over the same two years of 97.5 d. Individual plants were in flower for 5% and 95% of the period.

Depending on the size of the population and how abundantly it fruits, this source of variation may be overcome by aiming to collect fruits of uniform maturity – provided there is some marker(s) available and sufficient time and/or hands to carry out selective collecting.

1.3. Variation in flowering time between inflorescences or solitary flowers on an individual plant

Data on this aspect of reproductive phenology are uncommon. In teak (*Tectona grandis* L.f.), each individual tree is in flower for between 5 and 10 weeks (Palupi and Owens, 1997). The flowering periods are longer in *Ferocactus*. In the bimodal *F. cylindraceus*, the majority of flowers on each plant are borne for 7 of the 20 weeks in which flowers are produced; in the unimodal *F. wislizeni*, the majority of flowers on each plant are borne in the first 7 of the 13 weeks in which flowers are produced (McIntosh, 2002). Within the *Poaceae*, individual plants are constructed from repeated modular reproductive units (tillers) that do not mature and reproduce at the same time. In an experimental glasshouse study carried out in France using the annual cultivated *Pennisetum typhoides* (Burm.f.) Stapf & C.E. Hubb. of North Africa, individual plants were, on average, constructed from 5.3 tillers. The male phase of flowering, which started 3 d after the female phase, lasted for 49 d and seed maturity occurred 5 weeks after male flowering. Thus, the same range of variation in seed maturities (49 d) could be expected in the same individual plant (Sandmeier and Dajoz, 2000). However, in another member of the *Poaceae*, *Echinochloa oryzicola* (Vasinger) Vasinger, the

behaviour of different tillers on the same plant was less clear. Here the spread of recorded median ripening dates between each tiller was less than the spread of flowering dates (Yoshioka *et al.*, 1985).

1.4. Variation in flowering time within a single inflorescence

Whilst it may be possible for many species to select fruits of a certain maturity from different inflorescences, selecting fruits from within a single inflorescence is likely to be more difficult. This source of variable maturity is likely to be particularly prevalent in certain plant families; for example, the inflorescences of members of the *Poaceae*, *Cruciferae*, *Apiaceae*, or *Compositae*. For example, within the central inflorescence (raceme) of red cabbage (*Brassica oleracea* Group Capitata), a 30 d difference has been estimated between the oldest and youngest seeds (Still and Bradford, 1998). In lettuce (*Lactuca sativa* L.), flowering was spread over 70 d in the uppermost branch (Soffer and Smith, 1974).

In *Perilla frutescens* (L.) Britton, the indeterminate inflorescences are under photoperiodic control – flowering in response to short days – and differ between the northern and southern extremes of the species distribution. Southern plants produce fewer, more sparsely branched inflorescences than northern plants but have more flowers per inflorescence. Consequently, a similar number of seeds are produced by southern and northern plants, but possibly with different ranges of maturity (Preston, 1999).

Variability of inflorescence architecture has been observed within and between patches of *Euphorbia nicaeensis* All. along a 4 km transect in the Hérault, France and large differences in flowering times were reported between the three different architectural types (Al-Samman *et al.*, 2001). The inflorescence of *Echinochloa crus-galli* (L.) P. Beauv. becomes more branched and complex as its length increases (Norris, 1992). Hence the variation in flowering time could also be expected to increase. In teak, blooming of the 800 to 2,800 flowers within a single inflorescence (panicle) occurred between 14 and 42 d (Palupi and Owens, 1997).

Are variations in flowering phenology necessarily sustained through subsequent seed development? The answer depends upon the species in question. In lettuce, whilst flowering is spread over 70 d, more than 90% of the seeds produced originate from the flowers opening over the first 15 d of the flowering period. In both *Pennisetum typhoides* and *Echinochloa oryzicola*, earlier flowering tillers contributed greater seed numbers to the final population. In other species, such as *Salvia verbenaca* L., neither fruit set nor seed/ovule ratios varied significantly across inflorescences (Navarro, 1998). Thus the fruiting pattern will follow the flowering pattern. The same is true for cabbage; the difference in age is reflected all the way through development, so that even at 61 d after flowering, the seeds from the youngest 20% of siliques remained at high moisture content (*c.* 30%; *c.* 99% eRH). At the same time, those from the older 80% of siliques had already dried to around 10% moisture content (*c.* 60% eRH) (Still and Bradford, 1998).

In *E. oryzicola*, the ‘ripening’ (change in colour of the paleas and lemmas) of caryopses varies in a way that is consistent with the variation of flowering patterns. The outcome of this is that, at best, 8% of the seeds within a single plant ripen on the same day. At the time this maximum is reached, 55% of the annual seed harvest will be yet to ripen, half in the next 6 d and the remainder over the following 11 d (Yoshioka *et al.*, 1985).

1.5. Variation between individual ovules within a fruit

When the fertilisation of one flower leads to many seeds, the evidence suggests that the fertilisation of ovules is neither simultaneous nor random. Because of the ease of study this is reported most frequently in the *Fabaceae* (e.g., Rocha and Stephenson, 1995; Hossaert and Valero, 1998). The probabilities of fertilisation, subsequent abortion and maturation of the seed varies with the position of the ovule in the legume in a regularly repeated way. More recently, variation of this kind has been found to be reflected in the seed performance of *Cucumis sativus* L. when harvested sequentially from the time of pollination through to the developmental stage at which seeds would usually be harvested in normal seed production practice. However, after a further 5 weeks of in-fruit maturation, the differences in seeds harvested from the styler and peduncular fractions of the fruit had narrowed, suggesting that prolonged maturation until all seeds were of ‘equal biological rather than chronological maturity’ was beneficial (Jing *et al.*, 2000).

1.6. Variation in seed development between years

The variation in seed development between seasons is well documented. Given adequate water availability, seed development in annual herbaceous crops appears to be highly dependent on temperature. In *Pisum sativum* L. and *Glycine max*, the differences observed in timing of the seed development patterns at different nodes in the same year and between years, could be largely overcome by considering the cumulative day degrees after sowing, rather than calendar data (Dumoulin *et al.*, 1994; Munier-Jolain *et al.*, 1998). This has also been suggested for Alpine populations of *Gentianella germanica* (Willd.) Boerner ssp. *germanica* (Wagner and Mitterhofer, 1998); a number of grasses (Berdahl and Frank, 1998); and the buttercup squash (Harvey *et al.*, 1997). Colder seasons lead to longer developmental periods, whilst warmer seasons lead to shorter periods. The larger differences between the flowering phenology of *Silene nutans* recorded in the wild compared with the garden experiment mentioned earlier could be explained by the differences in the microclimates of the wild populations. The difference in flowering dates at different altitudes recorded for *Hormathophylla spinosa* could also be explained in this way.

Flowering synchronicity has also been reported to vary between years. In *Lotus corniculatus* the proportion of the population showing a flowering synchronicity of greater than 70% varied between 0.05 and 0.65 in the three years of study (Ollerton and Lack, 1998). This is in contrast to *H. spinosa*, where at least 70% of the population showed synchronicity values above 70% for all four years of study (Gomez, 1993).

2. Dealing with Immaturity in a Collection

So what are the implications to the seed collector of variation in developmental dates, between and within populations and between and within individuals of a single population?

There are two observations which are worth noting before discussing this further. Firstly, the degree of these variations is large in relation to the range of seed maturation periods. Secondly, dramatic seed maturation effects resulting in a several fold increase in longevity take place within the capsule of foxglove over a period of *c.* 16 d (Figure 6.4). This 16 d period is generally less than that for any other variation in phenology described above. Assuming the same is true for most species, the task of the collector is to select fruit/seeds of both similar and appropriate developmental stage from the wide range of variation that is available, if the longevities of conserved seed collections are to be maximised. Effort spent maximising the longevity of seed for banking will be repaid by reducing the lifetime costs of conserving the seed. The risks of genetic selection during storage will also be reduced to acceptable levels. For more detailed discussion of the latter topic see Smith (1995).

Clearly, no matter how well planned their collecting trips, seed collectors are likely to be faced with the problem of how to treat seeds, which are advanced enough in their development to be viable, but are not yet fully mature. One option may be to allow the fruits to ripen *ex planta*. In *Mesembryanthemum crystallinum* L., the maternal tissue of the fruit remains alive after the rest of the maternal plant has died. Seed formation is reported as the final phase of development, taking place when the capsules are the only viable plant part and when there is no water uptake by the parent plant. (Adams *et al.*, 1998).

This raises the possibility that fruits and their developing seeds may be semi-autonomous structures even if removed from the maternal plant and even before the vascular connections are closed. The behaviour of detached foxglove capsules also suggest this is so. There were considerable increases in seed quality when immature, intact capsules were allowed to dry slowly in the field (Hay *et al.*, 1997). Capsules collected as early as 8 d before mass maturity would develop seeds that were fully viable. However, though these capsules were left to develop for the same total period as those matured on the plant, the subsequent longevity of these seeds was reduced to *c.* 50% of those allowed to develop naturally on the mother plant. Improvements in the longevity of extracted seeds of foxglove harvested at different developmental stages also occur as a result of slow-drying for 7 d (Figure 6.9). The higher the RH at which the seeds were placed for drying, the greater were the improvements in longevity. When drying was delayed by placing the seeds over water for 4 d before immediate rapid drying in the a seed bank dry-room, their P_{50} was close to that of seeds harvested 4 d later. Seed behaviours consistent with these findings in foxglove have also been reported for six other mesic and dryland species possessing both dry and fleshy fruits (Hay, 1997).

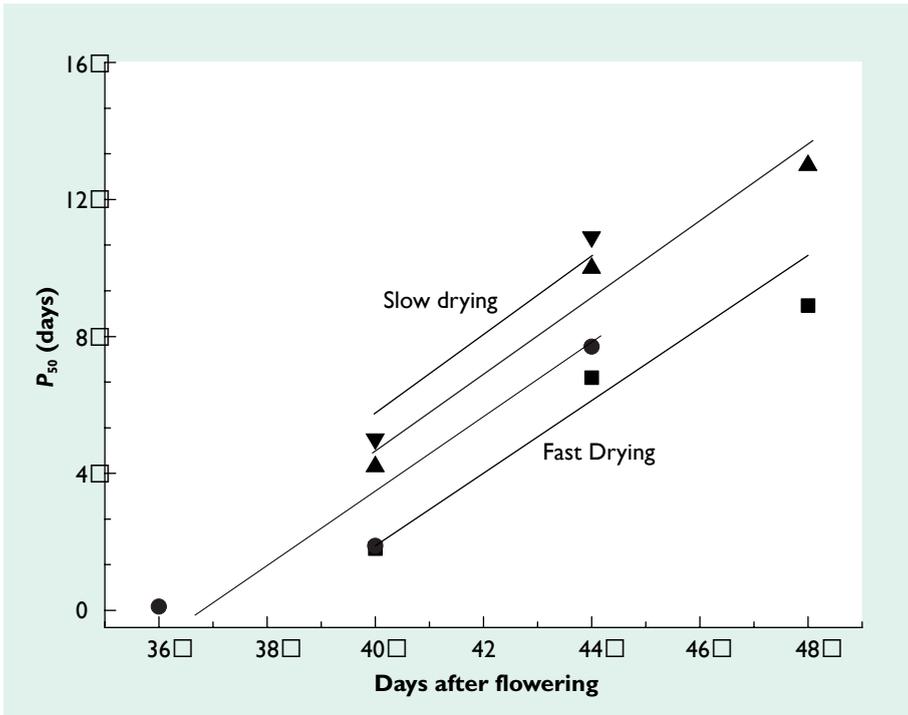


Figure 6.9 The longevity (P_{50}) of foxglove (*Digitalis purpurea*) seeds harvested at different stages of development and subjected to different drying treatments. Seeds were dried in a seed bank dry-room (15% relative humidity) (squares) or initially slow-dried at c. 30% relative humidity (circles) or c. 80% relative humidity (up-triangles) at 15°C for 7 d or initially placed over water (down-triangles) at 15°C for 4 d, before being transferred to the dry-room.

Similarly, the seeds from immature fruits (capsules) of the tropical tree *Cedrela odorata* L. have been shown to improve in both their desiccation tolerance and subsequent longevity in storage if the seeds are dried slowly within the capsules for up to 20 d following harvest (Lima *et al.*, 1998).

A slightly different behaviour is seen in the fleshy fruits of *Malus domestica* Borkh. in which the longevity of seed held inside the fruit shows no measurable decline when held at 16°C for 90 d. However, measurable losses in viability were detected in seeds held at the same temperature for the same length of time fully imbibed on agar, at high moisture contents between moist polyfoam, or allowed to dry slowly in cotton bags. The likely longevities (P_{50} values) were estimated to be 120, 210 and 185 d respectively (Dickie, 1988).

Box 6.3 Assessing and Managing Seed Maturity in the Field

The observations reported in this chapter may be consolidated into field advice which is built around the four stages of seed development presented in Box 6.2. For further details for drying techniques see Probert, 2003 – Chapter 19.

Stage 1: Formation and Differentiation

Diagnostics (dry and fleshy fruits): fruits green; seed contents watery; embryo hard to see even with a hand lens; eRH = 100%; vascular connection with parental plant intact.

Advice: Do not collect; seeds unlikely to be able to achieve desiccation tolerance (even with slow-drying / ripening); embryo rescue techniques likely to be more applicable.

Stage 2: Maturation and Reserve Accumulation

Diagnostics (fleshy fruits): fruits green and/or beginning to change; fruit turgid; seed coat colour beginning to change; seed reserves solid; eRH = 100%; seeds close to maximum dry weight; embryo relatively easy to find in most seeds with hand lens; vascular connection with mother plant intact for most seeds.

Advice: Do not collect unless there are compelling reasons (e.g., imminent threat of plant extinction or logistical difficulties). If seed is collected, keep intact fruit aerated in a porous bag or other suitable container. Do not clean the seed in the field unless the logistics of keeping them in the fruit are unmanageable. If field cleaning cannot be avoided, keep seeds aerated, at high RH but without allowing them to fully imbibe.

Diagnostics (dry fruits): As for fleshy fruits, with the additional observation that, in the case of dehiscent fruits, there will be the first signs of dehiscence.

Advice: Do not collect unless there are compelling reasons. If the fruits are fleshy at this stage, such that they are likely to slow the rate of drying significantly and provide enough photosynthate and water to allow seed development to continue, treat as fleshy fruits. If the fruit tissues are insubstantial (i.e., non-fleshy) so that the fruit is unlikely to provide sufficient photosynthate, collect whole inflorescence/stem into a bag or container that will restrict moisture loss but ensure ventilation so making sufficient oxygen available for seed maturation to progress. Do not field clean this seed.

Stage 3: Post Abscission Ripening

Diagnostics (fleshy fruits): Fruit coat continues to change colour; all fruit colour changes are completed in this phase; fruits remain turgid; seed coat colour may continue to change; seed reserves solid; seed eRH between 99 and 85%; all seeds at maximum dry weight; embryo equally easy to find in all seeds; vascular connection to mother plant severed in all seeds.

Advice: Keep seed in fruit; keep fruits aerated; do not field clean the seeds, unless the logistics of keeping them in fruit are unmanageable. If field cleaning cannot be avoided, keep seeds aerated at high relative humidity without allowing them to fully imbibe.

Diagnostics (dry fruits): As for fleshy fruits plus, where applicable, signs of dehiscence.

Advice: Keep intact fruit aerated in a cotton bag; allow the fruit to dry under ambient conditions until eRH is less than 85%; do not field clean the seed.

Box 6.3 Continued

Stage 4: Dispersal/Post-Harvest Ageing and Repair

Diagnostics (fleshy fruits): There is no evidence that fleshy fruits achieve this stage in the wild, until after dispersal. Some fruits soften and lose turgidity prior to dispersal by animal vectors.

Advice: Keep intact fruit aerated, if this is not possible, due to the breakdown of the fruit, either in the field or before return to the seed bank, extract the seeds from the fruit and dry under aerated ambient conditions until eRH is less than 85%. Seed development will be as complete as can be achieved practically. Now treat seeds as those from dry fruits.

Diagnostics (dry fruits): Fruit coat continues to change colour; all fruit colour changes are completed in this phase; fruits lose turgidity; all seed coat colour changes complete within this stage; all changes in seed reserves texture complete in this stage; seed eRH less than 85%; all seeds at maximum dry weight; embryo easy to find in all seeds; vascular connection to mother plant severed in all seeds; all dehiscent fruit types showing signs of dehiscence; seeds beginning to be dispersed.

Advice: All seed development now complete; seed cleaning in the field is no longer damaging; in cultivated species and serotinous species there are risks of loss of seed viability with time, following the completion of the previous phase; ensure the seeds that you collect have recently matured. Allow the seeds to dry further if ambient conditions are favourable.

Others have suggested that the ripening of immature seeds by slow- or delayed-drying is likely to be most effective if the rate of drying imposed is as close as possible to that which the seeds would experience *in situ* (Hong and Ellis, 1997). This would be difficult to determine and then control precisely and thus a general treatment would be of more practical use.

3. Dealing with More Than One Species

So far, in this chapter, seed maturity has been considered only at the level of a single species. However, illuminating data, from a seed collectors view, are available regarding the reproductive phenology of woody species at the level of a vegetation type. In all cases, the fruiting periods between species are separated such that on a single visit, the collection of seeds of all species at optimal maturity would not be possible. In Burkina Faso, four visits would be needed to optimally collect the 21 species observed (Devineau, 1999). A similar number of visits would be needed in Northern Australia for woody species of the four different tropical savannah types investigated (with between nine and 14 species for each savannah type; Williams *et al.*, 1999). Even for the seven species of the same genus (*Acacia* L.) occurring on the Nylsvley Nature Reserve in South Africa, three trips would be necessary to include the separate fruiting periods observed (Milton, 1987). Two species of *Prunus*, *P. spinosa* L. and *P. mahaleb* L. sharing the same thorny scrub habitat on the North West Iberian peninsula show remarkably different behaviours. Despite the overlapping spring flowering period, the appearance of mature fruits is separated by 3½ months (Guitian *et al.*, 1993).

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