Physiology and Biochemistry of Seeds

in Relation to Germination

In Two Volumes

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Viability, Dormancy, and Environmental Control

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"They also serve who only stand and wait"

Milton

Dedicated to our wives and children, Christine, Alex and Janette Bewley, and Marianne, Pauline, Nicola and Martin Black, whose continued patience and encouragement contributed in no small way to the production of this book.

Acknowledgements

It might be thought appropriate that this volume, in which considerations of dormancy occupy a major part, should emerge some years after Volume 1! But like the dormant seed whose seeming torpor can conceal a vigorous metabolism we, too, have not been inactive. Readers who already have some familiarity with the subject matter of this book will be aware of the vast and relevant research literature that is available. We have attempted to gather from this the essential features of seed viability, dormancy and environmental control of germination. In doing so, we have inevitably omitted very many research contributions and we do not claim to present an encyclopedic account; but we hope that the result is a fair statement of modern knowledge of these areas of plant physiology, useful to advanced undergraduates, graduate students, teachers and established research workers. We are grateful to many who have contributed to the production of this book: to those who kindly allowed us to use their published material and to Profs. E. H. Roberts and E. B. Dumbroff for reading and commenting on certain sections. Grants from the British Council, the Canada Council, the Department of Biology and the University at Calgary, made possible in situ collaboration between the authors in Calgary. One of us (J. D. B.) was in receipt of an award from the Natural Sciences and Engineering Research Council of Canada which is gratefully acknowledged. Erin Smith in Calgary had the unenviable task of producing the typescript – a job which she did quickly and efficiently: for this she receives our deepest gratitude. Karen Larsen and Joanne Papp provided invaluable assistance with the indexing. Finally, to our publishers we say "thank you" for being so patient and helpful.

Contents

Contents of Volume 1

Chapter 1. Viability and Longevity

1.1. The Life-Span of Seeds

In his publication of 1908, Ewart [88] divided seeds into three categories on the basis of their life-span under optimum conditions. These categories were (1) microbiotic – seeds whose life-span does not exceed 3 years; (2) mesobiotic – those whose life-span ranges from 3–15 years; and (3) macrobiotic – whose life span ranges from 15 to more than 100 years. This classification of longevity is not particularly satisfactory, however, and it has not been widely adopted. For many seeds the most favourable storage environment has not been determined, and until we possess this information (for different cultivars and harvests also) the categories have little meaning. As storage conditions are improved for any given seed, it may change from micro- to mesobiotic, or even to the macrobiotic class. For a comprehensive list of seeds whose known viability range (not necessarily under optimal conditions) extends up to a hundred or more years, see the review by Harrington [11]. A few examples are presented in Table 1.1. A fuller discussion of factors affecting longevity in storage is to be found in Section 1.2. Here we will first answer the intriguing question: how old are the oldest viable seeds? We will then consider the longevity of seeds buried in soil.

Table 1.1. Viability-span of some orthodox [a] seeds in storage

Plant species	Longevity and (% germinated)	Storage environment
Papaver rhoeas-corn poppy	10 years (53)	Laboratory
Brassica napus-rape	10 years (12)	Laboratory
Cucurbita pepo-squash, marrow	10 years (55)	Laboratory, sealed
Glycine max-soybean	13 years	Storage
Picea glauca-white spruce	15 years (40)	−4 °C, sealed
Cannabis sativa-hemp	19 years	Laboratory, sealed
Nicotiana tabacum-tobacco	20 years (92)	Laboratory, sealed
Lactuca sativa-lettuce	20 years (86)	−4° C, 8% R.H.
Phaseolus vulgaris-dwarf bean	22 years (30)	Laboratory
Allium cepa-onion	22 years (33)	Dry, laboratory
Fragaria spp.-strawberry	23 years (89)	$<5°$ C, dry
Hordeum vulgare-barley	32 years (96)	Storage
Medicago sativa-alfalfa	78 years (22)	Laboratory
Anthryllis vulneraria-kidney vetch	90 years (4)	Laboratory

[a] See Section 1.2.2
Excerpt from Harrington, 1972 [11]

1.1.1. The Oldest Seeds – from the Pharoah's Tomb to the Incendiary Bomb

Discoveries of apparently intact cereal grains during archaeological excavations of ancient Egyptian sites aroused great public interest when they were first described last century. Reports that "mummy" grains would germinate and produce seedlings were given considerable credence and even today, in some quarters, the myth lingers on. Typical of early reports is one from *The Gardener's Chronicle* of Nov. 11 1843, excerpts of which are presented in Figure 1.1. The evidence in that paper cannot now be disputed without prejudice, nor, of course, can the original work be authenticated! Nevertheless on the basis of other, and later reports which have repeatedly failed to substantiate the viability of ancient grains, it would appear that the (anonymous) author, and the "most exact and conscientious" Mr. Martin Farquhar Tucker, were probably victims (one presumes not perpetrators) of a hoax or deception of some kind. More recent reports, published over the past 40 years, show that ancient cereal grains have undergone severe morphological and physiological degradation with accompanying loss of viability. Barley grains from King Tutankhamun's tomb (ca. 1350 B.C.) were found to have been extensively carbonized, although still retaining their original shape [54]. The grain, with its scutellar and axial regions, was still intact, but the whole grain was much lighter and less dense than living grains of modern barley. Assays revealed the presence of riboflavin and nicotinic acid (20%–30% of the amounts present in new barley), together with increased acidity. Phytin had been completely lost. Some ancient wheat grains have been reported as being completely carbonized [119], and therefore non-viable, but keeping their original shape. An ultrastructural investigation of the non-viable embryos of wheat grains from Thebes (dated 3000–2000 B.C.) and from the grain silos of Feyum (ca. 4400 B.C.) has shown a remarkable conservation of cell fine structure [97]. Structures present were lipid bodies, nuclei, nucleoli, chromatin, some plasmalemma, and possibly mitochondrial remnants. On hydration, the plasmalemma pulled away from the cell walls and the cytoplasm appeared degraded. Maize grains of no less than 600 years of age have been found to contain intact endosperm tissue similar, ultrastructurally, to modern maize. The embryos were discoloured and damaged, however [79]. We might note that, with respect to these and other ancient grains, we do not know whether loss of viability was a consequence of some preparative treatment they might have received prior to being laid in storage, or due to time itself.

Other claims of amazing longevity have been made for *Nelumbium nucifera* (1000–3000 years) [116]; *Lupinus arcticus* (over 10,000 years) [141]; *Chenopodium album* and *Spergula arvensis* (at least 1700 years) [134]. These will be discussed in the next section, since the seeds were recovered from soil.

The longevity of a viable seed of *Canna compacta* has been put at about 600 years [114, 160]. The specimen was collected from a tomb in Santa Rosa de Tastil, Argentina, enclosed in a *Juglans australis* nutshell forming part of a rattle necklace. Carbon-14 analysis of the nutshell and surrounding charcoal remnants was used to derive the probable age of the seed – insufficient seed material was available for direct analysis. While the evidence for the ascribed age is circumstantial, nevertheless it is compelling, for the only way in which the *C. compacta* seed could have arrived inside the intact *J. australis* nutshell is for the seed to have been inserted there through the still-developing nutshell, while it was soft. Then the nutshell hardened and dried with the seed inside, forming a rattle. Hence the seed must have been at least the same age as the shell.

Herbarium specimens of *Albizzia julibrissin* collected during Sir George Staunton's mission to China in 1793 and deposited in the British Museum, London, germinated following attempts to quench a fire started by an incendiary bomb which hit the Botany Department of the Museum in September 1940 [46]. Three of the resulting seedlings were planted at the nearby Chelsea Physic garden but two eventually succumbed to enemy action during a subsequent bombing raid in May 1941! Some oat and barley grains found in a sealed glass tube within the foundation stone of Nuremberg City Theatre 123 years after burial were still capable of germination (21% and 12% respectively). Wheat grains buried in the same locale were completely non-viable [49].

Becquerel [56] tested the viability of old collections of seeds from the Museum of Natural History in Paris in 1906 and 1934. The longevity of some species are presented in Table 1.2. With the exception of *Stachys* (Labiatae) and *Lavatera* (Malvaceae) all long-lived seeds are members of the Leguminosae. The life-span of 158 years attributed to *Cassia multijuga* appears to be the record amongst seeds whose history can be wholly authenticated. A seed of *Nelumbium* spp. from the Sloane Collection of the British Museum was successfully germinated in 1855 (this seed was then 150 years old) but when, in 1926, 12 more seeds were tried, none germinated. In 1942 one further seed was imbibed, and it germinated [46]. Unfortunately, it is not clear from the report that this seed was from the same stock as that which germinated in 1855. There are several other reports of herbarium specimens remaining viable for 80–120 years (see [4, 10]). We should emphasize that the life-span attained by these long-lived seeds comes from arbitrary storage conditions. Museums are usually heated, and maintained at low relative humidities; hence the seeds probably had a low moisture content. Longevity might have been enhanced if different storage conditions had been used. Also, the longevity of other seeds may have been missed because of inadequate germination conditions, e.g. failure to scarify hard-coated seeds before imbibition.

1.1.2. Life-Span of Seeds Buried in Soil

Let us first consider those claims for extended longevity in soil. The most extreme of these is for the arctic lupin, viable seeds of which were discovered in 1954 near Miller Creek in the Yukon Territory, Canada. These seeds were removed from rodent burrows containing the remains of a nest, faecal matter, and the skull of a lemming species which apparently inhabited that particular area some 10,000 years previously [141]. Dating done on nests and remains of arctic ground squirrels found in similar conditions in Central Alaska, have shown them to be over 14,000 years old. On the basis of this and other highly circumstantial pieces of evidence, it was deduced that the seeds were at least 10,000 years old and that they (and the lemming!) were trapped in the burrow by a landslide or a deep layer of volcanic ash which caused the prehistoric tundra to be buried in spring or early summer. This, in turn, prevented the soil from thawing and caused the permafrost to rise, No subject in Vegetable Physiology is more interesting, both for theoretical and practical reasons, than the power which seeds undoubtedly possess, under certain circumstances, of preserving their vitality for an apparently indefinite period. It is doubtless true that many of the statements on this subject, to be found in books, are apocryphal; but certainly some are founded in fact, such as the famous case of the Raspberry-seed taken along with the coins of the Emperor Hadrian from an ancient barrow in Dorsetshire, the offspring of which is now to be seen in the Gardens of the Horticultural Society. None among the so-called instances of this excessive longevity have excited more doubt and discussion than what is called Mummy-Wheat; that is to say, Corn taken from mummies, and therefore of the highest antiquity, which has grown when sown. Every year produces cases of this sort about the harvest season, and even this season at least 20 specimens have been sent us of Wheat-ears, purporting to have had a mummial —pardon the word—a mummial origin; and strange to say, they have all proved to belong to the Egyptian Wheat, or *Blé de Miracle*, called by Botanists *Triticum compositum*. We have never, however, succeeded in satisfying ourselves that the Corn from which such Wheat is said to have been produced was really taken from mummy-cases. There is always some defect in the evidence; as was the case with the Tynningham Wheat, mentioned in the *Mark Lane Express* of Oct. 9, 1842, which had been raised from seed *said* to have been produced in Egypt, from plants *said* to have grown from grains *said* to have been taken from a mummy-case. Now all such statements may be true, but there is no proof that they are so; and when we are told that Onions taken from similar receptacles have also grown, *which is impossible*, we may be pardoned for requiring very decisive evidence before we accord our belief in those prodigies. Nevertheless they *may* be true; because we have before us an instance, in the evidence concerning which we find no flaw whatever. We have had it on our table for some months, and produce it now, in order to satisfy the many inquiries that are made about such things.

The history of this Wheat was given by Mr. Martin Farquhar Tupper, a most exact and conscientious man, in the *Times* of September, 1840; and to that gentleman we are indebted for the additional facts which we are now able to communicate.

Sir Gardiner Wilkinson, when in the Thebaid, opened an ancient tomb (which had probably remained unvisited by man during the greater part of 3000 years), and from *some alabaster sepulchral vases* therein took with *his own hands* a quantity of Wheat and Barley that had been there preserved. Portions of this grain Sir G. Wilkinson had given to Mr. Pettigrew, who presented Mr. Tupper with 12 grains of the venerable harvest. In 1840 Mr. Tupper sowed these 12 grains, and to show the care with which he preserved their identity we shall quote his own account of his proceedings thereupon. "I ordered," he says, "four gardenpots of well-sifted loam, and, not content with my gardener's care in sifting, I emptied each pot successively into an open newspaper and put the earth back again, morsel by morsel, with my own fingers. It is next to impossible that any other seed should have been there. I then (on the 7th of March, 1840), planted my grains, three in each pot, at the angles of an equilateral triangle, so as to be sure of the spots where the sprouts would probably come up, by way of additional security against any chance seed unseen lurking in the soil. Of the 12 one only germinated, the blade first becoming visible on April 22; the remaining 11, after long patience, I picked out again; and found in every instance that they were rotting in the earth, being eaten away by a number of minute white worms. My interesting plant of Wheat remained in the atmosphere of my usual sitting-room until change of place and air seemed necessary for its health, when I had it carefully transplanted to the open flower-bed, where it has prospered ever since. The first ear began to be developed on the 5th of July; a second ear made its appearance, and both assumed a character somewhat different from all our known varieties. Their small size and weakness may, in one light, be regarded as collateral evidence of so great an age, for assuredly the energies of life would be but sluggish after having slept so long; however, the season of the sowing—spring instead of autumn—will furnish another sufficient cause. The two ears on separate stalks were respectively 2½ and 3 inches long, the former being much blighted, and the stalk about 3 feet in height."

"If, and I see no reason to disbelieve it," says Mr. Tupper, in conclusion, "if this plant of Wheat be indeed the product of a grain preserved since the time of the Pharaohs, we moderns may, within a little year, eat bread made of Corn which Joseph might have reasonably thought to store in his granaries, and almost literally snatch a meal from the kneading-troughs of departing Israel."

Here we have no link lost in the chain of evidence. Sir Gardiner Wilkinson himself opened the tomb, and with his own hands emptied the alabaster vase; of its contents he gave a portion to Mr. Pettigrew, who gave it to Mr. Tupper, who himself sowed it, watched it, and reared it. What better proof can we require? Unless it be alleged that the grains, after all, may have been changed somewhere on the road between the Thebaid and Mr. Tupper's garden. But, upon this point, Mr. Tupper expressly says, in a passage that we have not quoted, that the grains which he sowed were brown and shrunk; which is a just description of some that we too have seen from Sir Gardiner Wilkinson, but which would not apply to any modern Wheat. They looked, indeed, as if they had been scorched.

But there are other proofs, less direct, but equally conclusive, as to the antiquity of the seed sown by Mr. Tupper. Out of twelve grains one only grew; that one produced but two ears—small, blighted at the base, and yielding altogether only 27 grains. Mr. Tupper has favoured us with a drawing of one of them. But in 1841, the second year, when the Wheat was recovering its constitutional vigour, the ears were perfect, and averaged 4½ inches each. In 1842, the renovation being complete, some of the ears measured 7½ inches in length. This, as Mr. Tupper observes, corroborates the idea of a re-awakening from so long a sleep, as if the Wheat had been gradually returning to its pristine vigour. One of these ears of 1842 is now before us, and is so like a good sample of Colonel Le Couteur's Bellevue Talavera, that even the experienced eye of that gentleman is unable to detect a difference. It proved a most abundant bearer: 18 grains in Mr. Mitchell's Nursery Garden, Brighton, having produced 625 ears, which Mr. Hallett of Brighton considers to have contained on an average 55 grains. And this (685, multiplied by 55, divided by 18) gives a productiveness equal to two thousand and ninety-three fold.

But with the quality of this Wheat we do not wish to concern ourselves just now. The important question is, what were the circumstances which preserved the growing power of Sir Gardiner Wilkinson's Wheat from the days of the Pharaohs down to our own time. For if that can be ascertained, a light will necessarily be thrown upon the very important art of preserving seeds artificially. To us it appears that we must ascribe the result entirely to the DRYNESS of the air where the Wheat was kept. And we believe that dryness will have been the true cause of similar results in all other instances. Such is the conclusion at which we long since arrived. ("Theory of Horticulture," pp. 79 and 189). Daily experience confirms our opinion; and reasoning, in the absence of experience, would almost have led to it. Decomposition, which in seeds is the cause of death, can only occur in a damp atmosphere; therefore to keep off a damp atmosphere is to prevent decomposition, and consequently to arrest the approach of death. And yet how little is this regarded by persons interested in such matters. In a damp country like England no precaution should be neglected to ventilate, at least seed-rooms, if not seeds themselves. And yet what is the practice? The seedsmen pack them in large sacks or huge casks, in close ill-ventilated granaries; and gardeners place them in drawers or bags in the damp and miserable sheds with which some masters so thoughtlessly provide them; farmers in damp barns or outhouses. What can possibly happen with such management except the speedy destruction of vitality, especially when we know how badly our home-grown seeds are in almost all seasons ripened, and how much free moisture they necessarily contain. What wonder that French seeds, ripened in a dry climate and preserved in dry buildings, should often be found so much better than English seed? Our climate offers so many impediments to the preservation of seeds that we cannot afford to neglect a single precaution; and we trust Mr. Tupper's Pharaonic Wheat will have the effect of turning those whom these observations may concern to wiser and better ways.

Mummy-Wheat in the first year of its revivification.

Fig. 1.1. An excerpt from The Gardener's Chronicle of Nov. 11, 1843 on "mummy" wheat

Table 1.2. Becquerel's record of old seeds

Species	Date collected	Seeds growing in 1906	Seeds growing in 1934	Determined longevity (years)	Probable longevity (years)
Mimosa glomerata	1853	5 out of 10	5 out of 10	81	221
Melitotus lutea	1851	3 out of 10	0 out of 10	55	–
Astragalus massiliensis	1848	0 out of 10	1 out of 10	86	100
Cytisus austriacus	1843	1 out of 10	0 out of 10	63	–
Lavatera pseudolobia	1842	2 out of 10	0 out of 10	64	–
Dioclea pauciflora	1841	1 out of 10	2 out of 10	93	121
Ervum lens	1841	1 out of 10	0 out of 10	65	–
Trifolium arvense	1838	2 out of 10	0 out of 10	68	–
Leucaena leucocephala	1835	2 out of 10	3 out of 10	99	155
Stachys nepetifolia	1829	1 out of 10	0 out of 10	77	–
Cytisus biflorus	1822	2 out of 10	0 out of 10	84	–
Cassia bicapsularis	1819	3 out of 10	4 out of 10	115	199
Cassia multijuga	1776	–	2 out of 2	158	–

Translated from Becquerel, 1934 [56]

thus maintaining the lupin seeds in a dry and continually frozen state. Alternative geological explanations were not considered, and proof for such longevity of these lupin seeds obviously is missing.

Doubt must also surround the claims for longevity of one to three thousand years for the seeds of the Indian or Sacred lotus, *Nelumbium nucifera*. Many of these seeds (or more strictly, fruits, which are extremely hard-coated) were recovered from peat under drained lakes of the Pulantien basin of South Manchuria. The age of the seeds was initially estimated by Ohga as 160–250 years using indirect evidence, particularly the known history of the drained lakes, although others have taken the view that the geological history of the Pulantien basin indicates an age of many thousands of years. But direct carbon dating of the seeds shows them to be indistinguishable in age from modern seeds! [94, 95]. Viable lotus seeds also have been found associated with the remains of a prehistoric boat, some 20 feet below the surface of a lake at Kemigawa, near Tokyo. The wood from the boat has been dated at about 3000 years, but this yields no evidence for the age of the lotus seeds, which could have been from modern lilies buried by natural sinking into soft lake sediments moved by the action of currents.

Ødum [134] surveyed a considerable number of archaeological sites in Denmark and in Skåne, Sweden and noted the appearance in recently excavated soils of plants which have not grown in such regions for many years previously. Using archaeological dating he claims that some weed seeds must have remained viable for between 100 and 600 years, and seeds of *Chenopodium album* and *Spergula arvensis* for about 1700 years. Evidence of seed age is indirect and weak, and until dating of the seeds themselves is obtained, the claims must be treated with due scepticism. To quote Ewart [88], such "observations are good evidence of the readiness of dispersal of certain seeds, but as evidence of their longevity are quite untrustworthy".

A number of buried seed projects have demonstrated survival for considerably shorter periods of time. These have been discussed in detail by Barton in her book on longevity, and in Crocker's review of the same subject [4, 10]. A study of buried weed seed, due to last for 50 years, was initiated in 1972, and the results of germination and viability tests after 2.5 years of burial have been published [84]: of 20 species buried, only 4 species maintained over 50% viability. A much earlier experiment, started by Duvel in 1902, involved the burying of seeds of 107 species of wild and cultivated plants. Of these, 71 species germinated after 1 year, 61 after 3 years, 68 after 6 and 10 years, 51 after 16 and 20 years, 44 after 30 years and 36 after 39 years. Seeds of cultivated plants, especially cereal grains and legume seeds, perished quickly in the soil, while seeds of wild plants, especially persistent weeds like docks, lambsquarters, plantains, daisies, poke, purslane and Jimson weed, retained their vitality well. Such weeds therefore cannot be controlled by ploughing the seeds under because the seeds outlive any crop rotation. We should note here that many persistent weed species do not possess hard coats. The poor survival of seeds of many species (and particularly the cultivated species) might not be related to their loss of viability, in the conventional sense. For survival in moist soil a seed not only has to maintain viability, but also has to possess a dormancy mechanism, otherwise it will germinate, degrade, and hence not be accounted for in the early viability tests. In these classical burial experiments it has not been possible to distinguish clearly between how many seeds were lost through germination in situ, and how many lost viability.

The longest controlled burial experiment to date is that of W. J. Beal who, in 1879, selected seeds of 23 different species of plants common in the vicinity of Michigan Agricultural College in East Lansing, mixed 50 seeds of each species with moist sand in unstoppered bottles and buried them in a sandy knoll. At regular intervals since, bottles have been unearthed and viability of the seeds tested. The spring of 1970 marked the 90-year period of this seed viability experiment, and the results of this, and previous tests are presented in Table 1.3. Under the germination conditions used (which may not have always been ideal) only *Verbascum blattaria* seeds remained viable after 90 years, although many species did persist for 25–30 years, and a few seeds of *Oenothera biennis* and *Rumex crispus* for 80 years. Thus the values of longevity for buried seeds of known age and maintained under controlled conditions are far shorter than for seeds dated by indirect, circumstantial evidence.

1.2. Viability of Seeds in Storage

Over the past few decades there have been hundreds, if not thousands, of research papers published on this subject, and we have consulted but a fraction of these in the preparation of this account. Instead, we have relied heavily on comprehensive summaries incorporated into books [4, 22, 32] and recent reviews [3, 5, 13, 15, 17, 20, 21, 23–31] – the reader should consult these for more detailed information and for further references. This account is therefore a synopsis which, we trust, includes most of the points essential for the reader to gain some feel for the subject.

Table 1.3. Viability of buried seeds in W.J. Beal's buried-seed experiment, 1879–1970

Name of species tested [a]	Duration of life-cycle (years)	5th year 1884	10th year 1889	15th year 1894	20th year 1899	25th year 1904	30th year 1909	35th year 1914	40th year 1920	50th year 1930	60th year 1940	70th year 1950	80th year 1960	90th[e] year 1970
Agrostemma githago	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Amaranthus retroflexus	1	+	+	+	+	+	+	0	+	0	0	0	0	0
Ambrosia artemisifolia	1	0	0	0	0	0	0	0	+ [d]	0	0	0	0	0
Anthemis cotula	1	+	+	+	0	+	+	0	0	0	0	0	0	0
Brassica nigra	1	0	+	+	+	+	+	+	+	+	0	0	0	0
Bromus secalinus	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Capsella bursa-pastoris	1	+	+	+	+	+	+	+	0	0	0	0	0	0
Erechtites hieracifolia	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Euphorbia maculata	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Lepidium virginicum	1	+	+	+	+	+	+	+	+	0	0	0	0	0
Malva rotundifolia	1 or 2	+	0	0	+	0	0	0	0	0	0	0	0	0
Plantago major	per. [b]	0	0	+	0	0	0	0	+	0	0	0	0	0
Polygonum hydropiper	1	+	+	+	+	+	?	0	0	+	0	0	0	0
Portulaca oleracea	1	+	+	+	+	+	0	0	+	0	0	0	0	0
Setaria glauca	1	+	+	+	0	+	+	0	0	0	0	0	0	0
Stellaria media	1	+	+	+	+	+	+	0	0	0	0	0	0	0
Trifolium repens	per. [b]	+	0	0	0	0	0	0	0	0	0	0	0	0
Verbascum thapsus [c]	2	+	?	+	+	0	0	+	0	0	0	0	0	0
Oenothera biennis	2	+	+	+	+	+	+	0	19 (38)	19 (38)	12 (24)	7 (14)	5 (10)	0
Rumex crispus	2	+	+	+	+	+	+	+	9 (18)	26 (52)	2 (4)	4 (8)	1 (2)	0
Verbascum blattaria [c]										31 (62)	34 (68)	37 (74)	35 (70)	10 (20)

[a] The + signs following each species, both in the upper and in the lower parts of the table, indicate that one or more seeds of that species germinated for the year shown. The number indicates the number of seeds germinating, while the number in parentheses indicates the percent germinated

[b] Perennial

[c] There is some question concerning the identification of *Verbascum* plants in the early period (1884–1920) as *V. thapsus* rather than *V. blattaria*

[d] In previous years incorrect germination conditions might have been used; for further comment see [55]

[e] For 100 years see: Kivilaan and Bandurski, Am. J. Bot. **68**, 1290 (1981)

It has long been known that the factors which most influence the longevity of seeds in storage are temperature, moisture and oxygen pressure. In general, (1) the lower the temperature and lower the moisture content the longer the period of viability and, (2) for many species, the higher the oxygen pressure the shorter the period of viability. There are exceptions to these generalizations, in particular those seeds which are known as unorthodox or recalcitrant [26], which cannot withstand drying. Few studies have been carried out on those seeds and hence we will discuss them briefly before considering the larger literature on the more commonly occurring orthodox seeds.

1.2.1. Recalcitrant Seeds

Some seed species must retain a relatively high moisture content in order to maintain maximum viability. Even when these recalcitrant seeds are stored under moist conditions their longevity is often quite short, and only occasionally exceeds more than a few months. Some species that produce recalcitrant seeds are to be found in Table 1.4. Included are a number of the large-seeded hardwoods (e.g. spp. of *Corylus*, *Castanea*, *Quercus*, *Aesculus*, *Salix* and *Juglans*) and important plantation crops such as *Coffea arabica*, *Cola nitida*, *Theobroma cacao* and *Hevea brasiliensis*. Seeds of most aquatic species also rapidly lose viability in dry conditions (e.g. *Zizania aquatica*).

From a commercial standpoint, the inability to store seeds of these and other recalcitrant species is a serious problem. While propagation by cuttings from the mature plant is possible for some species, and is the accepted practice, it is desirable to retain a viable seed stock to preserve maximum genetic diversity. Unfortunately, as well as conventional methods of dry storage being inappropriate for the maintenance of recalcitrant seeds, in certain cases low temperature storage may also be detrimental to their survival – this applies in particular to the seeds of tropical species. Even within the same genus some seeds appear to be more sensitive to low temperatures than others, e.g. *Shorea ovalis* seeds have to be stored above 15° C, but *S. talura* seeds can be kept at 4° C [155] (Table 1.4). Grains of *Zizania aquatica* present a different storage problem: they must be kept moist at low temperatures, and under these conditions they eventually lose dormancy and sprout.

Care must be taken to prevent deterioration of moist seeds through growth of contaminating micro-organisms. To cite but one example: at 5° C grapefruit seeds are best stored in open containers in a very humid room; under such conditions they maintain a moisture content of 18% [53]. Mould was found to appear at an earlier date on seeds stored in sealed containers, but whether this led to, or was a consequence of, deterioration is not known. On the other hand, grapefruit seeds dried to 60% of their fresh weight at laboratory temperatures maintain their viability better in sealed storage than in open storage.

Determination of optimal storage conditions for recalcitrant seed species is generally empirical, and little has been done to define the quantitative relationship between environmental parameters and viability. In certain cases seeds may have been reported as being killed by drying, and therefore classified as recalcitrant, when it is possible that the seed was actually orthodox and that the drying method