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LONG-TERM SEED STORAGE

OF MAJOR TEMPERATE FRUITS

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PREFACE

The conservation of genetic resources of temperate fruits has, until recently, relied on the maintenance of field genebanks (also called "clonal repositories" or "living collections"). There have de facto been mainly breeders' collections; as such they have been and are important for samples showing specific characteristics and for continuing characterization and evaluation. Such collections are rarely representative of the wide spectrum of genetic variability which needs to be conserved.

Recently the IBPGR has turned its attention to these problems. Clearly, genetic variation should be conserved for posterity by seed storage, although this may pose additional practical problems for breeders. Other conservation methods, e.g. the use of in vitro cultures, are complementary to the field genebanks: the number of stocks maintained in each would be limited to materials that are justified scientifically for inclusion in the collection. (Pollen storage is more applicable to breeding than for conservation.)

The IBPGR has in press a report on the feasibility of in vitro techniques for the conservation of temperate fruit germplasm.

Other aspects of conservation, e.g. through in situ reserves might well add security to the conservation strategy; however, such schemes are still in their early stages and they are often plagued by lack of clear objectives. The IBPGR aims to clarify some of these issues in the near future.

In recent years the IBPGR has supported research on seed storage for conservation. Two major handbooks are in press. The present booklet is issued, under the general editorship of Dr. R.H. Ellis of the University of Reading, UK, to encourage curators to take seriously their responsibilities for conservation and initiate seed storage of representative genepools as soon as possible.

INTRODUCTION

R.H. Ellis

This report provides information on the storage and germination of seeds of Prunus spp., Malus spp. Pyrus spp. and Vitis spp. The articles on Malus/Pyrus and Vitis were first published in the FAO/IBPGR Plant Genetic Resources Newsletter, 50: 53-61, and Vitis 22: 211-219, respectively, whilst that on Prunus has been prepared especially for this report. The problem of substantial seed dormancy is common to all 4 genera.

The style of the 3 articles differs slightly, but one difference which requires explanation is the use of various terms to describe the horticultural practice of exposing moist seeds to either high or low temperatures in attempts to overcome seed dormancy (prior to transfer to another regime in which seed germination is expected to occur). Warm stratification is the term usually applied to describe exposure of imbibed, or imbibing, seeds to a high temperature - usually 25° to 30°C, but occasionally 20°C - prior to transfer to a lower temperature, whereas stratification or pre-chilling are the terms usually applied to describe exposure of imbibed, or imbibing, seeds to a low temperature - within the range 0° to 10°C, and usually between 3° and 5°C - prior to transfer to a higher temperature.

The article on Vitis does not provide a review of information on seed storage characteristics. Experience of storage with seeds of Vitis spp. is limited to short-term storage - that is between extracting the seeds and commencing investigation on seed dormancy. Seeds of several lots have been stored at the University of Reading at 0°C and 7% moisture content (fresh weight basis) for various periods up to 9 mo without loss of viability. Evidence in the literature that grape seeds can be stored dry at sub-zero temperature is limited - e.g. 4 mo at -10°C (Manivel and Weaver, 1974), 3 weeks at -7°C (Harmon and Weinberger, 1959) (these references are provided in the Vitis article) - but is sufficient to confirm that Vitis spp. possess orthodox seeds which can be maintained within long-term seed stores operating at IBPGR preferred conditions.

It may be worthwhile here in the introduction to note a point which will become evident from detailed reading of this report. A misconception has developed which assumes that seeds of the species covered in this report cannot be stored dry because of the difficulties experienced in overcoming seed dormancy and the misinterpretation of the results of investigations on seed dormancy. The following examples for Vitis demonstrate how easy it is for the unwary reader to be misled by the literature. In the past stratification treatments (defined above) of as long as 9 mo have been used to break seed dormancy (and even these extreme periods of treatment have had only limited success). Consequently "storage" periods of 3 mo at 0°C have shown that it is preferable for seeds to be stored moist (fully imbibed) rather than dry - the former resulting in 52% germination and in the latter treatment the seeds failing to germinate (Yeou-Der et al, 1968). Not surprisingly, storage of imbibed seeds at sub-zero conditions (-7°C, 9 weeks) is detrimental (Harom and Weinberger, 1959) - as it is in all orthodox species. Hence there arises the widely stated, but erroneous, opinion that Vitis seeds should neither be dried, nor stored at sub-zero temperatures. In both cases, however, the treatments were in fact applied as treatments to remove seed dormancy and reported as such, not treatments to maintain seed viability. The articles on Prunus, Malus and Pyrus provide similar examples where the same mistake has been made.

PRUNUS SEED GERMINATION AND STORAGE

R.H. Ellis and T.D. Hong

Introduction

One difficulty which has arisen from the classification of seed storage behaviour as either orthodox or recalcitrant (Roberts, 1973) is that a tendency has developed for species to be mistakenly classified as possessing recalcitrant seed storage behaviour whenever difficulties have arisen in handling the seeds and/or producing plants from the seeds. Orthodox and recalcitrant seed storage behaviour have been clearly defined. In orthodox seed, longevity in dry storage is increased by reducing moisture content and/or temperature. In contrast, recalcitrant seeds cannot be dried below a relatively high moisture content without loss in viability, and in consequence they do not conform to the rules of orthodox seed storage behaviour. Examples of the problems which can result in species with orthodox seed storage behaviour being mistakenly classified as recalcitrant have been provided elsewhere (Roberts, King and Ellis, 1984).

Despite no Prunus species being listed as recalcitrant by King and Roberts (1979) or short-lived by Harrington (1972), 1 species (Prunus pensylvanica) being listed as long-lived by Harrington (1972), and King and Roberts' conclusion that Prunus spp. are largely orthodox in behaviour, a belief has developed, mistakenly, in some quarters that some or all Prunus species show recalcitrant seed storage behaviour. The IBPGR Advisory Committee on Seed Storage, at its meeting at the University of Reading in April 1984 requested that an article on the germination and storage of seed of Prunus species be prepared to clarify the seed storage characteristics of Prunus and provide information on suitable germination test procedures.

The following, which relies heavily on the literature cited, is an attempt to meet that request. For additional information the reader is referred to the major review on Prunus seed provided by Grisez (1974), the information on handling ornamental tree seeds provided by Gordon and Rowe (1982), and the information on fruit tree breeding provided by Janick and Moore (1975).

Extraction

It is generally recommended that seeds be extracted from fully mature fruit rather than immature fruit, e.g. Grisez (1974), because the period of rapid embryo growth occurs relatively late during fruit development (Tukey, 1934a, 1934b, 1934c). Thus embryos in immature fruit can be underdeveloped, and they may also show more pronounced dormancy. This makes them more difficult to germinate. It is also easier to extract seeds from ripe fruit. Grisez (1974) has provided a table of the colour of ripe fruits of Prunus species. Fruit can be left to ferment in water prior to extraction. This softens the fruit, making extraction easier but it tends to reduce viability, e.g. Fogle (1958), and is better avoided. Gordon and Rowe (1982) describe two designs of machines which can be used to depulp seeds, but hand extraction is convenient for small numbers of seeds. It is desirable to clean seeds of all pulp and juice by washing in water as soon as possible after extraction (Gordon and Rowe, 1982). A number of workers have separated heavy seeds where the embryos may be better developed by selecting only those seeds which sink in alcohol or salt solutions, e.g. Cummings, Jenkins and Dunning (1933). However there is little need for this (Grisez, 1974), and it may be counter-productive because drier seeds and those with air bubbles within them may germinate well but float (Cummings et al., 1933). Almonds (Prunus amygdalus) are ready to harvest when the hull (mesocarp) splits (Kester, 1969a).

Dormancy, stratification and germination

Seed dormancy in Prunus species is pronounced and specific dormancy-breaking treatments are almost always required before seeds will germinate. The seed (embryonic

axis and cotyledons surrounded by the testa) is contained within a stony endocarp. Consequently it has often been thought that dormancy is imposed by the endocarp. This is not the case, however. Although the endocarp can delay germination, extracted seed embryos may exhibit dormancy (Grisez, 1974; Lin and Boe, 1972; Michalska, 1982), whilst the cotyledons and testa also contribute to dormancy (Lin and Boe, 1972).

Mechanical and/or chemical treatments to the endocarp are rarely advantageous, and can be deleterious (Grisez, 1974). However, the complete removal of the endocarp can reduce the time taken to germinate and increase the proportion of seeds germinating, e.g. Giersbach and Crocker (1932), or reduce the severity of dormancy-breaking treatment required to promote germination, e.g. Toit, Jacobs and Strydom (1979). Removing endocarps from moist seed, however, can be damaging and result in microbial contamination during subsequent germination tests (Havis and Gilkeson, 1949). Many treatments have been applied to Prunus seed in attempts to promote the germination of dormant seeds. Since a review of Prunus seed dormancy has been prepared for forthcoming IBPGR seed dormancy 2-volume handbooks, 1/ a comprehensive list of such treatments has not been provided here.

In practical terms the most important seed dormancy-breaking treatment for Prunus is pre-chilling, where imbibed seeds are subjected to a low, but not freezing, temperature. Pre-chilling has been more frequently described as stratification, layerage and sometimes even after-ripening. Stratification and layerage are apt terms to describe the horticultural practice of exposing seeds in alternate layers between moist peat or sand to ambient (low) winter temperatures - with the treated seeds being sown in the subsequent spring to produce seedlings. Here, however, the term pre-chilling is used to imply the exposure of imbibed seeds to low temperatures, but not the method of exposure.

On occasion in the past the beneficial effect of pre-chilling in promoting the subsequent germination of Prunus seeds has been thought to be mechanical since the stony endocarps have sometimes been seen to split during the treatment. Indeed it was not unknown for seeds to be treated to freezing temperatures since it was assumed that the freezing action would assist in endocarp splitting. Although, of course, it is possible to split endocarps by mechanical means, endocarp splitting during pre-chill treatments results from radicle growth. Pre-chilling at temperatures between 0° and 10°C can be effective, but 3° to 5°C is the most suitable range (Giersbach and Crocker, 1932; Suszka, 1967). In fact it is quite satisfactory for the seeds to be left to germinate at between 3° and 5°C (Suszka, 1964, 1967; Kester, 1969b), but under normal propagation conditions seeds are removed to a higher temperature in order to ensure balanced hypocotyl/radicle growth (at low temperatures radicle growth is likely to be greater than hypocotyl growth), and to reduce the time taken to germinate. However, the germination temperature should not be too high because secondary dormancy may be induced, for example at 25°C (Michalska, 1982). At this point it should be noted that horticulturalists germinating Prunus seeds for rootstocks (to receive grafts/buds from selected clones) require simultaneous germination to produce large numbers of seedlings of a similar size. To provide this they may pre-chill at around 0°C for a considerable period (or pre-chill at 3° or 5°C for some time and then reduce the temperature to 0°C), and then subject the pre-chilled seeds to warm temperatures in an effort to achieve simultaneous emergence and uniformly developed seedlings. In other words the treatment at 0°C is intended not only to break dormancy but also to delay radicle and hypocotyl growth until the seeds are transferred to a higher temperature. With very long pre-chill treatments the risk of inducing secondary dormancy when the seeds are subsequently exposed to high temperatures is reduced, in which case alternating temperature germination regimes at relatively high temperatures can provide a further stimulation to germination, for example at 21°/27°C (10h/14h) (Lockley, 1980). Other successful alternating temperature regimes for germination are 3°/5°C or 5°/20°C

1/ Handbook of Seed Technology in Genebanks. Volume I. Principles and Methodology, and Handbook of Seed Technology in Genebanks. Volume II. Compendium of Specific Germination Information and Test Recommendations.

(16h/18h), both of which have been reported to result in higher germination than constant temperatures of 3° or 5°C for Prunus avium (Suszka, 1967).

Although, as noted above, exposure of imbibed seeds to warm temperatures can induce secondary dormancy, and although there is seldom any benefit to be gained from such treatments to non-pre-chilled seeds, a short duration exposure of seeds to such temperatures (that is, a warm stratification treatment) before pre-chilling can be of substantial benefit in promoting the germination of dormant seeds of most Prunus species (Suszka, 1962, 1964, 1967). Suszka has clearly shown that a 14-day treatment at 20°C prior to pre-chilling at 3°C is promotory for the majority of seed lots from many different sources of Prunus avium (Suszka, 1964, 1967), Prunus cerasifera, Prunus domestica, Prunus fruticosa, Prunus mahaleb, Prunus padus, and Prunus persica (Suszka, 1967). The promotion can be considerable. For example, seeds of Prunus spinosa pre-chilled for 18 weeks and then tested for germination for 10 weeks gave only 14% germination, whereas seeds given a 2-week warm stratification treatment and then pre-chilled and tested as above gave 80% germination (Gordon and Rowe, 1982). The usual warm stratification treatment temperature is 20° to 25°C (Gordon and Rowe, 1982; Grisez, 1974; Suszka, 1964, 1967), although in 1 case 30°C has been used (Laidlaw, 1983).

In Prunus avium Suszka (1967) and Michalska and Suszka (1980a) have shown that in addition to germination being promoted by a 2-week warm stratification treatment at 20°C prior to pre-chilling at 3°C, the proportion of seeds germinating is further increased by 1 or more additional short duration warm stratification treatments interspersed between pre-chilling treatments. Although 20°C is used for the first warm stratification treatment, a temperature of 25°C is more effective than 20°C for the subsequent warm stratification treatments (Suszka, 1967). In the original studies a 2-week treatment at 25°C given after a 2-week pre-chill at 3°C (which itself was applied after the first treatment of 2 weeks at 20°C) was the most promotory treatment (Suszka, 1967). In an extremely detailed investigation with 1 seed lot of Prunus avium the following alternating warm stratification/pre-chill treatments resulted in virtually full germination - a considerable promotion of germination above that obtained from a single warm stratification treatment followed by pre-chilling: 2 weeks at 20°C, then 4 cycles of 2 weeks at 3°C and 2 weeks at 25°C, then 3°C for the remainder of the treatment; or 2 weeks at 20°C, then 4 cycles of 4 weeks at 3°C and 2 weeks at 25°C, then 3°C for the remainder of the treatment; or 2 weeks at 20°C, then 2 cycles of 8 weeks at 3°C and 2 weeks at 25°C, then 3°C for the remainder of the treatment; or 2 weeks at 20°C, then 2 cycles of 14 weeks at 3°C and 2 weeks at 25°C, then 3°C for the remainder of the treatment (Michalska and Suszka, 1980a). Of these 4 alternatives the first is to be preferred for genebank purposes since full germination was achieved within the shortest period - about 9 mo overall (Michalska and Suszka, 1980a). Indeed, although the treatments at 25°C increased the total proportion of seeds germinating, the 25°C treatments also delayed the germination of the least dormant seeds within the population (Michalska and Suszka, 1980a). In subsequent similar investigations with seeds of Prunus cerasifera, Prunus domestica and Prunus spinosa, no increase in germination resulted from similar intermittent exposures to 25°C during pre-chilling at 3°C (Michalska and Suszka, 1980b). In all 3 species, however, germination was promoted by a 2-week warm stratification treatment at 20°C prior to pre-chilling (Michalska and Suszka, 1980b). Despite the absence of any promotion to the proportions of seeds germinating resulting from interrupting the pre-chill treatment with warm stratification treatments, there were sharp increases in the proportions of viable, but ungerminated, seeds at the end of the tests (detected by the indigo carmine embryo staining test - see Table 2) for Prunus cerasifera and Prunus spinosa (Michalska and Suszka, 1980b). It is not exactly clear why warm stratification, either prior to or both prior to and during pre-chill, proves so beneficial. Apart from any possible loss of dormancy per se, it is also possible that the period(s) of warm stratification enables underdeveloped embryos to develop further, or prevents seeds from dying during prolonged pre-chill treatments. Another possibility, suggested by Dr. A. Gordon, is that Prunus seeds imbibe more rapidly in an initial warm stratification regime - than they would in a pre-chill environment - and consequently respond to the subsequent pre-chilling treatment sooner than would otherwise be the case. Since difficulties with imbibition can often cause problems in seed germination tests the initial progress of imbibition in a seed lot of

Prunus avium was monitored at 2 temperatures. The results are described in a later section.

Table 1 provides a summary of recommendations of the appropriate duration of warm stratification (where it is considered necessary) and pre-chill treatments. It will be seen that the combined treatment periods can be substantial. One way of reducing these periods is to crack and remove endocarps before commencing these treatments (Toit et al., 1979). Endocarps should only be cracked and removed after the seeds have been dried (Havis and Gilkeson, 1949). Reid, Nicholls and Layne (1979) have described a mechanical device for cracking stones of Prunus species, but such devices can damage a small proportion (3 - 12%) of the seeds. Two reliable methods of extracting seeds from stones by hand are described in the next section. Of course a further problem for genebank staff will be that the pre-chill period required by individual accessions will vary considerably. It is suggested here that the pre-chill treatment be considered as the germination test regime for genebanks with checks at regular (weekly) intervals. The pre-chill/germination test environment can then be maintained for sufficient time until germination is deemed to be complete. In some cases this may require a further 30 - 60 d at circa 5°C beyond the pre-chill periods shown in Table 1. Where seeds are being germinated to produce seedlings (that is where germination is not merely required to estimate viability) it is suggested that the emerging seedlings be transferred at regular intervals to a higher temperature, say 15° to 20°C. In some accessions endocarp removal alone may enable seeds to germinate over a wide range of temperatures, but the seedlings resulting from germination at higher temperatures may be dwarf - see the section on alternative methods of seedling production.

When receiving a new Prunus accession genebanks will have to answer a number of points regarding the most appropriate method of germinating the seeds. Is a preliminary warm stratification treatment necessary? Is it necessary to interrupt the pre-chill treatment with several short duration warm stratification treatments? Is endocarp removal worthwhile? Table 1 provides some help in answering these questions, but it is unlikely to be enough. The following preliminary investigation at accession receipt is suggested where sufficient seeds (and staff to carry out the work) are available. When the accession has been dried and placed under long-term storage conditions, randomly sample 350 seeds from the accession. Randomly divide this sub-sample into 7 groups of 50 seeds and subject 1 group to each of the treatments described below (the germination tests may require 6 mo or more).

1. Estimate viability using either the tetrazolium test or the excised embryo test (see the next section)
2. Test for germination at 3° to 5°C
3. Treat the imbibed seeds at 20°C for 2 weeks, and then test for germination at 3° to 5°C
4. Treat the imbibed seeds at 20°C for 2 weeks, then subject to 4 cycles of 2 weeks at 3° to 5°C, and 2 weeks at 25°C, and then test for germination at 3° to 5°C
5. As for 2, but remove the endocarps from the dry seeds first
6. As for 3, but remove the endocarps from the dry seeds first
7. As for 4, but remove the endocarps from the dry seeds first

Remember to inform those who receive samples from the accession of the most suitable treatment for promoting germination.

The rules of the International Seed Testing Association suggest that seeds of Prunus species should be pre-chilled for 3 - 4 mo at 3° to 5°C (ISTA, 1976, 1985) but those of the Association of Official Seed Analysts provide no recommendation of a suitable dormancy breaking treatment (AOSA, 1981). The respective prescribed germination test procedures are a constant temperature of 20°C or a diurnal alternating temperature of 20°/30°C (16h/18h) applied for 28 d (ISTA, 1976, 1985), and a constant temperature of 18° to 22°C applied for 10 - 14 d (AOSA, 1981). Clearly (e.g. see Suszka, 1967), these prescriptions are inadequate for genebanks. However, the recommendations of ISTA and AOSA provide alternative procedures for assessing viability in Prunus species which are to be used in preference to germination tests - the

tetrazolium test and the excised embryo test (ISTA, 1976, 1985; AOSA, 1981).

Alternatives to the seed germination test to estimate viability

Although, of course, it is essential that genebanks are able to produce seedlings from Prunus accessions in store when required, the very long treatment periods in Table 1 which may be required to achieve full germination make it worthwhile to consider alternatives to the germination test as a method of estimating the viability of Prunus accessions in store.

The principle of the excised embryo test is that embryos are excised and maintained under conditions suitable for germination for sufficient time to allow signs of non-viability to develop. Viable embryos either show evidence of growth or remain firm and fresh. Non-viable embryos show signs of decay. A summary of a suitable procedure for Prunus follows, but the reader is referred to the following publications for a more detailed insight into the excised embryo test: Flemion (1936, 1937, 1938, 1941, 1948), Heit (1955), ISTA (1976), Tukey (1944) and Tukey and Barrett (1936).

1. Split the stony endocarp of the dry seed with a knife, scalpel, vice or pliers. There is generally a crevice where the 2 halves of the endocarp are not so tightly cemented together. The fulcrum of a cutting edge (preferably a strong blade, since scalpel blades may break) can be inserted into this crevice, twisted and the endocarp split apart. This is comparatively easy for P. avium but more difficult to achieve with P. domestica. Alternatively, using a vice or pliers, apply steadily increasing pressure at the broadest point of the longitudinal axis until the endocarp splits.

2. Remove the seeds and soak in water (in slowly running water, in standing water below 15°C, or standing water at room temperature with 2 or more changes per day) for 2 - 3 d. Alternatively imbibe the seeds between moist paper media for 5 - 7 d.

3. Now, under moderately sterile conditions in a clean draught-proof room, hold the seed by the radicle end and push the seed coat off the cotyledon end with the fingers of the other hand, and then remove the seed coat from the radicle end (but do not allow embryo or seed coat to dry out).

4. Immediately place the seeds on moist filter paper and incubate at 20°C under normal conditions of light and moisture for up to 14 d. Evaluate daily.

5. Embryos which germinate are obviously viable, whilst those with 1 or more cotyledons exhibiting growth or greening, and those which remain firm and do not discolour are also classified as viable. The greening is due to the proximity of the filter paper which removes leachate. Embryos which rapidly develop severe mould and deteriorate or decay, those which degenerate, and those which discolour are classified as non-viable, with the exception of those where discoloration occurs within 24 h of excision and is thought to be due to mechanical damage during excision.

The principle of the (topographical) tetrazolium test is that the colourless, water soluble, triphenyl tetrazolium chloride is reduced by dehydrogenases present within live embryo tissues to the red, insoluble, formazan so that the areas of live tissues within each embryo can be mapped and thereby an assessment made of the viability (before the test) of the embryo. The problem with the test is that this assessment is subjective and requires considerable experience. A summary of a suitable procedure follows but the reader is referred to publications of the International Seed Testing Association (ISTA, 1976, 1985) for more detailed information.

1. Split the dry stony endocarp as already described.

2. Remove the seeds and place between moistened filter paper overnight after scratching the seeds on the surface of the cotyledons opposite the radicle.

Table 1. Summary of recommended warm stratification and pre-chill periods for Prunus seeds

Species	Recommended period (weeks)		Author(s)
	Warm stratification	Pre-chill	
Prunus alleghaniensis	0	21	Grisez (1974)[1]
Prunus americana	0	13-21	Giersbach and Crocker (1932)
Prunus amygdalus	0	9	Kester (1969a)
Prunus angustifolia	0	9-18	Grizez (1974)[1]
Prunus armeniaca	2	27	Suszka (1967)[2]
Prunus armeniaca	2	11-18	Gordon and Rowe (1982)
Prunus avium	2	18	Gordon and Rowe (1982)
Prunus avium	2	30	Suszka (1964)
Prunus avium	2	27-33	Suszka (1967)[2]
Prunus avium	0[3]	22-26	Fogle and McCrory (1961)
Prunus avium	10[4]	28[4]	Michalska and Suszka (1980a)
Prunus besseyi	0	18	Grisez (1974)[1]
Prunus cerasia	0-8	12-16	Evenari, Konis and Zirkin (1948)
Prunus cerasifera	2	27	Suszka (1967)[2]
Prunus cerasifera	2	18	Gordon and Rowe (1982)
Prunus cerasifera	2	32	Michalska and Suszka (1980b)
Prunus cerasus	0	20	Havis and Gilkeson (1949)
Prunus domestica	2	27	Suszka (1967)[2]
Prunus domestica	2	35	Michalska and Suszka (1980b)
Prunus dulcis	0	9	Gordon and Rowe (1982)
Prunus emarginata	0	13-18	Grisez (1974)[1]
Prunus fruticosa	2	27	Suszka (1967)[2]
Prunus ilicifolia	0	13	Grisez (1974)[1]
Prunus insititia	0	12-16	Grisez (1974)[1]
Prunus laurocerasus	0	9	Gordon and Rowe (1982)
Prunus lusitanica	0	9	Gordon and Rowe (1982)
Prunus mahaleb	2	27	Suszka (1967)[2]
Prunus munsoniana	0	12-14	Tukey (1924)
Prunus padus	2	30	Suszka (1967)[2]
Prunus padus	2-4	18	Gordon and Rowe (1982)
Prunus pensylvanica	9	13	Grisez (1974)[1]
Prunus pensylvanica	4[5]	14[5]	Laidlaw (1983)

Table 1. Summary of recommended warm stratification and pre-chill periods for Prunus seeds (cont'd)

Prunus persica	0	14-15	Crocker and Barton (1931)
Prunus persica	2	18	Gordon and Rowe (1982)
Prunus persica	2	27-34	Suszka (1967)[2]
Prunus pumila	9	17	Grisez (1974)[1]
Prunus sargentii	2-4	18	Gordon and Rowe (1982)
Prunus serotina	4-8	17	Huntzinger (1971)
Prunus serotina	2	27	Suszka (1967)[2]
Prunus serotina	2-4	18	Gordon and Rowe (1982)
Prunus spinosa	0	24	Grisez (1974)[1]
Prunus spinosa	2	18	Gordon and Rowe (1982)
Prunus spinosa	2	32	Michalska and Suszka (1980b)
Prunus subcordata	0	13	Grisez (1974)[1]
Prunus tomentosa	0	9-13	Tukey (1924)
Prunus virginiana	0	17-23	Grisez (1974)[1]
Prunus virginiana	2	18	Gordon and Rowe (1982)
Prunus virginiana	0	16-24	Lockley (1980)

Notes

1 See Grisez's review for original references

2 The first seeds are reported to begin to germinate between weeks 11 and 14 or the pre-chill treatment, except in extreme cases where the delay can be as great as 18 weeks

3 No warm stratification treatment, but pre-soak in 400 ppm GA_3 before the pre-chill treatment

4 Complete treatment as follows: 2 weeks at 20°C, then 4 cycles of 3°/25°C (2w/2w), then 3°C for 20 weeks

5 Complete treatment as follows: 2-day pre-soak in 0.05 M calcium nitrate, then alternate between 5 d at 30°C and 5 d at 5°C for 30 d, then pre-chill for 60 d at 5°C with a further 30 d at 1° to 2°C, then pre-soak in 0.05% GA_3 for 1 d, and then germinate at 30°C

3. Provided the seeds have imbibed and swollen (if they have not then return to the imbibition medium for a few hours more), remove the seed coat and endosperm (where present) as described above.

4. Immerse the seeds in a 1% buffered solution of triphenyl tetrazolium chloride (10g of tetrazolium in 1 litre of buffer, where the buffer is prepared by mixing together two-thirds of a litre in which 6.052g of monobasic potassium phosphate have been dissolved with one-third of a litre in which 3.959g of dibasic sodium phosphate have been dissolved) for 18-20 h at room temperature, or 4-8 h at 30°C. Alternatively, immerse the seeds in a 0.5% tetrazolium solution for 10-12 h at 30°C.

5. Rinse the seeds with water and maintain in water at 3 - 5°C until they can be evaluated.

6. Viable embryos are classified as those completely stained (red), plus those in which unstained tissue is limited to the tip of the radicle or a small proportion of the surface area of the cotyledons provided the area of dead tissue is at the opposite end of the embryo to the embryonic axis.

If either procedure is to be used as a rapid viability test for Prunus seeds then it is essential for the operator to have developed sufficient expertise and to have calibrated the test. Calibration requires the comparison of the results of the test against estimates of viability derived from germination tests for many accessions of different viability of the species under consideration from which dormancy has been removed. Thus although the 2 procedures are comparatively rapid, a good deal of preparatory work is essential before they can be applied with confidence.

Alternatives to seed germination as a method of seedling production

Embryos extracted from endocarps as already described can, if wished, be cultured in a sterile artificial medium to produce seedlings rapidly (Davidson, 1933; Turkey, 1934d). This can be particularly useful for breeders in cases where the embryo is comparatively underdeveloped (Davidson, 1933). The reader is referred to Tukey (1934d) for details of such procedures and only a brief summary is provided here. Davidson (1933) found sterile sand culture to be preferable to agar culture and used a nutrient solution with the following molar proportions of salts: 0.00633 M monobasic potassium phosphate, 0.0073 M calcium nitrite, 0.00237 M magnesium sulphate, and 0.0014 M ammonium sulphate; plus 0.5 ppm iron as ferrous sulphate and 1% glucose. Tukey (1934d) found agar culture to be preferable to liquid culture and used the following medium (concentrations in grams per litre): 0.68 g/l potassium chloride, 0.17 g/l calcium sulphate, 0.17 g/l magnesium sulphate, 0.17 g/l tribasic calcium phosphate, 0.17 g/l ferric phosphate, 0.14 g/l potassium nitrate, 6.5 g/l agar, and 5 g/l glucose. As with the excised embryo test, 20°C is a suitable incubation temperature but seedlings cultured or germinated in warm conditions from non-pre-chilled embryos or seeds may be dwarf (Baillon, 1882; Davidson, 1933; Flemion, 1934; Tukey, 1934d); 6 - 8 weeks treatment of these seedlings at around 5°C enables the seedlings to resume normal growth after their return to warmer temperatures (Flemion, 1934; Tukey, 1934d).

Drying and storage

It would appear that confusion regarding the seed storage characteristics of Prunus species has mainly arisen as a result of these species' requirement for a considerable period of pre-chilling to remove dormancy. Many of the original investigations with dried Prunus seeds were carried out to determine whether dry seed storage resulted in loss in dormancy.

For example, Tukey (1924) reported that all treatments in which peach seeds were dried resulted in no germination whereas undried seeds which had received a substantial pre-chill treatment did germinate. That is, drying was not successful in breaking dormancy, but this was not an investigation of the effect of drying on viability. Moreover, in the same paper Tukey showed that previously dried seeds of sour cherry

(Prunus cerasus) germinated provided the seeds were given a sufficient subsequent stratification (pre-chill) treatment and stated that the observation that cherry stones should not be dried was erroneous (Tukey, 1924).

Similarly, although Havis and Gilkeson (1949) reported that "it seems essential that the seeds be kept moist during storage" since seeds stored dry for up to 8 mo did not germinate, once again the failure was one of not promoting loss in dormancy. Indeed it is clear from this investigation that Prunus seeds are not damaged by desiccation since the "successful" dormancy-breaking treatment involved drying the seeds for 3 d and then re-imbibing - to assist in the cracking of stones (Havis and Gilkeson, 1949).

A further note concerning desiccation which is relevant has been provided by Haut (1932). He reported a deleterious effect on germination of drying after a pre-chill treatment, whereas drying and then re-imbibing before pre-chilling was not deleterious and concluded that embryos in a completely after-ripened condition was susceptible to injury from desiccation (Haut, 1932). Genebanks will only pre-chill seeds after drying and storage. Thus the relevant point to note is that Haut provides further evidence demonstrating that Prunus seed are not damaged by desiccation. Nevertheless a possible explanation of Haut's observations is provided later.

Finally, another comment which may require some explanation is the suggestion that seeds of mazzard cherry (Prunus avium) should not be stored at -1°C and 10% moisture content for any longer than 32 weeks (Suszka, 1978). It is important to note that the reason for this limited storage period has nothing to do with loss in viability. It refers to the practicalities of distributing previously pre-chilled seeds which can then be immediately sown on arrival at their destination: the promotive effect on germination of a pre-chill treatment prior to drying begins to be lost after about 32 weeks storage in the above environment (Suszka, 1978).

Thus although moist Prunus seed storage has been repeatedly advocated for short-term storage (over 1 winter), drying is not damaging and is essential for medium- and long-term storage (Holmes and Buszewicz, 1958; Grisez, 1974). Despite this advice 1 factor which causes concern to some is the prevalence of literature in which Prunus seeds have not been dried. These are mainly investigations into seed dormancy, which have been carried out with freshly-extracted seeds - where there has been no requirement for storage. However, in some investigations Prunus seeds have been routinely dried without problem prior to investigating seed dormancy. The species successfully dried include: Prunus amygdalus and Prunus amygdalus x Prunus persica (Kester, 1969b); Prunus laurocerasus (Simancik, 1970); Prunus persica (Paul and Biggs, 1963); and Prunus virginiana (Lockley, 1980); but in these reports few details were provided. Suszka, however, has provided more detailed information. He has successfully dried Prunus armeniaca to 6.0-6.4% moisture content, Prunus avium to 9.3-11.0%, Prunus cerasifera to 7.4-8.1%, Prunus mahaleb to 8.1-8.3% and Prunus serotina to 7.2-8.1% with no loss in germination (Suszka, 1962). In a subsequent investigation seeds of Prunus avium were successfully dried to 7.5% moisture content (Suszka, 1967). Finally, Toit et al., (1979) have successfully dried seeds of Prunus persica to 8% moisture content.

The surface of Prunus seeds will dry within a few hours of extraction from the fruit. Typical equilibrium moisture contents of seeds exposed to temperate laboratory atmospheres for about 6 d is between 12 and 15% (Huntzinger, 1971). At 30°C a ventilated oven will dry Prunus seeds from 14% to around 5% within about 3 h (Huntzinger, 1971), but the use of a drying room at 15°C with about 10% RH and good air circulation is recommended for genetic resources conservation (Cromarty, Ellis and Roberts, 1982). In a genebank drying room Prunus avium stones dry to below 6% moisture content (wet weight basis) within 4 d (Fig. 1). It is worthwhile allowing the stones or seeds to remain in the drying room for a few more days after average seed moisture content has fallen below 6% moisture content, however, to ensure that the moisture content has equilibrated within and between the stones. In freshly-collected stones the seed moisture content is considerably higher than that of the stones from which they have been extracted (Figs. 1 and 2) and - not surprisingly - the extracted seeds dry more rapidly than whole seeds (Fig. 1). In a genebank drying room the time taken by seed accessions to dry to the moisture content range suitable for

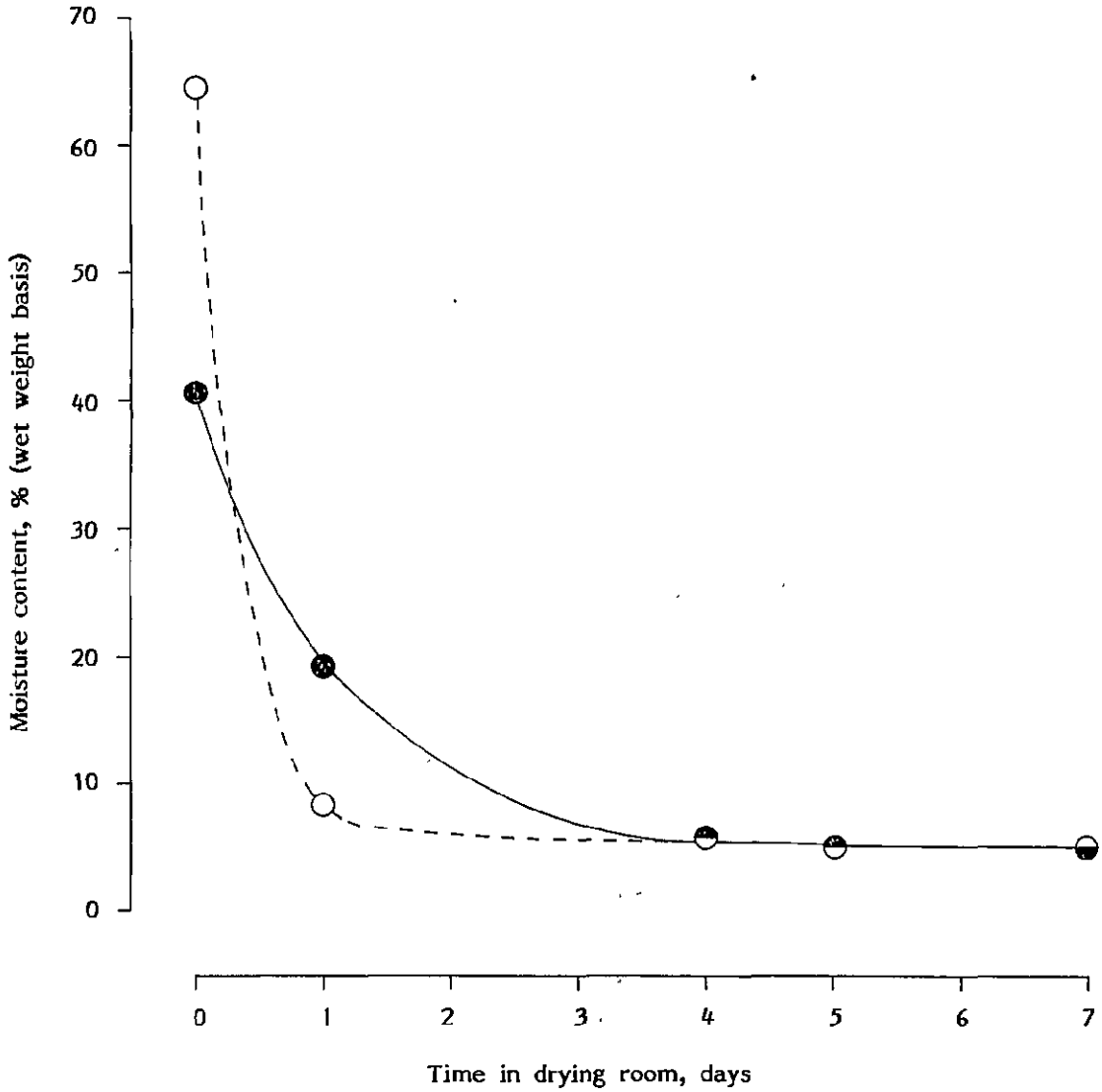

Fig. 1. Drying curves of freshly-extracted seeds of Prunus avium with endocarps (closed circles) and without endocarps (open circles) in a drying room maintained at 15°C with 15% RH. Seeds were washed in distilled water after extraction and then blotted to removed excess moisture. Seed moisture content was determined by drying at 105°C for 17±h. (Re-drawn from data provided by R.D. Smith, Royal Botanic Gardens Kew, UK.)

long-term storage (4-6%) will vary - largely due to differences in seed size. Cromarty et al. (1982) have provided a seed drying nomogram which can be used to estimate the drying period required. For a list of 1,000 seed weights for Prunus species (to be used in the seed drying nomogram) see Ellis (1984).

According to Grisez (1974) there is general agreement that excessive drying of Prunus seeds is detrimental, but what is excessive is not usually defined. Moreover, this qualification is reported to concern only short-term storage (Grisez, 1974). It seems likely that the problem underlying the suggestion that excessive drying is detrimental for short-term storage is the time taken by the dried seeds to imbibe moisture subsequently. Dry, circa 8% moisture content, intact Prunus seeds may take

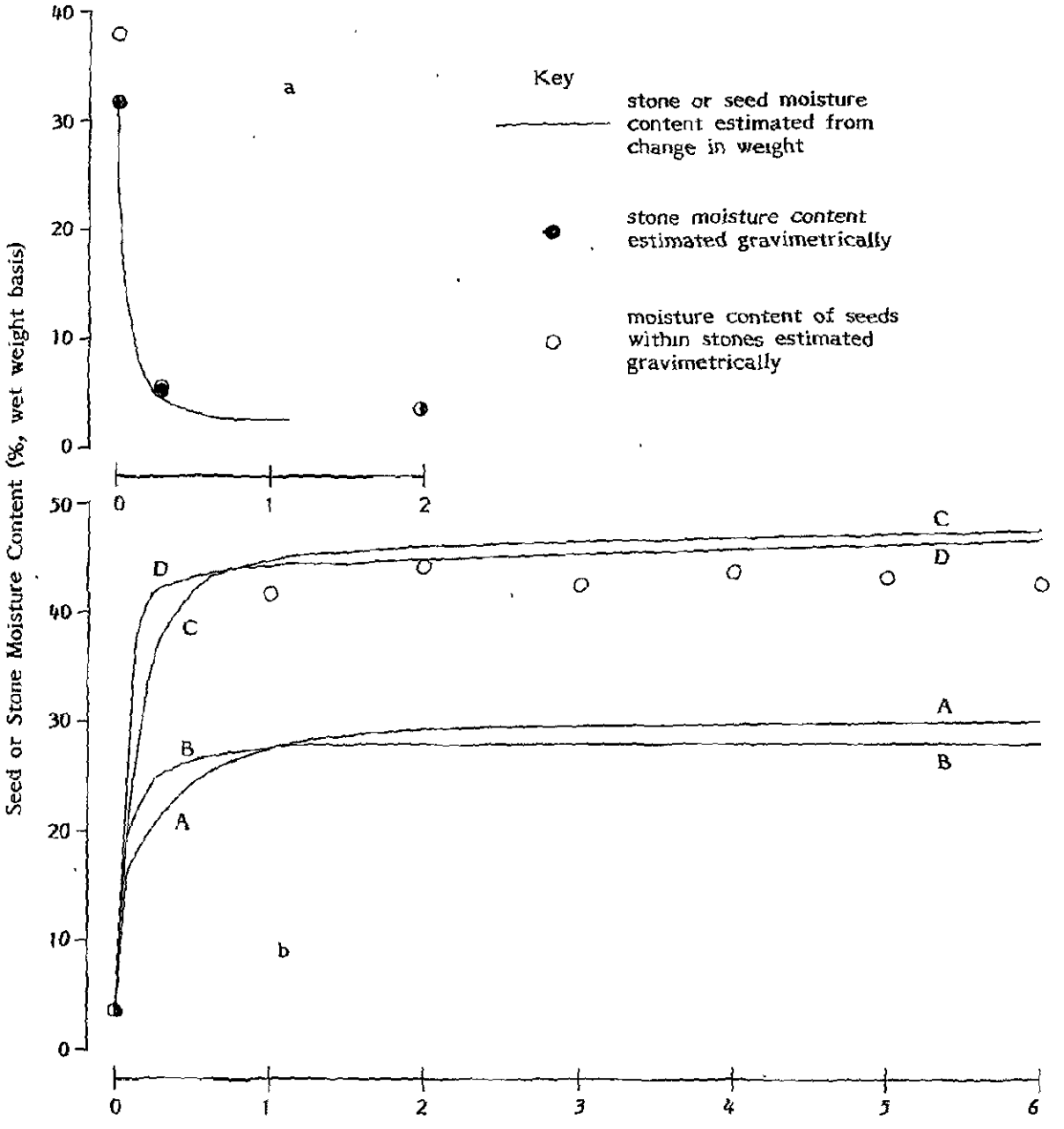

Fig. 2. Change in moisture content of Prunus avium stones and seeds in a drier at 28°C with 10-20% RH (a) and between moist rolled paper towels (b) at 3°-5°C (A,C) or 20°C (B,D). In b curves A and B represent stone moisture content, the open circles the moisture content of seeds imbibed within these stones, and curves C and D the moisture content of seeds imbibed without endocarps.

between 40 and 60 d to become fully imbibed (Toit et al., 1979); removing the endocarp can halve this time (Toit et al., 1979). The extended period of imbibition may explain why Haut (1932) was unable to germinate pre-chilled seeds after drying whereas Suszka (1978) was able to. That is in Haut's investigation the dried seeds may not have had sufficient time to imbibe (imbibition was presumed by Haut to have occurred within a 2-day soak) before the germination tests were concluded. Some indication that this explanation of Haut's results may be plausible has been provided by Blazek, Kares and Kloutvor (1981). They have shown that soaking Prunus seeds prior to pre-chilling

reduces the pre-chill treatment period required to promote germination. Although the endocarp does appear to regulate (delay) water uptake (Toit et al., 1979, Fig. 2), the problem is not one of hardseededness (where in legumes, for example, the testa prevents seeds from imbibing): Tukey (1924) reported that the endocarps of Prunus persica, Prunus avium, Prunus cerasus, Prunus munsoniana and Prunus domestica were permeable to moisture. See also the later section on the imbibition of dry seeds of P. avium. Finally Suszka provides evidence which confirms that the reported problems concerning drying are related to dormancy and the efficacy of dormancy-breaking treatments, not viability. He has shown that drying Prunus seeds does appear to result in increased dormancy - since viability is maintained during drying whereas germination - after a standard dormancy-breaking treatment - is reduced (Suszka, 1967).

Although moist storage is often used for short storage periods, dry storage of peach seeds under uncontrolled conditions may be adequate for the short-term storage of seeds for rootstock production. After 1 or 2 years dry storage, subsequent field emergence declined by only 8-12% and 16% respectively in one investigation by Brase (1949). Moreover, although the seedlings from the dry stored seeds were initially weaker than those from fresh seeds, by budding time seedlings from the different sources could not be distinguished (Brase, 1949). However, after 3-years' dry storage in uncontrolled conditions, viability (estimated by the excised embryo test) had been lost completely (Brase, 1949). Suszka (1964) recommends sealed storage at 3°C and 8-12% moisture content where storage of Prunus seeds is required for more than 8 weeks.

For storage over the medium-term (5 years) Bonner (1978) recommends that seeds of Prunus serotina be stored at 0° to 5°C with 5-10% moisture content, whilst Gordon and Rowe (1982) recommend 3° to 5°C with 12% moisture content for Prunus avium, Prunus padus and Prunus spinosa. Holmes and Buszewicz (1958) state that seeds of Prunus species can be stored dry satisfactorily for 2 or 3 years and probably longer if the temperature is low, but provide no specific recommendation. However, they do note that sub-freezing storage is necessary for the long-term storage of seed of Prunus virginiana. One puzzling (at first sight) recommendation has been provided by Huntzinger (1971): before cold, dry storage Huntzinger recommends that seed be kept in warm storage for 4 weeks or more. This is intended as a warm stratification treatment (Huntzinger, 1971). Consequently it is recommended that genebanks ignore this suggestion and that the warm stratification treatment, if required - see Table 1, be provided as a component of the germination test/dormancy-breaking procedure after storage.

The reported germination of Prunus seeds following various periods of dry storage under different environments are summarized in Table 2. A cautionary note is required regarding Prunus seed moisture contents. Figures are generally for whole stones, but Suszka (1970) has reported that at equilibrium the moisture content of extracted seeds can be 3% less than the stone moisture content. This is a rather surprising observation since embryos extracted from fresh seeds are at a higher moisture content than the whole seeds (Figs. 1 and 2). In the main the species shown in Table 2 are limited to those where seed is traded for the timber industry or for environmental forestry. The absence of the top fruit Prunus species from this list is a result of the lack of demand for long-term storage of these species in the commercial seed industry: the only requirement has been for short-term (overwinter) storage by breeders either for breeding programmes or for rootstock production.

The results of Giersbach and Crocker (1932) demonstrate the advantage of reducing storage temperature - at least to 7° or 10°C (Table 2). The results of the investigation reported by Huntzinger (1971) and Grisez (1976) are somewhat variable. Nevertheless their results clearly show that it is better to store seeds at 4-6% than at 11-13% moisture content: at the higher moisture content and 0.5° to 5°C loss in viability was complete after 5-years' storage (Table 2); and at -18° to -14°C it appears that 11-13% moisture content was sufficiently high for freezing damage to have occurred and immediately killed the majority of seeds (Table 2). At the lower moisture content there is less evidence of loss in viability under both temperature regimes - the germination test results (only 100 seeds each test) being somewhat erratic - but after 8-years' storage there is a suggestion that -18° to -14°C may be a more preferable storage temperature than 0.5° to 5°C since germination is 10% higher in the former

Table 2. Germination of seeds of Prunus species after dry storage reported in the literature

Author(s)	Species	Storage Period	Temperature	Moisture Content	Germination %
Giersbach and Crocker (1932)	*P. americana*	18 months	7°-10°C	dry	70
"	*P. americana*	53 months	7°-10°C	dry	45
"	*P. americana*	18 months	laboratory	dry	72
"	*P. americana*	53 months	laboratory	dry	16
"	*P. americana*	18 months	30°C+	dry	62
"	*P. americana*	53 months	30°C+	dry	0
Solovieva (1966)	*P. avium*	7 years	-5°C	c.10%?	91-97
Solovieva (1978)	*P. avium*	15 years	-5°C	c.10%?	98
Suszka (1970)	*P. avium*	55 months	1°C	11%	84
"	*P. avium*	55 months	1°C	11%	(88)*
Michalska (1982)	*P. avium*	8-12 months	3°C	9-11%	98-100
Michalska and Suszka (1980c)	*P. avium*	207 days	-3°C	8.6%	(100)*
"	*P. avium*	214 days	-3°C	9.0%	(99)*
"	*P. avium*	570 days	-3°C	8.6%	(100)*
"	*P. avium*	571 days	-3°C	9.5%	(94)*
"	*P. avium*	935 days	-1°C	8.3%	(99)*
Michalska and Suszka (1980d)	*P. avium*	213 days	-3°C	9.0%	(99)*
"	*P. avium*	214 days	-3°C	8.9%	(98)*
"	*P. avium*	568 days	-3°C	8.6%	(100)*
Laidlaw (1983)	*P. pensylvanica*	2 months	-18°C	dry	95
Heit (1967)	*P. pensylvanica*	6 years	1°-3°C	low	74
"	*P. pensylvanica*	10 years	1°-3°C	low	76
Huntzinger (1971)	*P. serotina*	1 year	-18°to-14°C	4-6%	52
"	*P. serotina*	2 years	-18°to-14°C	4-6%	81
"	*P. serotina*	3 years	-18°to-14°C	4-6%	81
Grisez (1976)	*P. serotina*	5 years	-18°to-14°C	4-6%	47
"	*P. serotina*	8 years	-18°to-14°C	4-6%	66
Huntzinger (1971)	*P. serotina*	1 year	-18°to-14°C	11-13%	4
"	*P. serotina*	2 years	-18°to-14°C	11-13%	7
"	*P. serotina*	3 years	-18°to-14°C	11-13%	1
Grisez (1976)	*P. serotina*	5 years	-18°to-14°C	11-13%	4
"	*P. serotina*	8 years	-18°to-14°C	11-13%	0
Huntzinger (1971)	*P. serotina*	1 year	0.5°-5°C	4-6%	63
"	*P. serotina*	2 years	0.5°-5°C	4-6%	81
"	*P. serotina*	3 years	0.5°-5°C	4-6%	90
Grisez (1976)	*P. serotina*	5 years	0.5°-5°C	4-6%	69
"	*P. serotina*	8 years	0.5°-5°C	4-6%	56
Huntzinger (1971)	*P. serotina*	1 year	0.5°-5°C	11-13%	72
"	*P. serotina*	2 years	0.5°-5°C	11-13%	88
"	*P. serotina*	3 years	0.5°-5°C	11-13%	77
Grisez (1976)	*P. serotina*	5 years	0.5°-5°C	11-13%	0
"	*P. serotina*	8 years	0.5°-5°C	11-13%	0

* Viability determined by indigo carmine embryo staining test (2 hours in 0.05% solution at 20°C).

(Table 2). Moreover, it is clear from Solovieva's results that long-term storage at sub-zero temperatures and low moisture contents for seeds of Prunus species is feasible. Indeed it is suggested that there is a little point in carrying out regular monitoring tests of Prunus accession viability under IBPGR preferred long-term storage conditions unless a reliable test procedure for estimating viability has been developed. (That is, errors in the germination test procedures are more likely than loss in viability in the first few years.)

Although Table 2 demonstrates that long-term Prunus seed storage is feasible and that the IBPGR preferred storage conditions (minus 18°C or less with 5 ± 1% moisture content) are appropriate for these species the information available at present (Table 2) does not enable the likely regeneration interval to be estimated. Nevertheless there is clearly no need for Prunus accessions of high viability which are stored hermetically under IBPGR preferred conditions to be monitored for viability within the first 10 years.

Investigation of the progress of imbibition in seeds of Prunus avium at 3° to 5°C and 20°C

In nursery sowings of freshly-harvested but dry stones of P. avium and P. padus, Gordon (personal communication) has observed that a 4-week warm stratification treatment at 20°C prior to pre-chilling can be an extremely effective dormancy-breaking treatment. During the warm stratification treatments he has observed that a high proportion of the stones split and has suggested that the effectiveness of warm stratification may result from an increased rate of seed imbibition. The aim of the investigation described below was to determine whether warm stratification did affect the rate of imbibition.

Freshly-collected fruits of P. avium were provided by Dr. Gordon. The stones were extracted by hand, repeatedly rinsed to remove flesh and juice, blotted dry and then placed in a seed drier maintained at between 28° to 32°C with 10 - 20% RH for 8 d. The stones were regularly weighed to monitor loss in moisture. The dried stones were sealed in a laminated aluminium foil packet and maintained at 20°C for 6 d to allow moisture to equilibrate within and between the stones. Moisture contents of stones and seeds from which the endocarps had been removed were determined gravimetrically by drying ground material in an oven at 103° ± 2°C for 17 h. For very moist seeds or stones a 2-stage procedure was employed: the seeds or stones were first dried for 24 h at room temperature prior to grinding and drying as above. Moisture contents are expressed on the wet weight basis. The stones dried to below 5% moisture content within 1.75 d, the seeds within the stones losing moisture only slightly less rapidly (Fig. 2a). After equilibration seed and stone moisture contents did not differ (Fig. 2a).

The dried stones were then divided randomly into 8 sub-lots of approximately 130 stones each. In 1 sub-lot the seeds were extracted from the stones with a knife using the method described earlier. These seeds without endocarps were then divided into 2 groups, weighed and then placed between moist rolled paper towels within polyethylene envelopes, either at 20°C or at 3° to 5°C. Several times each week for 6 weeks the seeds were removed from the paper towels, weighed and then replaced on the paper towels. After 6 weeks seed moisture contents were determined gravimetrically. A further sub-lot of dried stones was placed between moist paper towels within polyethylene envelopes at 3° to 5°C and regularly weighed and the moisture content of stones, endocarps and seeds without endocarps was determined after 6 weeks. Finally the remaining 6 sub-lots were placed between moist paper towels within polyethylene envelopes at 20°C and regularly weighed. Each week for 6 weeks 1 sub-lot was removed and the moisture content of stones, endocarps and seeds without endocarps determined gravimetrically.

Both stones and seeds without endocarps imbibed moisture more rapidly at 20°C than at 3° to 5°C during the first week, but thereafter moisture contents at the lower temperature were marginally higher (Fig. 2b). No stones split during the entire investigation. Table 3 shows the gravimetric determinations of seed, stone and endocarp moisture contents after 6 weeks imbibition. The seed moisture contents were 3 - 5% lower for seeds imbibed within endocarps (see also the open circles in Fig. 2b) than

those imbibed after endocarp removal. The moisture content of seeds imbibed within endocarps at 3° to 5°C was approximately 2% higher than those at 20°C. These results suggest, then, that warm stratification treatments only marginally increase the rate of imbibition during the first week and that this factor cannot account for the promotion of germination obtained from the use of such treatments. It should be noted, however, that the warm stratification treatments applied in nursery sowings differ slightly from those applied here. In this investigation a temperature of 20°C was maintained in a cooled incubator and the temperature and moisture regimes were constant. In the nursery sowings the seed beds were heated from below (bottom heat) and changes in ambient temperature would have been likely to result in fluctuations in both temperature and moisture. Also, in the practice described the commercial seed lots were only dried to 12-14% moisture content and this factor may also account for the difference between this investigation and the commercial practice.

Table 3. Moisture contents (wet weight basis, determined gravimetrically) of Prunus avium stones and seeds imbibed within and without endocarps after 6 weeks imbibition on moist rolled paper towels at 20°C and at 3° to 5°C

Imbibition temperature	Stones	Endocarps	Seeds	
			imbibed within endocarps	imbibed without endocarps
3°C to 5°C	31.3	23.4	44.6	47.7
20°C	29.6	23.0	42.5	47.9

Summary of recommended procedures

1. Delay harvest until fruit is ripe, extract seed from fruit and clean off pulp and juice by washing copiously in running water, except in almond which should be harvested when the hull splits.

2. Prunus species have orthodox seed storage characteristics and must be dried for long-term storage: surface dry endocarps, and then dry seeds in a drying room (spread seeds out) at 15°C with 10% RH until moisture content is reduced to between 4 and 6%.

3. Seal the dried seeds in hermetic containers and store at -20°C.

4. Allow sufficient time for seeds to imbibe during germination tests; endocarp removal (carry out with dry seeds) will reduce this delay to germination.

5. Dormancy-breaking treatments are almost certainly required: the pre-chill treatment (3° to 5°C) can be considered as an extended germination test treatment, but as many as 6 mo may be required (Table 1); in addition a single prior warm (20° to 25°C) stratification treatment (Table 1), or several cycles of warm stratification/ pre-chilling may also be necessary; a simple - but lengthy - procedure is provided for determining a suitable germination test regime for each accession.

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SEED STORAGE AND GERMINATION OF APPLE AND PEAR 1/

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Introduction

The purpose of this report is to provide information on apple and pear seed storage and germination in order that:

(1) The potential role of seed storage in conserving genetic variation in these species can be assessed;

(2) A suitable methodology is available to those who wish to maintain apple and pear seed collections.

Although this report relates to storage of seed in conventional genebanks and the routine monitoring of viability, it is pointed out that for material such as apples and pears, field genebanks (clonal repositories) are an essential adjunct for genetic conservation programmes.

Dormancy stratification and germination

Apple and pear seeds extracted from fresh, mature fruit are normally dormant and fail to germinate when tested for germination (e.g. Bakke et al., 1926; Westwood and Bjornstad, 1967). This dormancy can be overcome in both species by stratification treatments where the moist seeds are exposed to low temperatures (about 5°C) for up to 6 mo (Abbot, 1955; Crocker and Barton, 1931; Dall'Orto et al., 1978; Harrington and Hite, 1923; Karnatz, 1969; USDA, 1961; Westwood and Bjornstad, 1968). On return to higher temperatures the seeds germinate within a few days and the longer the period of stratification the sooner germination occurs (e.g. Bakke et al., 1926; Sanada et al., 1980a). In fact, radicle emergence does eventually occur at 0° to 5°C (Crocker, 1928; Sanada et al., 1980a) but higher temperatures are required for hypocotyl growth.

Many authors have demonstrated that seeds from different crosses and sites require different stratification periods to break dormancy. For example, apple seeds extracted from fruits harvested later required shorter stratification periods than seeds from fruits harvested earlier (Sanada et al., 1980b); similarly those extracted from decaying fruits generally required shorter stratification periods than those extracted from healthy fruits (Pustovoitova and Rudnicki, 1977). Seeds of pear cultivars from warm winter climates required less chilling than those from colder climates (Westwood and Bjornstad, 1968). In addition, the former cultivars had higher optimum chilling temperatures (7° to 10°C) than the latter (3° to 5°C), but stratification at 5°C did eventually (150 d) result in full germination of seeds of all 14 pear species investigated (Westwood and Bjornstad, 1968).

It has been suggested that apple and pear seeds require stratification for 90 - 120 d (USDA, 1961). However, the stratification period required to break dormancy is greatly influenced by the temperature at which the seeds are subsequently set to germinate (e.g. Sanada et al., 1980a). This is because secondary dormancy can be induced when the moist seeds are placed in ambient laboratory temperatures or higher. This was first demonstrated by Abbot (1955) with apple seeds. He showed stratification to be a reversible process because dormancy could be re-imposed by subjecting an incompletely stratified seed to a warm temperature (Abbot, 1955). Between the low temperatures at which dormancy is removed and the high temperatures at which dormancy is

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re-imposed there is a compensation temperature of 17°C at which the rate of both reactions is apparently balanced because of the capacity of the seed to germinate remains constant (Abbot, 1955). Abbot's results showed that the stratification period could be halved if apple seeds were germinated at 17°C instead of 25°C. These results have been confirmed by others (e.g. Perino and Come, 1977; Sanada et al.,1980b; Visser, 1956b). Secondary dormancy induced by these high temperatures can also be removed by further stratification (Sanada et al., 1980b).

Removing the covering structures can enable the excised embryos from apple and pear seeds to germinate at ambient temperatures (Flemion, 1934; Omura et al., 1978; Westwood and Bjornstad, 1968). The coverings of the embryos in mature seed consist of 3 layers. A thick brown fibrous outer seed coat with an open hilum; a thin translucent inner seed coat of very dense structure and without openings; and a delicate creamy white cellular tissue slightly thicker than the inner coat and apparently endospermous. All 3 seed coats can be removed easily from the imbibed seed either with forceps or by applying pressure to the seeds between the thumb and index finger. However, the seedlings obtained from non-stratified excised embryos exhibit abnormal (dwarf-like) growth (Flemion 1934; Harrington and Hite, 1923). Normal growth is resumed after some 8 mo of this abnormal growth, but the dwarfing can be avoided either by stratifying the seed or by exposing the seedlings to low temperatures (Flemion, 1934).

For many apple seed lots extracted from fruits at harvest, seed coat removal alone is insufficient to avoid dormancy, but the periods of stratification required to break dormancy can be substantially reduced by seed coat removal (Abbot, 1955; Visser, 1954, 1956a). For example; to achieve 50% germination when tested at 25°C, intact apple seeds required 65 d stratification, 42 d if germinated as embryo and endosperm, but only 30 d if germinated as excised embryos (Visser, 1954). Removal of about 10% of the endospermous layer (either at the top of the cotyledons or the base of the hypocotyl) was almost as effective as complete removal (Visser, 1954). Although in one investigation it did not matter whether the embryos were excised before or after stratification (Visser, 1956a), the balance of advantage lies with stratifying seeds intact and excising the embryos after stratification. This is done because, firstly, it sometimes gives higher germination than excising the embryos before stratification (Visser, 1954), secondly because germination proceeds more rapidly if the embryos are excised after stratification (Visser, 1956a), and thirdly, because the seeds are less prone to fungal deterioration (Visser, 1956a; Ellis, unpublished).

If the technique of embryo excision and the avoidance of secondary dormancy are combined, the stratification period required can be substantially reduced. 120 d of stratification at 5°C were necessary to give 100% germination of intact apple seeds at 25°C, 65 d were necessary for 100% germination of intact seeds at 17°C, but only 20 d stratification were required for 100% germination of the excised embryos at 17°C (Abbot, 1955).

Stratification and seed coat removal are not the only techniques that have been investigated for their effectiveness in promoting the germination of dormant apple seeds, but none has been found that approach the effectiveness of these combined treatments. Harrington and Hite (1923) investigated whether dry storage broke dormancy by after-ripening, but found no effect. Thévenot and Come (1978) found that it was possible with high temperature treatments (30-35°C) on moist seeds to reduce dormancy when the seeds were subsequently tested at 20°C but that these treatments were not totally effective. Sanada et al., (1980b) found that neither thiourea (0.05, 0.1, 0.5, or 1.0%), potassium nitrate, (0.05, 0.1, 0.5, or 1.0%), hydrogen peroxide (1, 3, 5 or 10%), ethephon (0.1, 0.2, 0.5 or 1.0%), kinetin (1, 5, 10, 50 or 100 ppm), nor gibberellic acid (5, 10, 50, 100, or 200 ppm) had any effect on promoting the germination of dormant apple seeds. Soaking in 150 ppm gibberellic acid or 5,000 ppm thiourea for 36 h followed by 28 d stratification was effective in promoting the germination of wild pear seeds (Dhillon and Sharma, 1978) and soaking in ethephon (250 ppm) with 60 d stratification promoted apple seed germination (Sinha et al., 1977), but it is not clear in either case whether the promotion was the result of the growth regulator treatments or the stratification treatments. However, in certain circumstances GA_3 can substitute for a small period of the stratification requirement

of dormant pear seeds (Westwood and Bjornstad, 1968). Although it is clear that growth regulators can cometimes promote the germination of dormant apple and pear seeds, their influence appears to be inconsistent and thus unreliable.

Badizadegan and Carlson (1966) reported the following method as a rapid viability test for apple seeds. Concentrated sulphuric acid was used to rupture and partially remove the seed coats. The seeds were then washed and soaked in a solution of 10 mg/l of N^6 benzyl adenine for 24 h. As an alternative a solution of 15 mg/l N^6 benzyl adenine with 150 mg/l gibberellic acid was also used. The seeds were then placed on moist filter papers in petri dishes and grown under continuous light at room temperature. Apparently viable embryos showed some germination whereas non-viable embryos remained unchanged or deteriorated (Badizadegan and Carlson, 1966). It is not clear whether the "viable embryos" were capable of germination and continued growth following this treatment, and there is some evidence from other sources that this would be unlikely following concentrated sulphuric acid treatments (Sanada et al., 1980b).

Moist filter paper, sterile sand, sand/peat mixtures or (less frequently) peat are the usual media for stratification and germination tests. In the latter media, seeds should not be sown more than 1 cm deep (Tylkowski, 1978). If it is necessary to protect the seeds from fungal attack, treatment in 0.1 or 0.2% solutions of Benlate for 3-6 h has been reported to provide the most effective sterilization from fungi without causing injury to the seed (Sanada et al., 1980a).

It is also possible to germinate apple embryos in culture media (Nickell, 1951; Tukey, 1934). Nickell (1951) found that a 1% agar culture medium was suitable for excised embryos of weeping crab apple provided the following nutrients were added: 0.002M KNO_3; 0.003M $Ca(NO_3)_2$; 0.001M KH_2PO_4; 0.001M $MgSO_4$; 0.003M $CaCl_2$; 0.002M KCl; 0.001M $MgCl_2$; 2% sucrose; 100μg/l thiamin; 800μg/l pyridoxin; 800μg niacinamide; with trace elements B(0.1 ppm), Mn(0.1 ppm), Zn(0.3 ppm), Cu(0.1 ppm), Mo(0.1 ppm) and Fe(0.5 ppm). The pH of the culture medium was 4.6. Other workers have used Nickell's culture medium with apple seeds and found it to be satisfactory. Removal of either all or a part of the cotyledons had no effect on subsequent germination and growth in this culture medium (Nickell, 1951). Although these embryos were germinated at 25°C without stratification the seed were not dormant (Nickell, 1951). Dormant seeds would require stratification before culturing.

Drying and storage

Apple and pear seeds dry rapidly when exposed to low relative humidity: freshly extracted apple seeds (46% moisture content, fresh weight basis) dried to 9% moisture content after 11 d and to 7% moisture content after 21 d exposure to air (Bakke et al., 1926); at 15°C and 10% RH freshly extracted apple seeds dried from 37% - 8% moisture content in 42 h (Ellis, unpublished); in air, freshly extracted pear seeds dried from 50% - 5% moisture content in 8 d (Omura et al., 1978); and over silica gel, freshly extracted pear seeds dried from 50% - 3% moisture content in 8 d (Omura et al., 1978). The seed coats remain permeable to water after drying, and re-imbibe rapidly (e.g. Visser, 1954), that is, they do not exhibit hardseededness.

Early workers encountered difficulties with dried apple seeds: (Bakke et al., 1926) reported that air-dried seeds germinated poorly in all cases, whilst those which were air-dried and stored for a year failed to germinate; similarly, apple seeds stored dry for a year did not germinate, although air-dry storage for 2 mo was satisfactory (Harrington and Hite, 1923). Because of these early difficulties a belief developed that apple seeds should be stored moist (at temperatures close to 0°C) and not allowed to dry out (e.g. Conner, 1947).

It is probable that the difficulties encountered by these workers were caused by the induction of secondary dormancy during drying at ambient temperatures. The germination of apple seeds dried at room temperature for 6 weeks and then re-imbibed and stratified was not significantly different to that of seeds which had not been dried, a progressive reduction in germination as the drying period increased (Haut, 1932). Similarly, pear seeds dried after stratification showed reduced germination, whereas

pear seeds stratified after drying germinated fully (Westwood and Bjornstad, 1968). Secondary dormancy was induced in stratified apple seeds dried at 25°C, but not in stratified apple seed dried at 5°C (Kaminski, 1974). Moreover, it is possible that secondary dormancy is more difficult to break than the primary dormancy of freshly extracted apple seeds (Kaminski and Zagaja, 1974). Haut's results appear to confirm this; a short period of restratification of the apple seeds in which secondary dormancy had (presumably) been induced by drying at room temperature did not result in full germination (Haut, 1932).

Clearly, then, apple and pear seeds should not be dried at temperatures where secondary dormancy is induced in the moist seeds, that is, they should be dried at about 15° to 17°C or less. However, it should also be recognized that, as with other species, very moist apple and pear seeds are vulnerable to freezing damage at sub-zero temperatures. Hence it is not surprising that the drying of freshly extracted pear seeds in a freeze-drier resulted in almost complete loss of germination (Omura et al., 1978). Thus, the acceptable temperature range for drying is about 0° to 17°C. This rules out the use of both freeze-driers and heated-air driers. It is suggested that the drying regime of 15°C with 10% RH that has been recommended for long-term seed stores by Roberts, Cromarty and Ellis (1982) is suitable for drying apple and pear seeds.

The ability of apple and pear seeds to germinate when tested in suitable regimes is maintained during storage at sub-zero temperatures with low moisture contents (Table 4). The period of seed survival in both species is increased by reducing the storage temperature and/or the seed moisture content (e.g. Sanada et al., 1980a, 1980b; Omura et al., 1978). The information summarized in Table 4 is insufficient to enable the period

Table 4. Germination following short- , medium- or long-term storage of apple and pear seeds in various environments

Species	Storage environment: Temperature (°C)	Storage environment: Moisture Content (% f.wt)	Storage Period (years)	Germination (%)	Author
Apple	-7	4.5	0.5	100	Sanada et al., 1980a
Apple	-7	9.3	0.5	100	Sanada et al., 1980a
Apple	-7	29.6	0.5	100	Sanada et al., 1980a
Apple	-5	c.10?	5	94	Solovieva, 1966
Apple	-5	c.10?	7	96	Solovieva, 1978
Apple	0	9.3	0.5	100	Sanada et al., 1980a
Apple	5	4.5	0.5	100	Sanada et al., 1980a
Apple	5	9.3	0.5	100	Sanada et al., 1980a
Apple	5	17.6	0.5	100	Sanada et al., 1980a
Apple	5	21.1	0.5	100	Sanada et al., 1980a
Apple	10	8-10	2	90-95	Karaseva et al., 1981
Pear	-196	3.0	0.01	96	Omura et al., 1978
Pear	-196	4.3	3	89	Omura et al., 1978
Pear	-196	11.7	3	96	Omura et al., 1978
Pear	-20	4.3	3	76	Omura et al., 1978
Pear	-20	11.7	3	86	Omura et al., 1978
Pear	-5	c.10?	22	98	Solovieva, 1978
Pear	0	4.3	3	90	Omura et al., 1978
Pear	0	11.7	3	89	Omura et al., 1978

of longevity under IBPGR-preferred conditions (-18°C with 5% moisture content; IBPGR, 1976) to be estimated. Nevertheless, the longest period of storage investigated -- 22 years pear seed storage at -5°C with 10% moisture content (Solovieva, 1978) -- suggests that longevity will be within the range of those for accessions of other species currently maintained in long-term seed stores, and substantially greater than the period of the reproductive cycle of apple and pear species.

The 1000-seed weight range for apple and pear seeds at 5% moisture content is about 25-50 g and, on average, 1000 seeds can be assumed to occupy a volume of approximately 70 ml. Assuming that each accession consists of 4,000 seeds, each accession is packed in a 300 ml sealed container and only 25% of the volume of any store is occupied by the containers (to allow for a filing system,) a 600 l deep-freeze chest could accommodate about 500 accessions.

Conclusions

It is clear from this report of the relevant literature that the long-term storage of seeds of apple and pear species is highly practicable but that care is necessary to avoid dormancy preventing seed germination in viability monitoring tests and in seedling establishment after storage.

Experimental results

The procedure outlined in the summary has been tested with seeds extracted from windfall Worcester Pearmain apples. The experimental results so far have confirmed the soundness of this procedure. In addition, the following points are noted:

1. Removing the 3 seed coats after stratification is preferable to removal before stratification. In the latter case, fungal deterioration of the seeds caused a reduction in germination.

2. The 21-day stratification period is likely to be insufficient for some seed lots. It would be useful to know what period is required by the most dormant seeds; this period could then be used with confidence for all seed lots.

3. Radicle emergence and extension occurred quite rapidly in tests of 15°C after a period of stratification (approximately 50% of seeds had 1-4 cm long radicles after 7 d), but hypocotyl growth was difficult to detect. Now the optimum temperature for apple seed radicle growth is 15°C whereas that for hypocotyl growth is 25°C, but the temperature at which embryo germination occurs (at least within the range 5° to 25°C) does not affect subsequent radicle or hypocotyl growth rates in other environments (Perino and Come, 1979). Thus, it may be advisable to transfer germinating seedlings from 15°C to a slightly higher temperature (perhaps 20°C) to enable more balanced growth of both radicle and hypocotyl.

4. Apple seeds stored for 7 d in sub-zero temperature (-196°C, or -20°C with 8% moisture content) germinated equally well as seeds that were not dried and frozen.

Summary

A review of the literature relevant to apple and pear seed storage and germination is presented. The following procedures are suggested to those wishing to maintain seed collections of apple and pear species based on this review and preliminary laboratory investigations.

1. Fruit should be stored for the shortest period possible at 5°C prior to seed extraction.

2. Seeds should be transferred to a drying room (15°C, 10-15% RH) immediately after extraction from the fruit. Approximately 4-7 d exposure to this regime will reduce seed moisture content to 5% (fresh weight basis).

3. Dried seeds should be hermetically stored under IBPGR-preferred conditions (-18°C, or less, with 5±1% moisture content). On average a 300 ml container can accommodate a 4,000 seed accession, and a 600 l deep freeze 500 such accessions.

4. When removed from sub-zero storage the container should only be opened when the contents have reached ambient temperature.

5. For germination, the dry seeds should first be stratified by placing them in petri-dishes on moist filter paper and maintained at 5°C for at least 21 d. All 3 seed coats should then be removed from the imbibed stratified seeds, and the excised embryos placed in petri-dishes on moist filter paper and maintained at 15° to 17°C. Non-deteriorated excised embryos which fail to begin to germinate within 21 d at 15° to 17°C can be returned to 5°C for a further period of stratification and then be re-tested at 15° to 17°C. It may be necessary to transfer germinating embryos to 20°C if it is found that hypocotyl growth is inhibited.

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A NOTE ON THE DEVELOPMENT OF A PRACTICAL PROCEDURE FOR PROMOTING THE GERMINATION OF DORMANT SEED OF GRAPE (Vitis spp.) 1/

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Introduction

To be practical a seed dormancy-breaking treatment must be able to remove dormancy from almost all dormant seeds within all seed populations (different provenances and/or cultivars) of a species, be non-injurious to non-dormant or weakly dormant seed, be easy to apply and relatively quick to achieve full germination. Freshly extracted grape seeds show considerable dormancy. For those concerned with the breeding or genetic resources conservation of Vitis spp. this represents a considerable problem. In plant breeding it is advisable to remove dormancy physiologically when growing plants from seed in order to avoid any direct selection pressure against dormancy (to avoid vivipary) and to avoid any possible indirect selection pressure against other characters linked with dormancy. Moreover the more rapid the method of removing dormancy the better. For genetic resources conservation it is necessary to be able to remove dormancy to ensure that the genetic heterogeneity of an accession is maintained for use by breeders and to avoid confounding dormancy with non-viability in germination tests designed to monitor the viability of accessions in long-term storage.

Exposure of imbibed seed to low temperature for long periods (variously described in the literature as pre-chill, stratification or after-ripening) is moderately successful in breaking grape seed dormancy. Various temperatures and treatment periods have been used. Those reported as successful include 12 weeks at 5°C (Flemion, 1937; Scott and Ink, 1950; Harmon and Weinberger, 1959; Singh, 1961), 9 weeks at 0°C (Yeou-Der et al., 1968), and 8 weeks at 5°C (Chohan and Dhillon 1976). However, despite the considerable treatment period -- in itself a drawback -- such treatments alone are rarely totally effective in breaking seed dormancy. For example in the work of Singh (1961) treatment at 5°C for 12 weeks with seeds of 14 hybrid crosses resulted in a mean germination of only 40% and ranged from only 8% in 1 cross to a maximum 62% germination in the least dormant cross. Thus pre-chill treatments are not practical in the sense discussed here.

Various other dormancy-breaking treatments which have been successful with other species have been tested for their efficacy in removing dormancy in grape (e.g. Scott and Ink, 1950; Harmon and Weinberger, 1959; Yeou-Der et al., 1968; Manivel and Weaver, 1974). Of these, apart from the pre-chill treatments described above, only treatment with gibberellic acid (GA_3) has been found to be at least partly effective. GA_3 appears to be more suitable than other gibberellins for breaking grape seed dormancy (Pal et al., 1976). Very high concentrations of GA_3 (e.g. 8,000 ppm) are required if GA_3 is the sole method of breaking dormancy and this can result in very tall seedlings (Yeou-Der et al., 1968). Such deformities may result in reduced plant establishment. GA_3 concentrations can be reduced however, if treatment in GA_3 is combined with a pre-chill treatment. Applying GA_3 before the pre-chill treatment appears to be more effective in promoting germination than applying it after the pre-chill treatment (Kachru et al., 1972; Selim et al., 1981) although contradictory results have also been reported (Ran-Dhawa and Negi 1964; Randhawa and Pal, 1968). Although combining pre-chill and GA_3 treatments can result in much shorter overall germination periods compared to pre-chill alone, the GA_3 concentrations used appear to result in very high proportions of abnormal seedlings (e.g. Selim et al., 1981). Such seedlings are often incapable of developing into plants. Moreover there is little evidence to suggest that

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the combination of treatment in GA_3 and a short pre-chill promotes grape seed germination sufficiently. The best combined treatment of Kachru et al., (1972) was treatment in 2,000 ppm GA_3 for 48 h followed by pre-chill at 5°C for 30 d. The period and environment of the subsequent germination test was not given but 81% germination was recorded after this treatment for seed of the cultivar Black Muscat compared to no germination for a control treatment.

In this paper we report the results of 3 simple experiments in which we have attempted to improve the above procedure by the action of an additional dormancy-breaking agent - hydrogen peroxide - which we have found to be particularly effective when combined with other agents for removing seed dormancy in rice (Oryza spp.) (Ellis et al., 1983).

Materials and methods

Seeds were extracted by hand from table grapes purchased locally, cleaned by washing in running tap water with a final wash in distilled water, blotted and then dried at 20°C with 10-15% RH for 10 d. The drying treatment reduced seed moisture content from 30% - 7% (fresh weight basis) -- moisture content being determined from ground seed dried at 130-133°C for 2 h. The dried seeds were hermetically stored at 0°C in laminated aluminium foil packets until the experiments were begun.

Seeds were extracted from both red (R) and white (W) table grapes. Those used in Experiments 1 and 2 were of 3 unknown cultivars of unknown origin (R_A, W_A, and W_B). Seed of 7 cultivars were used in Experiment 3:

R_B - cultivar Barlinka from South Africa;
R_C - cultivar Red Emperor from Chile;
R_D - cultivar Alphonse La Vallée from France;
R_E - cultivar Cardinal from Spain;
W_C - cultivar Uva Regina Puglia from Italy
W_D - cultivar Almeria from Spain
W_E - unknown cultivar from Italy.

Pre-chill treatments of 21 d at 3-5°C and the subsequent tests were carried out in moist rolled paper towels. The number of seeds tested varied substantially according to the total numbers available for each seed lot. Seedlings were evaluated according to the criterion of normal germination, i.e. only those seeds which produced morphologically normal seedlings were scored as germinated.

Pretreatments -- where carried out -- were at 20°C. Treatment in hydrogen peroxide (H_2O_2) preceded treatment in GA_3 solutions. Both pretreatments preceded the pre-chill treatment. For the H_2O_2 treatment 200 seeds were soaked in 160 ml of solution. For the GA_3 treatment seeds were soaked in twice their volume of solution. Each of these treatments were applied for 24 h and between the 2 treatments seeds were rinsed in distilled water and blotted.

Experiment 1 investigated the effect of 1 M H_2O_2, 2,000 ppm GA_3 and the 21 d pre-chill treatment on the germination of lots R_A, W_A and W_B in a factorial design. 25 seeds of W_A, 200 seeds of W_B and 120 seeds of R_A were tested at each treatment. The subsequent germination tests were at a constant temperature of 20°C and were concluded after 63 d.

Experiment 2 investigated the effect of a limited number of different temperature regimes on the germination of seed of lots R_A and W_B which had been previously pretreated in 1 M H_2O_2 and 2,000 ppm GA_3 and pre-chilled for 21 d. The temperature regimes tested were constant temperatures of 20°, 25°, 30° and 35°C and alternating temperatures of 20°/30°C and 20/3°C. In the latter 2 regimes the higher temperatures were applied for 8 h in each 24 h cycle. 120 seeds of lot R_A and 200 seeds of lot W_B were tested in each temperature regime. Germination tests were concluded after 63 d.

Experiment 3 compared germination of seed subjected to the combined treatments of pretreatment in 0.5 M H_2O_2, 1,000 ppm GA_3 and a 21 d pre-chill for lots R_{B-E} and W_{C-E}. Germination tests were carried out at an alternating temperature of 20/30°C (16/8 h) and were concluded after 42 d. Germination test size was 200 seeds for the combined treatment but only 50 seeds for the untreated (control) germination test in which few seeds were expected to germinate.

In a number of treatments the topographical tetrazolium test for viability was carried out on seeds which had failed to germinate during the test. This was done by bisecting the imbibed seeds with a sharp knife and, provided an embryo was present, incubating one-half of the bisected seed in 0.5% tetrazolium chloride solution for 2 h at 40°C, washing the embryos in distilled water and evaluating the staining patterns. In addition in the final experiment a tetrazolium test was carried out on a sample of the dried seed from each lot with the exception of lot R_B where insufficient seeds were available. These seeds were moistened on filter paper at 30°C for 16 h before bisection, but the staining period had to be extended to 3 h. The number of seeds available for this test was severely limited for certain seeds lots (Table 5).

Table 5. Viability of grape seed lots assessed by the topographical tetrazolium test, normal germination after 42 d in the test at 20/30°C for seed previously untreated or treated in 0.5 M H_2O_2 for 24 h, 1,000 ppm GA_3 for 24 h and pre-chilled at 3-5°C for 21 d, and viability of seeds remaining ungerminated at the end of these tests as assessed by the topographical tetrazolium test.

Cultivar	Tz test		Germination test			
			Untreated		Treated (H_2O_2 + GA_3 + pre-chill)	
	No. of seeds tested	Viability (%)	Normal germination (%)	Viability of seeds failing to germinate 1/ (%)	Normal germination (%)	Viability of seeds failing to germinate 1/ (%)
R_B	-- 2/	-- 2/	5	90	94 *** 3/	0 NS 4/
R_C	27	74	24	44	84 ***	0 **
R_D	32	69	22	52	70 ***	0 NS
R_E	9	100	0	90	94 ***	0 NS
W_C	79	74	0	60	62 ***	1 NS
W_D	100	97	2	88	90 ***	0 NS
W_E	19	95	6	88	91 ***	0 NS

1/ Expressed as the percentage of the original number of seeds tested for germination. (Thus this value can be added to that for normal germination to obtain an estimate of viability for the treated and untreated seeds at the end of the germination test.)

2/ No seeds available for the tetrazolium test.

3/ Probability of error for significant difference in normal germination between treated and untreated seeds (*** = $P < 0.005$).

4/ Probability of error for significant difference in total viability between treated and untreated seeds (NS [not significant] = $P > 0.2$, ** = $P < 0.01$).

Results and discussion

Fig. 3 shows that seeds of lots R_A, W_A and W_B were dormant, that all 3

dormancy-breaking agents tested had some effect in removing dormancy either singly or when combined with other agents, and that 2,000 ppm GA_3 was by far the most promotory single agent. Within lots R_A and W_B germination following the three-factor treatment, all 3 two-factor treatments and the GA_3 single factor treatment did not differ significantly ($P > 0.05$), but all of these results were significantly greater ($P < 0.005$) than either the single factor H_2O_2 treatment, the single-factor pre-chill treatment or the control treatment. For lot W_A, however, the germination of the three-factor treatment was significantly great ($P < 0.05$) than the other 3 treatments which included treatment with GA_3; and all 4 treatments which included treatment with GA_3 resulted in significantly greater ($P < 0.005$) germination than the remaining 4 treatments.

Consequently the three-factor treatment combining treatment in 1 M H_2O_2, 2,000 ppm GA_3 with a 21-d pre-chill prior to the germination test was the only treatment to provide a result which was not significantly less than any other treatment

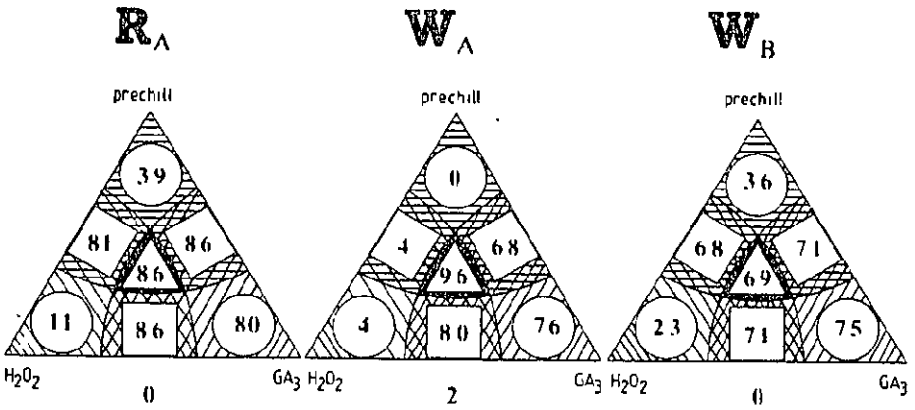

Fig. 3. The influence of soaking 1 M H_2O_2 for 24 h, soaking in 2,000 ppm GA_3 for 24 h and 21 d pre-chill at 3-5°C either singly or in combination on subsequent germination of 3 grape seed lots in test at 20°C over 63 d. For each seed lot the investigation was based on a simple 2^3 factorial design. The value at the base of each triangle is the result of a 63 d germination test at 20°C on untreated seed. Values contained within a circle at the apices of the triangles are for single-factor treatments; values contained within squares where arcs of 2 circles overlap are for 2--factor treatments; values in the central triangles where the arcs of all 3 circles overlap are for the 3--factor treatments. All results are presented as percentage normal germination.

in all 3 seed lots, treatment in 2,000 ppm GA_3 being the most effective dormancy breaking agent of the 3 when applied alone. However, subsequent tetrazolium tests on seeds of lots R_A and W_B which failed to germinate in test provided overwhelming evidence that the 2,000 ppm GA_3 treatment resulted in the death of a small proportion of the dormant seeds. For both lots none of the seeds that remained ungerminated following the combined treatment with all 3 agents were viable according to a tetrazolium test, whereas 18% and 14% of the original number of seeds tested were viable at the end of the germination test following the combined treatment of 1 M H_2O_2 and pre-chill, but only 2% and 3% were viable following treatment in 2,000 ppm GA_3 alone for lots W_B and R_A respectively. Thus (by adding these values to those given in Fig. 3) we estimate that lots W_B and R_A were at least 86% and 95% viable respectively, and that treatment in 2,000 ppm GA_3 either alone or in combination with 1 M H_2O_2 and pre-chill resulted in the death of between 9-17% ($0.03 > P > 0.0000.2$) and 9-12% ($0.008 > P > 0.0022$) of seeds respectively.

This phenomenon of the contradictory effects of certain dormancy breaking agents (i.e. promoting the germination of certain dormant seed whilst causing the death of other seeds) is one which we have investigated quite extensively in rice (Ellis et al. 1983). In rice our solution was to use many dormancy-breaking agents in combination,

but with each agent at low concentrations in order to avoid toxic effects. Here we adopted a similar tactic, reducing the concentrations of both H_2O_2 and GA_3 from 1 M - 0.5 M and from 2,000 ppm - 1,000 ppm respectively in Experiment 3.

Much of the work on overcoming seed dormancy in Vitis spp. has, apparently, used uncontrolled environments for the subsequent germination tests. This is surprising since the temperature of the germination regime influences the expression of seed dormancy quite considerably in a wide range of species. One exception is the work of Yeou-De et al., (1968). They tested grape seeds for germination in a diurnal alternating temperature regime of 18/30°C (the period spent at each temperature was not reported). In this investigation (Fig. 4) a similar diurnal alternating temperature regime of 20/30°C was consistently superior to the other, admittedly few in number, constant and alternating temperature regimes in which seed were tested for germination for both lots. Note also that the ranking of germination test temperatures with respect to final percentage germination was identical for seeds of both lots (Fig. 4). Although the differences in final percentage germination between the alternating temperature regime of 20°/30°C and the constant temperature regime of 25°C were not significant ($P > 0.2$) for either seed lot when expressed in terms of the total number of seeds tested, this is not surprising since very few of the viable seeds in each lot remained dormant at 25°C (viability of R_A and W_B was estimated to be 95% and 86% respectively). Expressing germination as the proportion of viable seeds which germinated showed germination at 20°/30°C to be significantly greater than germination at 25°C ($P < 0.005$ for seed of both lots). That is, dormancy was broken in a significantly greater proportion of seeds at 20/30°C than at 25°C. Consequently an alternating temperature regime of 20°/30°C was used to test seeds for germination in the final experiment. In addition since no further germination occurred after 21 d in any germination test (Fig. 4) the test period was shortened from 63 - 42 d. Of the 4 constant temperature regimes used 25°C consistently gave the most rapid germination and the highest proportion of germinating seeds (Fig. 4). It is therefore suggested that anyone wishing to germinate grape seeds but unable to provide an alternating temperature regime of 20°/30°C should use 25°C.

Five of the 7 lots tested at 20°/30°C showed considerable dormancy (Table 5). The combined treatment of a 24 h soak in 0.5 M H_2O_2 followed by a 24 h soak in 1,000 ppm GA_3 with a subsequent 21 d pre-chill at 3-5°C and germination testing at 20°/30°C resulted in a significant increase in germination over the control in all 7 seed lots. Moreover, virtually no dormant seed remained ungerminated at the end of the procedure

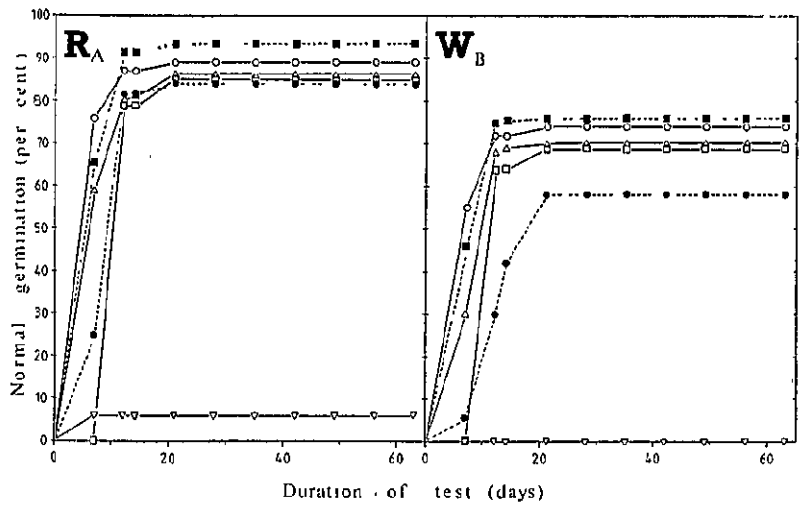

Fig. 4: Germination progress curves of 2 grape seed lots treated in 1 M H_2O_2 for 24 h, in 2,000 ppm GA_3 for a further 24 h, pre-chilled at 3-5°C for 21 d and then tested at 20°C (---), 25°C (O-O), 30°C (---), 35°C (V-V), 20/30°C (---), or 20/35°C (---) for 63 d. In the alternating-temperature treatments the first temperature quoted was applied for 16 h and the second temperature for 8 h in each 24-h cycle.

(Table 5), and comparison with the results of both the initial tetrazolium tests for viability and those after the germination test of untreated seed provide no evidence of any significant damage to seed resulting from the use of the combined dormancy breaking procedure.

There are a number of reports in the literature which demonstrate that the use of high concentrations of gibberellins can result in seedlings of increased height (e.g. Yeou-Der et al., 1976; Pal et al., 1976); in extreme cases seedlings may be too etiolated for subsequent growth and development. No such problems were encountered here; the combined treatment on the 7 seed lots had no noticeable effect on subsequent seedling height. Selim et al. (1981), using a number of compounds, including gibberellins, to break dormancy encountered considerable problems with high proportions of abnormal seedlings in all their treatments - including the control treatments. Again no such problems were encountered here. It is possible that the 30°C germination test temperature of Selim et al., (1981) was too high because there is some evidence here that 30°C should be regarded as a super-optimal germination temperature, the progress of germination at 25°C being both more rapid and complete (Fig. 4).

Although the evidence presented here is limited, nevertheless we believe that it is sufficient to demonstrate the advantage of this combined procedure for promoting the germination of grape seeds compared to either of the procedures suggested previously - 12 weeks pre-chill at 5°C (Scott and Ink 1950), or treatment in 2,000 ppm GA_3 followed by a pre-chill treatment (Kachru et al., 1972). Consequently we urge those involved in the breeding and genetic resources conservation of Vitis spp. to compare the efficacy of this procedure with that currently in use.

Although we are confident of the benefits of this procedure over those currently in use, this does not mean that a better procedure could not be developed given more time and a greater supply of seeds. We have 3 suggestions for anyone attempting to develop an even better dormancy breaking procedure. Firstly we are by no means certain that the pre-chill treatment need be as long as 21 d. Reduction to 14 or even 7 d may be possible. Secondly 0.01 M 2-mercaptoethanol added to the germination medium in our experience promotes the germination of dormant grape seeds. It may be possible for this agent to replace or be combined with 1 or more of the agents used here but we have been unable to test this. Finally Kachru et al., (1972) had considerable success in removing dormancy quickly and simply by allowing running water to drip through seeds in muslin bags for up to 16 d. One problem they found was that seeds began to germinate in the bags and were easily damaged such that these germinated seeds were incapable of further growth. As a practical procedure in genebanks, or elsewhere, a long washing procedure would be fraught with similar operational difficulties. Nevertheless it occurs to us that a shorter washing period of 2 d or so in combination with other dormancy breaking agents might result in a procedure as effective as that developed here.

Summary

The effect of hydrogen peroxide (H_2O_2), gibberellic acid (GA_3) and a short exposure of imbibed seed to 3-5°C (pre-chill) on grape seed germination were investigated factorially. All 3 factors had some effect, either singly or in combination with 1 or more of the other agents, in promoting the germination of dormant seed. 2,000 ppm GA_3 was the best single agent for breaking dormancy but tetrazolium staining revealed that this treatment caused the death of a small proportion of seed. Comparison of germination tests at 20°, 25°, 30° and 35°C showed 25°C to be the best constant temperature for rapid full germination. Seeds were also tested for germination in 2 diurnal alternating temperature regimes - 20°/30°C and 20°/35°C, the higher temperatures being applied for 8 h in each 24 h cycle. The alternating temperature of 20°/30°C proved better for testing seed for germination than either 20°/35°C or 25°C, the best constant temperature. The following germination test procedure was devised for grape seeds: a 24 h soak in 0.5 M H_2O_2, a further 24 h soak in 1,000 ppm GA_3, followed by a 21 h pre-chill at 3° to 5°C with subsequent testing for germination in a diurnal alternating temperature regime of 20°/30°C with the higher temperature being applied for 8 h in each daily cycle. This procedure was tested with dormant seed of 7

lots of diverse origin and found to be very effective in promoting the germination of dormant seed whilst not damaging to other seeds. In the opinion of the authors the above procedure represents a suitable practical dormancy breaking procedure for seed of Vitis spp.

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