Stratification, germination and emergence of mazzard seeds following 15- or 20-year storage

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Summary

Two seed lots of mazzard cherry (*Prunus avium* L.) seeds in stones with a 10–11 per cent moisture content (fresh weight basis) were stored in sealed containers at −3°C for 15 or 20 years. The seed lot stored for 20 years were stratified either in or without a substrate. Those stored for 15 years were stratified in a substrate. All seeds were kept at 25/3/25/3°C (2 + 2 + 2 + 14 weeks) or 3/25/3/25/3°C (6 + 2 + 2 + 2 + 14 weeks). The germination and seedling emergence of seeds stored for 15 years were maintained at a high level (mean 98 per cent). The two different thermal variants of stratification for these seeds had no effect on their germinability and seedling emergence. Germination and emergence from the seed batch stored for 20 years reached, on average, 70 per cent. Those seeds which were stratified at 25/3/25/3°C (2 + 2 + 2 + 14 weeks) were characterized by 78 per cent germination and 72 per cent emergence, whereas seeds kept at 3/25/3/25/3°C (6 + 2 + 2 + 2 + 14 weeks) germinated and emerged at 63 and 60 per cent, respectively. All the temperatures of germination and type of stratification (in or without substrate) tested for seeds stored for 20 years proved to be equally effective. Thus, desiccated mazzard seeds could maintain a high germinability after long-term storage in controlled conditions followed by different stratification methods.

Introduction

Many forest tree species produce large amounts of seeds only in some years. This necessitates the building up of seed reserves and their proper storage. Optimum conditions for long-term seed storage in most tree species of the orthodox category are ensured by a low moisture content (MC) of seeds ca. 10 per cent, fresh weight basis (fresh w.b.) and freezing (Suszka, 2000). Mazzard cherry (*Prunus avium* L., syn. *Cerasus avium* (L.) Moench) is found in central and southeastern Europe, in the Caucasus, and in western Asia. This is a valuable species in forest ecosystems, because its fruits, similar to those of other *Prunus* species, are readily eaten by birds (Yagihashi *et al.*, 1999). Mazzard cherry wood is of high quality, so it is used in furniture making. In nursery production, it is used as rootstock for cultivated cherries (Bugała, 1991). Its seeds are characterized by a deep physiological dormancy of the embryo (Suszka, 1967), which can be broken by stratification in or without a substrate, with two warm stages preceding the major cold stage, i.e. either 2 weeks at 25°C, next 2 weeks at 3°C, next 2 weeks at 25°C and then 12–16 weeks at 3°C, till the appearance of the first germinating seeds, or additionally with an initial cold stage at 3°C for 6 weeks, i.e. 3/25/3/25/3°C (Suszka, 1962, 1964, 1967; Muller and Bonnet-Masimbert, 1990; Muller, 1992; Muller and Bonnet-Masimbert, 1993; Suszka *et al.*, 1996; Suszka, 2000).

For *Prunus* seeds, drying is not damaging and it is essential for medium and long-term storage (Holmes and Buszewicz, 1958; Grisez 1974). The results of the investigations reported by Huntzinger (1971) and Grisez (1976) show that it is better for *Prunus serotina* seeds to store them at MC 4–6 than at 11–13 per cent. At the higher MC and storage temperature of 0.5–5°C, viability was lost completely after 5-year storage. An MC of 11–13 per cent was also too high for seeds of *P. serotina* to be stored at −18 to −14°C, because of freezing damage (Ellis and Hong, 1985). Seeds dried in stones to an MC of 9–10 per cent fresh w.b. can be stored for 4.5–5 years at −1°C (Suszka, 1964, 1970; Grisez, 1974; Muller and Bonnet-Masimbert, 1993) or for 7 (Soloveva, 1966) and even 15 years (Soloveva, 1978) at −3°C to −5°C without viability loss. Unfortunately, Soloveva (1966, 1978) does not give any data on the exact seed MC during storage. Chmielarz (2009) proved that there is no critical seed MC for deeply desiccated mazzard seeds (stone MC 1.6 per cent) under silica gel. Storage of seeds desiccated to an MC of 7.8 per cent for 2 years in liquid nitrogen did not decrease seed germination after thawing, in comparison to 2-year storage at −3°C (Chmielarz, 2009).

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Deterioration of the physiological condition of seeds stored in gene banks is a major problem that remains to be solved. According to the recommendations of the Food and Agriculture Organization/International Plant Genetics Resources Institute (FAO/IPGRI 1994), orthodox seeds of trees should be stored at −18°C after desiccation to an MC of 3–7 per cent. Walters and co-workers (Walters *et al.*, 2005) claimed that in spite of the extended shelf lives of seeds achieved under gene banking conditions (seeds of 168 plant species stored in National Center for Genetic Resources Preservation, Fort Collins, CO, at −18°C for 50 years), there is still a high variability in longevity among individual species and even individual samples.

The aim of this study was to analyse the influence of long-term seed storage for up to 20 years at −3°C, with the use of various types of stratification, germination and emergence conditions on seed germination and seedling emergence in the laboratory and in the greenhouse.

Materials and methods

Plant material

Mazzard cherry (*P. avium* L.) seeds were collected in 1985 (tree no. 15) and in 1990 (tree no. 14) in the cherry avenue in Kórnik Arboretum, Kórnik, 52°15′N 17°06′E, Poland. Seeds (in stones) were dried after collection at room temperature to an MC of 11 and 10 per cent fresh w.b., respectively. They were then stored in tightly closed containers at −3°C ± 1°C (glass bottles, sealed with a plug and sealing wax). The bottles (volume 1 l) were never opened during their storage for 15 years (tree no. 14) or 20 years (tree no. 15). In these bottles, 1500 and 2500 seeds, respectively, were stored. Initial germination and emergence percentage after seed collection were unknown. We extrapolated seed initial germination for these trees from earlier data for freshly harvested seeds (Suszka, 1976a, b).

Seed MC, 2,3,5-triphenyltetrazolium chloride and stratification tests

The MC of seeds (after drying in an oven at 105°C/24 h, 3 × 20 seeds) was determined in relation to fresh seed weight. After storage, seed viability was tested (4 × 50 seeds) by staining with 1 per cent solution of 2,3,5-triphenyltetrazolium chloride (TTC) according to International Seed Testing Association (ISTA, 1999). Seeds were next subjected to warm–cold–warm–cold stratification (25/3/25/3°C; 2 + 2 + 2 + 14 weeks) or a similar stratification with an additional cold stage at the beginning (3/25/3/25/3°C; 6 + 2 + 2 + 2 + 14 weeks). The stones were stratified in a moist substrate (Gordon and Rowe, 1982), composed of quartz sand and acid (pH 3.5–4.5) peat (1:1). Stones were mixed with the substrate at a volume ratio of 1:3 and placed in 0.2-l plastic bottles. Each bottle was covered with a plastic lid with three holes (each 0.5 cm in diameter) to protect the substrate with seeds against excessive drying but at the same time enabling gaseous exchange. During stratification, seed germination was counted every week (seeds with radicles 3 mm or more were regarded as germinated) and simultaneously water was replenished in the substrate. Stratification was carried out till the appearance of the first (5 per cent) germinated seeds.

The stratification without substrate at a controlled seed MC (28–30 per cent) in unsealed boxes used in our experiments was first applied in Nancy, France (Muller, 1992; Muller and Bonnet-Masimbert, 1993; Suszka *et al.*, 1996). Stratification without substrate was carried out for the same time as the control stratification in the medium (4 × 50 seeds), till the appearance of the first germinated seeds (with a radical 3 mm long, 5 per cent), both started in the same time.

Germination and emergence tests

For seeds stored for 20 years stratified without substrate, germination tests were conducted at a constant temperature of 3°C and at cyclically alternating temperatures of 3–15°C or 3–20°C (16 + 8 h). For other variants (seeds stored for 15 years and for 20 years – stratified with the substrate), only germination at 3–20°C was applied. Stones were mixed with the same substrate as during stratification. Seed germination was counted every week, and simultaneously, water was replenished. Germination and emergence tests lasted, respectively, 5 and 8 weeks. Seeds with radicles 3 mm or more were regarded as germinated.

For the emergence tests, seeds were sown in the substrate (moist sand with peat, as for the stratification and germination tests) to the depth of 1 cm in plastic plant propagators (Stewart Co., Croydon, UK) by using a plastic sheet with holes (Ø 1 cm) spaced 0.5 cm apart. The seeds were covered with quartz sand and next watered to the suitable MC (Gordon and Rowe, 1982). The propagators had transparent covers (with adjustable ventilation vents) and were kept in a growth room at cyclically alternating temperatures of 3–20°C (16 + 8 h). Seedling emergence was controlled every week, and only the seedlings with cotyledons above the medium and first leaves visible were counted.

Seeds were also sown in a greenhouse in plastic growing trays HIKO V-50 (40 cells each) to the depth of 2 cm. As a sowing substrate, in the growing trays, acid peat (pH 3.5–4.5) was mixed with perlite (at a ratio of 3:1), with addition of Osmocote® Classic Standard fertilizer (2.8 g/l) with controlled nutrient release for 5–6 months.

Final evaluation of non-germinated and non-emerged seeds

Seeds stored for 15 years germinated and emerged at a rate of nearly 100 per cent; thus, final evaluation (cutting test) of non-germinated and non-emerged seeds was performed only for seeds stored for 20 years. Non-germinated seeds (which did not germinate until the end of the germination test) were removed from the hard endocarp, and cut lengthwise with a knife, across cotyledons and the embryo axis. In the group of non-germinated seeds, the following

groups of seeds were distinguished: healthy, decayed and germinated abnormally. Healthy seeds had white, fleshy and shiny cotyledons and embryo axes. Seeds that had decayed partly or completely as a result of primary infection (i.e. originating from the seed) were classified as decayed seeds. Such seeds did not develop into seedlings. Seeds with small abnormal roots and visible small abnormal cotyledons were classified as seeds germinated abnormally; these seeds did not develop into seedlings.

Data on germination and seedling emergence were subjected to analysis of variance (one-way ANOVA) after arcsine transformation. The significance of results was assessed by Tukey test at $P < 0.05$. For the analyses, STATISTICA (StatSoft Polska, 1995–2005) software was used. Separate ANOVAs and Tukey tests were performed for germination and emergence.

In laboratory tests of germination and emergence, each experimental treatment included four replications of 50 seeds each.

Results

After stratification in the substrate at 25/3/25/3°C (2 + 2 + 2 + 14 weeks) or at 3/25/3/25/3°C (6 + 2 + 2 + 2 + 14 weeks), germination and emergence in the laboratory at 3–20°C (16 + 8 h) and in the greenhouse were both very high (i.e. on average 98 per cent) for the seeds stored for 15 years (tree no. 14), Table 1. Seeds stored for 20 years (tree no. 15) and which were then stratified in the substrate at 25/3/25/3°C (2 + 2 + 2 + 2 + 14 weeks) had a 77 per cent germination capacity, while seedling emergence reached 70 per cent in the laboratory. Those seeds stored for 20 years, which had not germinated and not emerged after stratification in the substrate were as follows: decayed (15–30 per cent), healthy (3–8 per cent) or germinated abnormally (1–9 per cent). Of those seeds stored for 20 years ungerminated and unemerged after stratification without substrate, 15–25 per cent were decayed, 1–7 per cent were healthy and 2–13 per cent germinated abnormally. In the greenhouse, seedling emergence decreased to 50 per cent for seeds stored for 20 years. The seeds stratified after 20 years with an additional cold stage at the beginning (3/25/3/25/3°C; 6 + 2 + 2 + 2 + 14 weeks) had a lower germination capacity (61 per cent), while seedling emergence reached 59 per cent in the laboratory and 51 per cent in the greenhouse. The seeds stored for 15 and 20 years maintained a high viability according to the TTC test (Table 1).

The seeds stored for 20 years and stratified without substrate at 25/3/25/3°C (2 + 2 + 2 + 14 weeks) were characterized by a 78 per cent germination capacity and 73 per cent seedling emergence. All the tested temperatures of germination after the substrateless stratification proved to be equally effective for such seeds, as seed germination reached 63 per cent at 3°C, 62 per cent at 3–15°C and 65 per cent at 3~20°C, while seedling emergence amounted to 61 per cent (Table 1).

Stratification in or without substrate did not affect the variation in germination and emergence (Table 1).

The seeds stored for 15 and 20 years and stratified in the substrate germinated vigorously after 1–4 weeks and seedling emergence was observed in the laboratory after 3–5 weeks (Figure 1).

Discussion

Long-term storage of orthodox seeds is defined as a period two to three times longer in duration than the mean interval between good seed crop years. The frequency of good seed crop years is dependent on species and environmental conditions. Mazzard cherry trees can bear fruit every year if conditions are favourable for effective pollination, including the absence of late spring frosts, strong winds and/or excessive rains (Janick and Moore, 1996). The seed storage periods tested in this study (15 and 20 years) can be regarded as long-term storage.

Research on long-term storage under controlled conditions (−3°C) of orthodox seeds from *Crataegus monogyna* L. and *Crataegus submollis* Sarg. (also Rosaceae) demonstrated

Table 1: *Prunus avium* L. Effect of seed storage (for 15 and 20 years); thermal stratification conditions (A: 25/3/25/3°C, B: 3/25/3/25/3°C); the means of stratification in a substrate or without substrate and germination conditions (3°C, 3~15°C, and 3~20°C) – only for seeds stored for 20 years – and seedling emergence conditions (in the laboratory or in a greenhouse) on seed viability (TTC), germination and seedling emergence

		Stratification		Germination			Emergence	
Storage time (years)	Viability TTC (%)	Thermal variant	Substrate	3°	3–15°	3–20°	3–20° laboratory	Greenhouse
15	97	A	Yes	–	–	98a	99A	98A
15	97	B	Yes	–	–	98a	97A	96A
20	95	A	Yes	–	–	77ab	70AB	50D
20	95	B	Yes	–	–	61cd	59CD	51D
20	95	A	No	78a	78a	78a	73A	–
20	95	B	No	63b	62b	65b	61B	–

A = stratification at 25/3/25/3°C (2 + 2 + 2 + 14 weeks); B = stratification at 3/25/3/25/3°C (6 + 2 + 2 + 2 + 14 weeks). Values within the same column within each seed lot followed by different letters are significantly different (Tukey test, $P < 0.05$), data for the germination and emergence were evaluated separately (lower case letters for germination and capital letters for emergence).

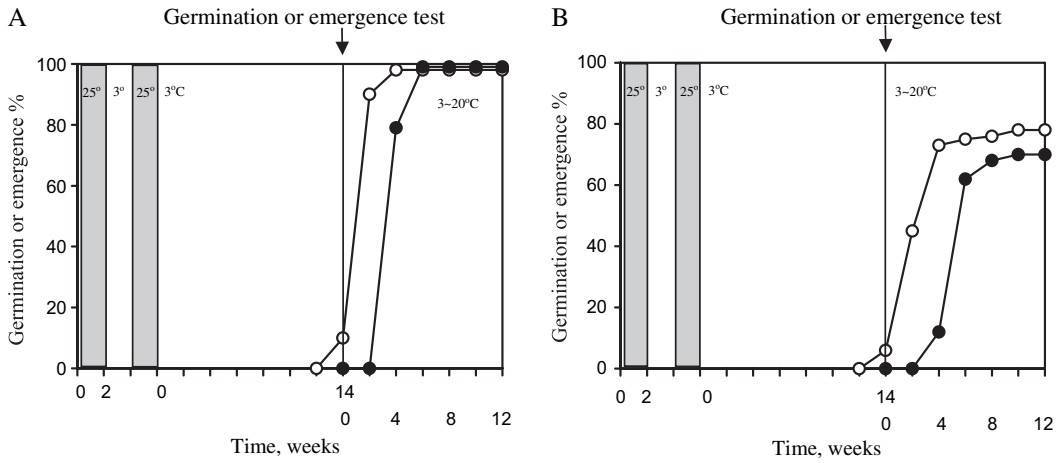

Figure 1. Prunus avium L. Effect of seed storage at −3°C for 15 years (A, tree no. 14) and 20 years (B, tree no. 15) on germination and emergence patterns. Stratification in a substrate at 25/3/25/3°C (2 + 2 + 2 + 14 weeks), followed by a germination test (white points) or emergence test (black points) at 3–20°C (16 + 8 h).

that seeds from both species remained viable following 10 years (*C. submollis*) and 20 years (*C. monogyna*) of storage, and seed germination and seedling emergence reached ~90 per cent (Bujarska-Borkowska, 2002, 2007).

Deep dehydration of orthodox seeds, when combined with freezing, slows down metabolic processes. Subsequent re-hydration and stratification at both suitable temperature and humidity initiate germination in non-dormant seeds or breaks dormancy in dormant seeds (Suszka, 2000). In our study, following long-term storage (15 and 20 years), seeds exhibited dormancy as freshly harvested seeds and required a similar pre-treatment for germination (Suszka *et al.*, 1996). Various authors report that after drying to an MC of 8–10 per cent, mazzard cherry seeds (in stones) can be stored for 4–5 years at 1°C to −3°C (Suszka, 1970; Huntzinger, 1971; Grisez, 1974; Grześkowiak and Suszka, 1983; Grześkowiak *et al.*, 1983; Tylkowski, 2006) and up to 7 years at −3°C to −5°C (Soloveva, 1966, 1978) without viability loss. Results of the present study confirm previous reports that mazzard cherry seeds after stratification in or without substrate at 25/3/25/3°C (2 + 2 + 2 + 14 weeks), without the necessity for 6 weeks of cold stratification at the beginning, can be stored with no or little loss of germinability for many years. Grześkowiak and co-workers observed (Grześkowiak *et al.*, 1983) a small decrease in mazzard cherry seed viability when stored (in stones) in tightly closed containers for 3 years at −18°C following dehydration (9–10 per cent, fresh w.b.). Stored pin cherry (*Prunus pensylvanica*) seeds frozen at −3°C to −5°C maintained high viability for 10 years (Dirr and Heuser, 1987).

Results of the present study demonstrated that mazzard cherry seeds dried to an MC of ca. 10 per cent fresh w.b. can be stored for 15 and 20 years with germination and seedling emergence remaining high after 15 and slightly less (emergence in a greenhouse) following 20 years, what should be expected because of vigour differences of different seed lots.

Initial data (seed germination, emergence or TTC) were not available for investigated trees from the years of seed collection: 1985 (tree no. 15) and 1990 (tree no. 14). However, overall mean seed germination in the laboratory (before storage) from three seasons (1967, 1968 and 1973) (Suszka, 1976a), with stratification at 20/3/25/3°C (2 + 2 + 2 + 14 weeks), and overall mean data from five seasons (1967, 1968, 1970, 1972 and 1973) with stratification at 20/3°C (2 + 14 weeks) were available and analysed in the study for both these trees (Suszka, 1976b). On the basis of these data (Suszka, 1976a, b), we extrapolated that the initial germinability of seeds collected from tree no. 15 (stored for 20 years) was approximately 5–10 per cent lower (already before storage) than that of the seeds collected from tree no. 14 (stored for 15 years). In research presented here, germination of seeds stored for 20 years is on average 20 per cent lowered. Therefore, 20-year storage would be likely to slightly decrease seed viability. Furthermore, a higher percentage of abnormally germinated seeds was observed in seeds stored for 20 years relative to seeds stored for 15 years. Similar to this, long storage of *P. avium* seeds and seeds of *Betula alleghaniensis*, *Picea mariana*, *Pinus banksiana*, *Pinus resinosa* and *Populus grandidentata* also showed a marginal decline in viability after 14–32 years of storage at −20°C (Simpson *et al.*, 2004), as well as *Picea abies* seeds stored for 29 years (Suszka *et al.*, 2005). In our experiments, it is possible that under greenhouse conditions, lower germination (70 per cent) and seedling emergence (50 per cent) were caused by a loss of seed viability during the 20 years of seed storage at −3°C. FAO recommends that orthodox seeds be stored at −18°C after desiccation to an

MC of 3–7 per cent (FAO/IPGRI, 1994). In our experiments, seed MC and storage temperature were too high for 20 years of safe seed storage. However, germinability of seeds stored for 15 years was very high (98 per cent) under these conditions. In our experiment, seeds were stored at a relatively high MC of 10–11 per cent. Huntzinger (1971) and Grisez (1976) reported decreasing values of germinability for *P. serotina* seeds desiccated to an MC of 11–13 per cent (in our experiments, *P. avium* seed MC of 10–11 per cent) and stored at 0.5–5°C or –18°C to −14°C for 1–8 years.

In conclusion, mazzard cherry seeds prepared with an MC of 10 per cent (fresh w.b.) and stored in tightly closed containers for 15 years at −3°C were characterized by very high germination and emergence (96–99 per cent) both in the laboratory and greenhouse. Mazzard cherry seeds with an MC of 11 per cent collected from another tree and stored for 20 years exhibited germination and emergence of 50–78 per cent. Seed dormancy after 20 years of seed storage was successfully broken at 25/3/25/3°C (2 + 2 + 2 + 14 weeks) by stratification both in and without substrate (1–8 per cent of healthy non-germinated seeds after stratification). Our results provide a basis for knowledge about long-term storage of mazzard cherry seeds and successful stratification methods and germination conditions (3–20°C/16 + 8 h) after such storage.

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