JOURNAL OF FOREST SCIENCE, *61*, 2015 (10): 417–421

doi: 10.17221/57/2015-JFS

Long-term storage of service tree (*Sorbus domestica* L.) seeds and induction of their germination

H. Prknová

Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

ABSTRACT: Service tree (*Sorbus domestica* L.) is a significant species with regard to the biodiversity of specific habitats. Its reproduction in natural conditions appears to be problematic and in the interest of preserving the countryside, forests and their biodiversity, it is necessary to apply controlled reproduction procedures. Therefore, when applying such procedures it is of crucial importance to optimize the storage and use of seed material. The objective of the present article is to evaluate the germination rates of seeds which were stored in the long term. 95% of the seeds preserved at low temperatures above zero in a normal refrigerator for 8 years germinated after stratification in wet sand. The germination rate of the same seeds when moistened without stratification was 0%. However, after removal of the seed coat, 76% of these seeds germinated without stratification. Storage of air-dried seeds in the cold (at temperatures above zero) is ideal and is better than freezing them to –20°C, as reported in the literature. Wet peat is completely unsuitable for stratification.

Keywords: stratification; cold storage; seed coat; inhibitors; dormancy

The service tree (*Sorbus domestica* L.) is considered to be a tree species introduced into Bohemia in the Middle Ages; in the territory of Moravia it is considered a native species. In relatively warm landscape units it is, or it may become, a significant species in terms of landscape ecology (Paganová 2008a,b; Paganová, Jureková 2012). But it also has the wood of high quality, the hardest of any European wood, and may be an element of open forests, so there is also an interest in it from a forestry perspective. For these reasons, it has also attracted considerable attention (Hrdoušek et al. 2014; Kopecký 2014), and has become the subject of conservation programs and multidisciplinary research (Arrillaga et al. 1991; Arrillaga et al. 1995; Mikic et al. 2008; Paganová 2008a,b; Ďurkovič et al. 2009; Ďurkovič, Mišalová 2009; Kamm et al. 2009; Brus et al. 2011; Miletić, Paunović 2012; Piagnani et al. 2012).

In the Czech Republic the service tree reproduces sporadically in some places, but at some locations it does not reproduce at all. Artificial propagation is not productive enough to allow the price of seedlings, which currently ranges from 5.5 to 43.3 € on the Czech market, to be considered as cheap. Both generative and vegetative propagation is of practical significance – grafting onto the rootstock of selected individuals of the same species and cloning by micropropagation.

The aim of this paper is to evaluate the suitability of examined techniques for long-term storage of service tree seeds, stored for 8 years in a refrigerator, with known origin of seeds, date of collection and storage conditions. Thanks to this unique material it was possible to analyse the quality of seeds and to formulate the following questions:

– Do the old seeds kept cool retain any germination capability, which would be reflected after stratification?

Supported by Ministry of Agriculture of the Czech Republic, Project No. QJ1530298.

Table 1. Conditions for the storage of *Sorbus domestica* seeds obtained by measurements inside the refrigerator (each line contains a synchronous measurement of temperature and humidity)

Measurement no.	1	2	3	4	5	6	7	8	9	10
Temperature (°C)	8.1	8.2	8.6	11.0	7.7	8.9	8.9	7.9	8.0	7.7
Relative humidity (%)	72	75	79	85	53	47	48	49	61	62

– If the seeds are still germinative, is their dormancy maintained by inhibitors in the embryo tissue or is the inhibition caused by the seed coat?
– Are mineral substrates (wet sand) and organic substrates (wet peat) equally suitable for stratification?

MATERIAL AND METHODS

The seeds came from 50-year-old specimens with registration number 1 in the Arboretum in Kostelec nad Černými lesy affiliated to the Czech University of Life Sciences Prague, growing in groups of three even-aged individuals of the same origin in departments D1 and D2. Seeds were removed from fruits harvested in 2006, thoroughly washed and after drying put into an Erlenmeyer flask. The flask was (not hermetically) sealed with aluminium foil and on 26/10/2006 it was placed in a refrigerator where it remained for 8 years. Heat and humidity conditions in the place where the flask was stored were repeatedly measured using a General Tools & Instruments PTH8708 Digital Temperature and Humidity Pen. The measurement accuracy is declared by the manufacturer as follows: temperature ± 1°C, relative humidity ± 5%. The temperature varied during the automatic switching of cooling from about 7.7 to 11.0°C and the relative humidity ranged from about 47 to 85% (Table 1).

After the previously explained treatment, seeds were subjected to three experiments. Stratification was carried out in wet sand, in the dark and at temperatures of 5 ± 2°C, from 9th November 2014 to 12th February 2015, i.e. 13 weeks and 4 days. Stratification was terminated when germination became apparent, even under these conditions. Then the seeds were placed on wet filter paper in a glass container, in diffused light at the natural day length, at temperatures of 20–22°C to continue the germination. They were treated with the fungicide Previcur against mildew. The resulting state was determined by counting the germinated seeds and documented by photographs. This experiment with a hundred seeds served as an indicator of the maximum attainable germination and as a comparative set for comparison with seeds subjected to the second and third experiments.

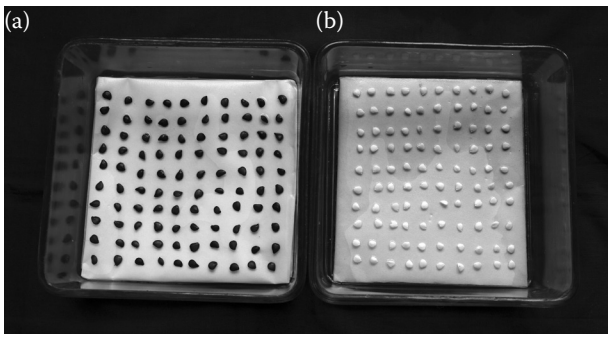

Fig. 1. Sowing on wet filter paper in a jar before closing: (a) intact unstratified seeds and shelled, (b) unstratified seeds, or bare embryos

The second experiment with two sets of a hundred seeds that were not stratified was performed as follows: the seeds were sown on wet filter paper and incubated in the same manner as in the first experiment. One series was carried out with unpeeled seeds and at the same time the second series was incubated from seeds with the seed coat removed, including the membranous endotesta (Fig. 1). The experiment was started on 2nd October 2014, when all the seeds from both series were soaked for 3 days in an excess of water, which facilitated the peeling of the seed coat. On 5th October 2014 they were placed on wet paper. On 13th October the dead seeds were distinguishable from germinating seeds according to any one of the following criteria: opening of the cotyledon, formation of roots, viridescence. Germinating seeds were counted and the state was documented by photographs. The frequency of germination of peeled unstratified seeds was compared by a chi-squared test with the frequency of seeds that were stratified in sand.

Pivot tables were produced to calculate the testing criteria, using the usual Equations (1) and (2):

$$n_{ei} = \frac{s_i \times t_j}{n} \quad (1)$$

$$\chi^2 = \sum_{i=1}^{4} \frac{n_{oi}^2}{n_{ei}} - n \quad (2)$$

where:
n_{ei} – expected frequency;
n_{oi} – observed frequency;
n – total number of seeds estimated;
s_i – sum of observed frequencies in a line;
t_j – sum of observed frequencies in a column.

The third experiment was conducted in the same way as the first experiment, but peat was used in place of sand in stratification. The outcomes of stratification in mineral (sand) and organic (peat) substrates were compared using the chi-squared test.

RESULTS AND DISCUSSION

The conditions for seeds exposed to the conditions prevailing in an ordinary refrigerator for 8 years are specified using 10 measurements at random intervals (Table 1).

The first experiment

Stratification is recommended as the best practice to induce germination (Miko, Gažo 2004; Paganová 2007; Benedíková 2009). A set of 100 seeds was stratified in the manner described and after less than 14 weeks, even at low temperatures and in the dark, 77% of the seeds germinated (Fig. 2b). They were subsequently placed on wet filter paper and then incubated for 4 days in the manner described above, in a jar in daylight and at higher temperatures. Germination was rapid, and so in this short time the final germination rate of 95% was achieved (Fig. 3). According to the findings of other authors this value is a maximum (Miko, Gažo 2004; Benedíková 2009). It is therefore useful as a comparative set for the evaluation of further experiments. In itself, however, it allows the surprising assertion that, even after 8 years, the seeds stored in the simplest way in a dry state in the refrigerator achieve the maximum life expectancy. It is therefore a better way of storing seeds than those reported in the peer-reviewed methodology for the propagation and cultivation of the service tree,

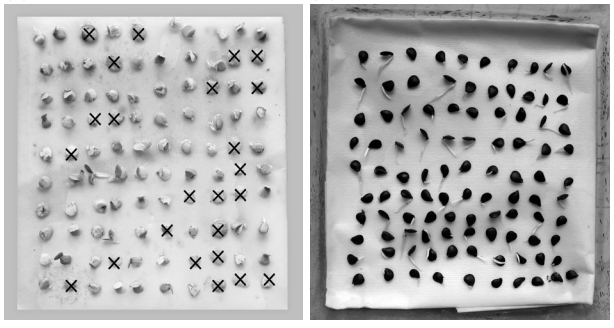

Fig. 2. Germination rate of shelled unstratified seeds 76%, observed in the second experiment (dead seeds are marked with a dagger) (a), status of seeds at the end of stratification, observed during the first experiment (this set was consecutively affected by incubation in heat and light) (b)

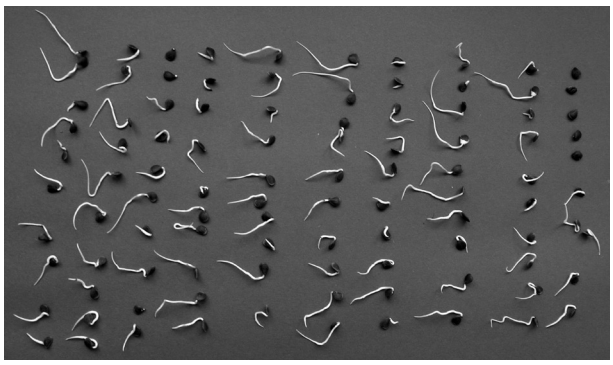

Fig. 3. Germination of the set from Fig. 2a increased after incubation of stratified seeds in heat and light (95% germination rate at the end of the first experiment)

in which the condition of the seeds frozen at –20°C already worsens during the third year (Benedíková 2009). Cryopreservation of seeds is usually carried out after they are dried by a rather strong desiccant agent, which seems to be rather destructive, unlike the more gentle air drying and storage of seeds at temperatures above zero. In search of an explanation, had been taken into account the climate diagrams (Walter, Lieth 1960) from the core area of occurrence of the service tree (Rotach 2003). From the examples of the sites in Larissa (Greece), Bologna (Italy) and Digne (in southern France) it can be seen that the average winter temperatures are above zero. The embryos in the air-dried seeds, with a natural water content in the tissue, clearly survive better in storage conditions closer to the environmental constitution of the species during the period of dormancy than when they are deep-frozen for a long period of time.

The second experiment

Other possibilities for inducing the germination were also explored, namely by simply removing the seed coat. It had been worked with the assumption that dormancy could be maintained by an inhibitor contained in the seed coat (amygdalin or abscisic acid). Intact seeds and seeds with the seed coating removed (both sets unstratified) were placed on wet filter paper. Both sets were simultaneously incubated in the conditions described. Not even a single intact seed germinated within one month. It was possible to assess the peeled seeds as early as on the eighth day, because the germinating seeds (see assessment criteria) clearly differed from the dead ones, which were already visibly affected by rotting despite treatment with a fungicide. The germination rate of shelled seeds was found to be 76% (Fig. 2a and Table 2).

Table 2. Pivot table of observed frequencies n_{oi} and calculated expected n_{ei} frequencies n_{ti} (in brackets) used for the chi-squared test

Seeds		Viable	Dead	s_i
Stratified and intact seeds versus unstratified and peeled seeds				
Stratified intact		95 (85.5)	5 (14.5)	100
Unstratified peeled		76 (85.5)	24 (14.5)	100
t_j		171	29	(n) 200
Survival of seeds affected by stratification				
Stratified in	sand intact seeds	95 (59)	5 (41)	100
	peat and then peeled seeds	23 (59)	77 (41)	100
t_j		118	82	(n) 200

t_j – sum of empirical frequences in a column, s_i – sum of empirical frequences in a line

A hypothesis was tested that the proportions of viable seeds for stratified (in sand) and unstratified but peeled seeds are the same (Table 2).

The calculated $\chi^2 = 14.559$ and by the probability $P = 0.05$ we find $\chi^2_{crit.} = 3.84$. $\chi^2 > \chi^2_{crit.}$ Therefore, we reject the hypothesis that the frequency of viable seeds for both groups is the same.

Peeling the seeds, i.e. exposing embryos, has been carried out by other researchers in differently conceived experiments (Arrillaga et al. 1992), but these authors believed that the germination was dependent on the use of an in vitro culture medium with mineral nutrients and 3% sucrose. According to the experiment presented here, germination is not supported or induced by the effects of a nutrient-rich medium, and can also take place in non-sterile conditions, provided with sufficient water. However, the ability of the seeds to germinate immediately after peeling is most useful in practice just for beginning the cultivation in vitro (Prknová, Kobliha 2008). Stratification of seeds remains the best method for intact seed germination in non-sterile conditions, whereas stratified seeds are unsuitable for germination in vitro even after sterilization, because they are highly contaminated by soil microorganisms. From this experiment, we receive the answer to the second question, and I can state that, to induce germination, enzymatic degradation of the inhibitor in the seed during stratification is not necessary. The crucial factor for inhibition is the seed coat. Its removal will terminate the inhibition at any time of the year, without the seeds being stratified. However, germination is significantly reduced in comparison with stratified seeds, and therefore we can assume that the embryo also presents a source of inhibition of seed germination, but a weaker source than the seed coat. Such is an answer to the second question posed in the introduction.

The third experiment

The seeds stratified in peat behaved differently from the seeds stratified in sand, as none of them germinated. However, after the seeds were peeled, 23% of the seeds displayed some viability. The obvious negative impact of the peat was further statistically confirmed. A hypothesis was tested that the proportions of viable seeds stratified in sand and in peat are the same (Table 2).

The calculated $\chi^2 = 107.152$ and by the probability $P = 0.05$ we find $\chi^2_{crit.} = 3.84$. $\chi^2 > \chi^2_{crit.}$ Therefore, we reject the hypothesis that the frequency of viable seeds for both groups is the same.

Therefore, peat is unsuitable for stratification. This surprising finding raises a question for further research: is an organic substrate also unsuitable for the germination of service tree seeds in natural conditions, and is the germination most successful on soils without a humus horizon?

CONCLUSIONS

We can recommend storing the service tree seeds so that they are air-dried and maintained at temperatures varying between 7.7°C and 11°C in a refrigerator. It was demonstrated that the maximum known germination rate was retained for 8 years.

Wet sand is an ideal substrate for stratification. After stratification at temperatures of about 5°C for 13 weeks dormancy was completely broken and the germination rate reached 95%.

Dormancy can also be broken without stratification, namely by peeling the seeds, by which a very satisfactory germination rate of 76% was also achieved.

References

Arrillaga I., Marzo T., Segura J. (1991): Micropropagation of juvenile and adult *Sorbus domestica* L. Plant Cell Tissue and Organ Culture, 27: 341–348.

Arrillaga I., Marzo T., Segura J. (1992): Embryo culture of *Fraxinus ornus* and *Sorbus domestica* removes seed dormancy. HortScience, 27: 371.

Arrillaga I., Lerma V., Pérez-Bermúdez P., Segura J. (1995): Callus and somatic embryogenesis from cultured anthers

of service tree (*Sorbus domestica* L.). HortScience, 30: 1078–1079.

Benedíková M. (2009): Metodické postupy a pěstování jeřábu oskeruše (*Sorbus domestica* L.). Lesnický průvodce, 3: 1–18.

Brus R., Ballian D., Bogunić F., Bobinac M., Idžojtić M. (2011): Leaflet morphometric variation of service tree (*Sorbus domestica* L.) in the Balkan Peninsula. Plant Biosystems, 145: 278–285.

Ďurkovič J., Lengyelová A., Čaňová I., Kurjak D., Hladká D. (2009): Photosynthetic performance and stomatal characteristics during ex vitro acclimatisation of true service tree (*Sorbus domestica* L.). Journal of Horticultural Science and Biotechnology, 84: 223–227.

Ďurkovič J., Mišalová A. (2009): Wood formation during ex vitro acclimatisation in micropropagated true service tree (*Sorbus domestica* L.). Plant Cell Tissue and Organ Culture, 96: 343–348.

Hrdoušek V., Špíšek Z., Krška B., Šedivá J., Bakay L. (2014): Oskeruše strom pro novou Evropu. Available at http://www.maskrajinasrdce.cz/files/Brozura%20oskeru%C5%A1e-web.pdf

Kamm U., Rotach P., Gugerli F., Siroky M., Edwards P., Holderegger R. (2009): Frequent long-distance gene flow in a rare temperate forest tree (*Sorbus domestica*) at the landscape scale. Heredity, 103: 476–482.

Kopecký V. (ed.) (2014): Service tree (*Sorbus domestica* L.) in the České středohoří Mountains. Děčín, Czech Union for Nature Conservation Děčín District, AOPK ČR – České středohoří PLA Administration: 39.

Mikic T., Orlovic S., Markovic M., Kovacevic B., Pilipovic A. (2008): Variability in service tree (*Sorbus domestica* L.) populations in Serbia. Forestry Journal, 54, (Supplement 1): 61–67.

Miko M., Gažo J. (2004): Morphological and biological characteristics of fruits and seed of the service tree (*Sorbus domestica* L.). Journal of Fruit and Ornamental Plant Research, 12 (Special ed.): 139–146.

Miletić R., Paunović S.M. (2012): Research into service tree (*Sorbus domestica* L.) population in Eastern Serbia. Genetika-Belgrade, 44: 483–490.

Paganová V. (2007): Generative reproduction of *Sorbus domestica* L. as a limiting factor of its wider utilization in conditions of Slovakia. Propagation of Ornamental Plants, 7: 199–203.

Paganová V. (2008a): Ecology and distribution of service tree *Sorbus domestica* (L.) in Slovakia. Ekológia (Bratislava), 27: 152–167.

Paganová V. (2008b): Ecological requirements of wild service tree (*Sorbus torminalis* [L.] Crantz.) and service tree (*Sorbus domestica* L.) in relation with their utilization in forestry and landscape. Journal of Forest Science, 54: 216–226.

Paganová V., Jureková Z. (2012): Woody plants in landscape planning and landscape design. In: Ozvavuz M. (ed.): Landscape Planning. Shanghai, Rijeka: 199–216.

Piagnani M.C., Zaccheo P., Crippa L. (2012): Micropropagation of service tree (*Sorbus domestica* L.): role of some factors on in vitro proliferation and rooting, and extra vitro acclimatization. Agrochimica, 56: 219–233.

Prknová H., Kobliha J. (2008): Micropropagation of *Sorbus domestica* L. for forestry practice – optimalisation of *in vitro* cultivation. Forestry Journal, 54: 393–402.

Rotach P. (2003): EUFORGEN Technical Guidelines for genetic conservation and use for service tree (*Sorbus domestica*). Rome, International Plant Genetic Resources Institute: 6.

Walter H., Lieth H. (1960): Klimadiagramm-Weltatlas. Jena, Gustav Fischer Verlag: 253.

Received for publication June 11, 2015
Accepted after corrections September 1, 2015

Corresponding author:

Ing. Hana Prknová, Ph.D., Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Kamýcká 1176, 165 21 Prague 6-Suchdol, Czech Republic; e-mail: prknova@fld.czu.cz