Testing of germination of spruce, pine and larch seed after 10 years from collection – Short Communication

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ABSTRACT: Germination capacity and germination energy are usually the most frequently used quantitative parameters of forest tree seed. With seed ageing both parameters decreased and the rate of the collapse is given by tree species, age of tree and its seed and biotic and abiotic factors. Relatively little attention has been paid to the age of seed. As it was found, the longevity of the main tree species remained relatively high, and spruce (*Picea abies* [L.] Karsten and pines (*Pinus sylvestris* L.) from the investigated areas across the Czech Republic maintained minimally one third of germination capacity or germination energy during the 10 years with the exception of larch (*Larix decidua* Mill.) where germination capacity decreased almost to zero after 10 years. Although the germination energy and germination capacity decreased significantly, it is possible to use the seed in the case of shortage of the seed of better quality.

Keywords: germination capacity; germination energy, viability; long-term storage

Germination capacity of seed comes from its genetic origin and environmental factors. Germination capacity is influenced by tree species, age of tree and its seed, climatic conditions during the pollination and biotic factors such as fungal or insect infestation. The age of the parent trees reduces germination capacity especially after thermal shocks, darkness, and leaf and humus exudates where seeds of older parent trees have lower germination capacity (Alvarez et al. 2005) Immersion of the seed in water enhanced germination energy (Himanen et al. 2012). Moreover, dormancy and germination are complex traits that are controlled by genes which are affected by both developmental and environmental factors. Little is known about the interconnected key molecular processes controlling dormancy and germination in response to hormonal and environmental cues (Koornneef et al. 2002). The light is another factor influencing the germination capacity – seeds of some tree species do not germinate in the dark (Leinonen, De Chantal 1998; Cho et al. 2014). Experiments with alternate red and far red light treatments indicate the need for long periods of exposure to red light for germination – this property of seeds may be related to the detection of light gap size and its differentiation from the normal flecks of sunlight in the forest (Vázquez-Yanes, Smith 1982). Allelopathy is another influencing factor. In some cases a release of water-soluble phytotoxic substances from the dwarf shrub *Empetrum hermaphroditum* has strong negative effects on germination and early root development of Scots pine (Zackrisson, Nilsson 1992). Water is crucial for germination because of water intake during swelling when the starch is split into the elementary sugars as an available source of energy. An amount of water keeping the seed in a non-germinating resting condition is 5–9% (Tommasiet al. 1999). The temperature for germination of pine, spruce and larch should be alternate temperatures 20 and 30°C. Age as an important factor for germination capacity has been published less frequently. Generally, increasing seed age is accompanied by lower germination energy, germination capacity and number of abnormally germinated seeds hard or fresh (Bewley, Black 1994). With increasing age the germination capacity, germination energy and absolute weight are decreasing. There is a significant effect of storage conditions on seed ageing: (1) the temperature and humidity, the lower the temperature and/humidity, the longer storage is possible (Bonner

1990); (2) drying and packaging and atmosphere composition (Shrestha et al. 1985; Niane et al. 2013).

There is information about conifer and broad-leaved seed storage longevity in the Czech Republic (CR); nevertheless, many of the studies are focused on the species not native to the CR (Simpson et al. 2004).

We expect that (*i*) seeds older than 10 years will have very low germination energy as well as germination capacity, (*ii*) there will be significant differences between tree species in a decrease of germination capacity, (*iii*) there will be significant differences in selected tree species between different regions.

The main purpose of the study was to establish whether the originally best germinating seed lots are expected to have a smaller decline of germination capacity and describe the loss of germination capacity in practical conditions. We used typical seed for forest regeneration in the Czech Republic.

MATERIAL AND METHODS

The cones of spruce (*Picea abies* [L.] Karsten), pine (*Pinus sylvestris* L.) and larch (*Larix decidua* Mill.) were collected during November and December 2001. During January and February 2002 the seeds were extracted (Table 1). The cones were dried at 40°C. The extracted seeds were cleaned and dewinged and the seed lots were stored in plastic ziplock bags, using the amount of 250–300 g of seeds per one region. Seed lots of all investigated trees were handled similarly. During April 2002 the germination tests were carried out and in 2012 the same tests were repeated under the same conditions. During the 10 years seeds were stored in a refrigerator at a temperature of 4–6°C. From each species 4 × 100 seeds were randomly detracted from 250 g lots and they were placed on moist filter paper (diameter 11 cm) in Petri dishes (diameter 12 cm) with 10 ml of water. Seeds were incubated in the growth room at 22 ± 2°C under cool-white (Philips) light at 65–70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16/8-h photoperiod. During the experiment continuous control of Petri dishes was applied, distilled water was provided to keep the filter paper wet. After 7 days all germinated seeds were counted and germination energy was estimated. After 21 days all seeds were counted and germination capacity and number of fresh seeds were estimated. Before the experiment selected seed samples were weighed for a comparison of weight changes (Denver Instruments SI-234, New York, USA). Differences between qualitative parameters in 2012 and 10 years ago were evaluated by the Statistica software. During data processing the following methods were used: Mann-Whitney U Test for statistical significant differences between the variables, chi-square test for evaluating if paired observations on two variables are independent of each other and Spearman correlation coefficient for strength and direction of the linear relationship between variables. Data were evaluated at the level $\alpha = 0.05$.

According to the place of origin the investigated seed lots were dispersed across the CR (Fig. 1).

RESULTS AND DISCUSSION

After 10 years the germination capacity and germination energy remained relatively high in all tree species although statistically significant differences

Table 1. Overview of the origin of tested seeds

Tree species (labelling)	Natural forest area	Altitudinal vegetation zone	Forest category
Pine (A)	29 – Nízký jeseník	3	A
Spruce (B)	3 – Karlovarská vrchovina	5	B
Larch (C)	33 – Předhůří Českomoravské vrchoviny	3	B
Pine (D)	9 – Rakovnicko-Kladenská pahorkatina	3	B
Spruce (E)	10 – Středočeská pahorkatina	3	B
Larch (F)	16 – Českomoravská vrchovina	5	A
Pine (G)	10 – Středočeská pahorkatina	3	B
Spruce (I)	26 – Předhůří Orlických hor	3	B

forest category according to Recommendation No. 481211: A – the most valuable forest stands, B – forest stands of above-standard economic value, C – forest stands of average economic value, D – forest stands economically inconvenient; the capital letters behind the tree species are abbreviations of the place of origin

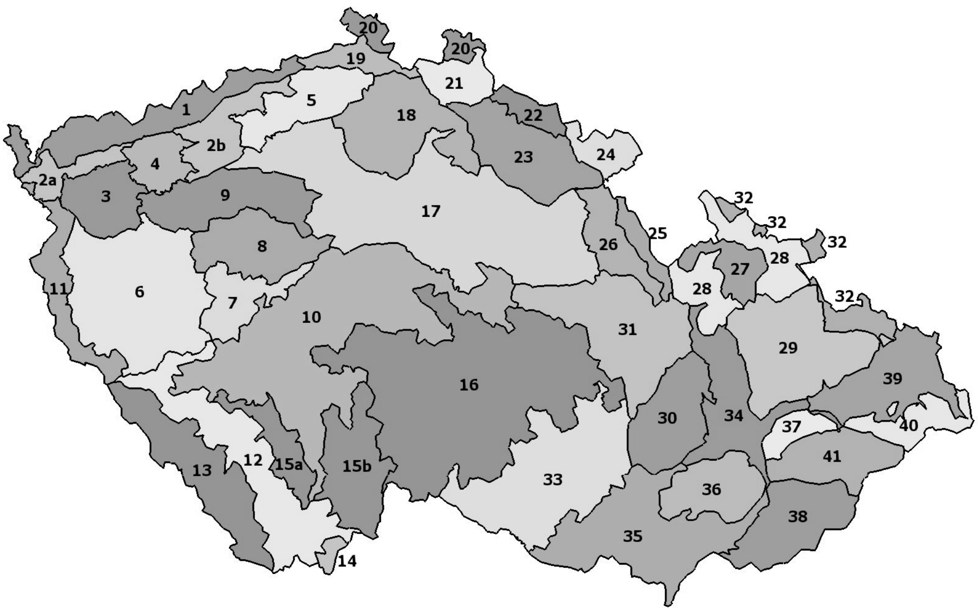

Fig. 1. Natural forest area – PLO (http://www.uhul.cz/nase-cinnost/oblastni-plany-rozvoje-lesu/prirodni-lesni-oblasti-plo)

were recorded between the values from 2002 and 2012 in all tree species (except of spruce germination energy in I region and larch germination capacity in F region). No significant decrease in absolute weight in all tree species was found out, which is probably related to oxygen consumption and depression of storage mean storage tissue. Decrease or increase of selected parameters related to the qualitative analysis in 2002 is shown in Table 2.

Germination decreases naturally during the seed lifetime due to ageing; the rate of changes is influenced by genetics, conditions during ripening and storage conditions (Anonymous 2013). Except larch from the Natural Forest Areas Předhůří Českomoravské vrchoviny and Českomoravská vrchovina with low germination capacity and germination energy in 2002 (under 30%) all spruces and pines maintained minimally one third of germination capacity or germination energy during the 10 years. A decrease of germination capacity and germination energy is presented together with fresh seed changes in Table 2. On the basis of the data reporting the longevity of 15 years in pine and spruce it is probably longer and it would be possible to use the seed in the case of shortage. Our results are in accordance with the results published in Anonymous (2013), where the 5–22 years old pine and spruce seed was used and there was still 70% germination capacity. According to our expectations the germination energy decreased to 80% in old seed samples in all species (Fig. 2). Nevertheless, the germination capacity decrease was strongly species and site dependent ranging from 10 to 80%. In all species an increase of fresh seeds was recorded, e.g. seeds which would germinate later after 21 days in healthy seedlings.

Table 2. Germination capacity, germination energy and weight tested in 2002 and 2012 in pine, spruce and larch seed from different regions (see regions A–F in Table 1)

Tree species/region	Germination energy (%)		Germination capacity (%)		Absolute weight (g) decrease from 2002 (100%) to 2012
	2002	2012	2002	2012	
Pine/A	**75**	**26**	**92**	**74**	90.6
Pine/D	**58**	**18**	**79**	**35**	97.2
Pine/G	**84**	**36**	**89**	**57**	94.8
Spruce/B	**83**	**41**	**94**	**57**	92.6
Spruce/E	**71**	**16**	**96**	**31**	98.0
Spruce/I	14	13	**89**	**45**	94.3
Larch/C	**24**	**5**	**29**	**7**	94.0
Larch/F	**21**	**11**	26	25	98.6

in bold – statistically significant differences

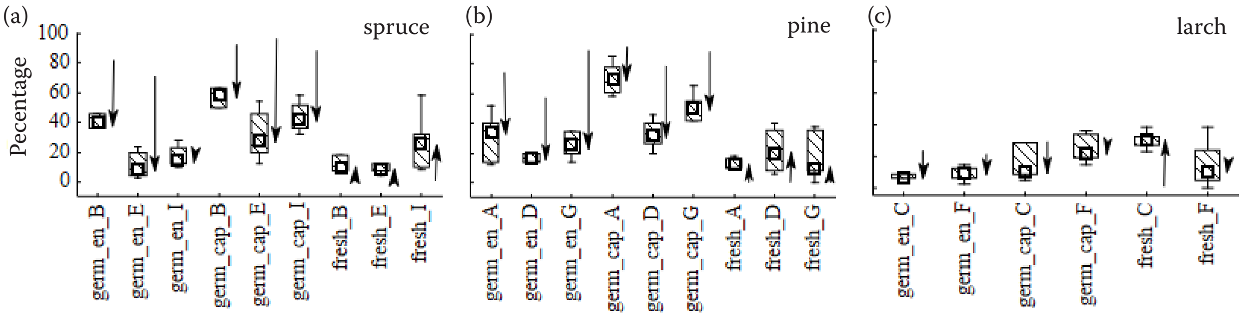

Fig. 2. Basic characteristics of spruce, pine and larch seed in 2002 (beginning of an arrow) and 2012 (median in the box with error bars as interval of confidence)

Germ_en ‒ germination energy, germ_cap – germination capacity, fresh – fresh seeds. Capital letters are abbreviations for the place of origin – see Table 1

Our hypothesis that there is a positive correlation between absolute weight decrease and germination energy decrease was not proved. Neither was a positive correlation between absolute weight decrease and germination capacity found by detailed statistical analysis. However, previous observations of individual seeds predicated such a correlation. Moreover, the chi-square test revealed that higher input values of germination capacity and energy did not ensure a milder decrease in these parameters after 10 years. To assume this issue 10-years-old or older seed material can be used in the case of shortage of better quality material.

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