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Contribution to seed ecology of *Sequoiadendron giganteum* (Lindl.) Buchholz growing in the Central European conditions

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Abstract

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The properties of Giant Sequoia (*Sequoiadendron giganteum* (Lindley) Buchholz) seeds originating from a single cultivated Sequoia tree in the second gene strain in the Czech Republic were examined. The maximum germination level was set at 1.8%. This type is a pyrophyte, but nonetheless the short-term effects of a temperature of 100°C simulating a natural fire are still lethal. The seeds were planted with the same success in one case using a fungicide, which is routine practice in nurseries, and in another case using a "biological method" relying on the positive effect of soil microflora. Both stratified and non-stratified seeds can germinate, but stratification increases the number of germinating individual trees to at least double.

Keywords: giant sequoia; germination; stratification; germination level; substrates

The Giant Sequoia (*Sequoiadendron giganteum* (Lindley) Buchholz) is cultivated in the Czech Republic only for decorative purposes. Due to its protected status, it is not used in commercial forestry even in the United States, but due to its large dimensions and quality of wood which is resistant to rotting, it has a huge potential for use.

The history of introduction of the Giant Sequoia into the Czech lands dates back to the end of the 19th century. In the Ratměřice municipality in Central Bohemia there are individual fertile trees which are more than 160 years old (Němec, Kyzlík 2003). However, the geographic latitude and climate of our territory differ greatly from the species natural range in California (Thomas et al. 2016). The individual tree cultivated already for 64 years in Kostelec nad Černými lesy, at the arboretum of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences in Prague – Truba breeding station, is the offspring of a Sequoia tree from Ratměřice. Thus it is a tree that continues a domestic generation, with genetic properties that make it suitable for life far away from the ideal ecological conditions for the species. This has been verified in a less favourable climate for the species than the sub-oceanic climate of Western and Southwestern Europe, where they have been cultivated and where the first such trees on the European continent have grown to maturity (Češka 2002). The situation is similar to that of other species originating from the Pacific region and widely used in European forestry, such as Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) and giant fir (*Abies grandis* (Douglas ex D. Don) Lindley) (Podrázský, Remeš 2007; Podrázský et al. 2016).

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Seeds originating from natural locations have very well examined properties (Thomas et al. 2016). But what are the properties of seeds of Czech origin, and how can they most easily be sown successfully? We attempted to find this out by solving the following partial problems:

(*i*) If it is known that a Sequoia tree can grow in habitats naturally influenced by fires, do such conditions cause a thermal shock to seeds and act as an inductor of germination, like in the case of seeds of known pyrophytes (*Drosophyllum* Link, *Roridula* Burman f. ex Linnaeus, *Banksia* Linnaeus f., etc.)?

(*ii*) Is it possible to replace the standard means of sowing stratified seeds (substrate of peat and sand, fungicide use) by an alternative biological method (substrate enriched with humus from coniferous forests, without the use of fungicide)?

(*iii*) Do both stratified and non-stratified seeds germinate?

(*iv*) If the seeds have not been stratified, then is it possible instead of having them germinate using the standard method of sowing to support them using soil microflora, the biological method of sowing?

MATERIAL AND METHODS

Seeds originated from a 64-year-old tree 26.5 m tall with the trunk measuring 90 cm in diameter at the breast height of 1.3 m, growing in Kostelec nad Černými lesy (Truba breeding station), the offspring of a tree growing in Ratměřice. The individual tree in Kostelec grows alone, while the closest fertile individual tree is 1.5 km away in a straight line. The average size of cones of this individual tree (based on a sampling of 100 cones) was 6.0 cm in length and 4.8 cm in width in the fresh closed condition. The cones were picked on 23 December 2015. The seeds were extracted at the Truba breeding station and stored in a refrigerator in a plastic sack.

Two variants of substrate were prepared for experiments:

(*i*) standard: Baltic peat + silica sand (grains of 0.5 to 1.5 mm), at a ratio of 3:1;

(*ii*) enriched with forest microflora and proportions used by cultivators for especially environmentally sensitive plants: Baltic peat + humus from a pine forest + silica sand (grains of 0.5 to 1.5 mm) + clipped green interior sections of peat + dried crushed peat + crushed charcoal at a ratio of 6:6:2:1:1:1 (Šrůtka 2005).

When the standard substrate was used, always after two weeks Previcur Energy fungicide (Bayer S.A.S., France) was applied by spraying, to prevent seedlings from falling over; we refer to this method of sowing as the "standard method". The use of the enriched substrate was not associated with the use of any chemical products; we refer to this method of sowing as the "biological method".

In February, the seeds were weighed for individual tests in plastic bags (40 g each). It was determined statistically that this amount represents 11,498 seeds with an insignificant deviation, which was the batch size for each of the plantings. Half of the seeds were stratified, while the other half was not. The stratification took place under moist sand, in the dark, at a temperature of 5 ± 2°C from 3 February 2016 to 12 April 2016 (10 weeks). During examination of the effect of thermal shock, before sowing the stratified seeds were placed on a sieve through which boiling water was poured.

The sowing took place on 13 April 2016 in routinely used plastic vegetable crates with holes and water penetrable geotextiles. The germination occurred in a greenhouse, at temperatures between 15 and 22°C. Sowing was done in such a way that the seeds were covered over with a 5 mm thick layer of silica sand with grains measuring 0.5 to 1.5 mm.

All variants of sowing were monitored for three months: Every two days the number of living (vitality +) and dead (vitality –) seedlings was recorded for each experimental variant.

In the comparison of the experiments, the resulting values were arranged in 2 × 2 contingency tables. The zero hypothesis was formulated and tested with the help of a χ^2 test, in the same manner as in the previous work focused on germination (Prknová 2015). If the fields of the 2 × 2 contingency table are labelled a, b, c or d, where $a + b + c + d = n$, then the testing criterion can be most easily counted according to Eq. 1:

$$\chi^2 = \frac{(ad - bc)^2 \times n}{(a+b)(c+d)(a+c)(b+d)} \qquad (1)$$

where:

a – number of living seedlings originating from the standard method,

d – number of dead seedlings originating from the biological method,

b – number of living seedlings originating from the biological method,

c – number of dead seedlings originating from the standard method.

RESULTS

The effect of thermal shock on seeds, caused by one-time brief pouring of boiling water, turned out to be 100% lethal. This factor, expected in a species growing in an environment where fires occur naturally as a possible inductor for germination, was therefore ruled out.

Seeds preadapted by stratification were subjected to further examination to determine whether they would better germinate during sowing using the standard method or using the biological method. A zero hypothesis was formulated: The methods do not differ significantly based on the number of living seedlings and mortality at the probability level of $P = 0.05$.

Based on Table 1, the testing criterion χ^2 was found to be equal to 0.06 and was compared with the critical value $\chi^2_{krit} = 3.84$. So it applies that $\chi^2 < \chi^2_{krit}$. Therefore, we accept the zero hypothesis at the probability level of $P = 0.05$. Germination of stratified seeds does not depend on the used sowing method, and either the standard method or the biological method can achieve the same results.

In the natural environment, an area where ecological conditions are favourable for germination thanks to a previous fire is naturally sown with seeds falling from cones in tree crowns at the top. Such seeds do not undergo any stratification. The question that needs to be answered is whether these seeds can germinate immediately. We also asked whether or not the natural soil microflora in a coniferous forest, with its numerous mycorrhizae, supports germination by breaching pods or even penetrating into the seeds.

An experiment serving to shed light on this question involved planting stratified seeds and then separately non-stratified seeds using both the standard and biological methods. The resulting numbers of successfully germinated (living) seedlings are shown in Table 2.

Table 1. Numbers of living and dead seedlings after sowing using two methods

Vitality	Method		Total
	standard	biological	
+	(*a*) 159	(*b*) 148	307
–	(*c*) 50	(*d*) 44	94
Total	209	192	(*n*) 401

The marking of cells with letters corresponds to Eq. 1; *a* – number of living seedlings originating from the standard method, *c* – number of dead seedlings originating from the standard method, *b* – number of living seedlings originating from the biological method, *d* – number of dead seedlings originating from the biological method

Table 2. Frequencies of successful germination when different sowing methods are used for stratified and non-stratified seeds

Treatment	Method		Total
	standard	biological	
Stratified	(*a*) 159	(*b*) 148	307
Not stratified	(*c*) 58	(*d*) 70	128
Total	217	218	(*n*) 435

The marking of cells with letters corresponds to Eq. 1; *a* – number of living seedlings originating from the standard method, *c* – number of dead seedlings originating from the standard method, *b* – number of living seedlings originating from the biological method, *d* – number of dead seedlings originating from the biological method

A zero hypothesis was formulated: the biological method and the standard method of sowing do not differ significantly at the probability level of $P = 0.05$ during germination of stratified and non-stratified seeds.

Based on Table 2, the testing criterion was figured as $\chi^2 = 1.52$ and compared with the critical value of $\chi^2_{krit} = 3.84$. So it applies that $\chi^2 < \chi^2_{krit}$. Therefore, we accept the zero hypothesis at the probability level of $P = 0.05$. Soil microflora supplied to the substrate in the ground from a coniferous forest is neither an inductor nor a stimulator of germination.

In the aforementioned data (Tables 1 and 2), an experiment can be sought with a maximum number of germinated seeds from a sampling of 11,498 seeds, and with the greatest production of actually living seedlings, which have not succumbed to mycosis (Table 1: *a*, *c*). When a total of 209 seedlings germinated (including those that later died), the figure was 1.8%. Of them, 159 living and further developing seedlings remained, which is 1.4% of the total number of planted seedlings (Fig. 1a). At the same time, it has to be pointed out that according to the statistical result, the biological method of sowing seeds is just as effective (Fig. 1b).

DISCUSSION

The five questions highlighted in the introduction can be cleared up based on the results. The first relates to the well-known fact that the habitats of Sequoia trees are influenced by fires, and this tree can be considered a pyrophyte (Stark 1968). It has very thick bark with excellent thermally insulating properties and cones holding seeds for

Fig. 1. Seedlings at an age of 11 weeks: classic (a), biological (b) method of sowing with stratification of seeds

many years and releasing them under the influence of heat, which are striking adaptations to their way of living. Yields from the *Banksia* species function similarly and include a range of known pyrophytes (Walter 1968). Some pyrophytes release seeds immediately after ripening, but only some of them germinate during the same year. Another portion becomes part of the seed bank and germinates only after receiving thermal shock during a natural fire (Juniper et al. 1989; Studnička 2006). In the case of Sequoias, it has been proven that the seeds themselves are not resistant to short-term effects of a temperature of 100°C. The massive points of cones creating a "sandwich structure" apparently function during a fire like thermal protection for seeds. Precisely the delay between a fire and the dispersal of seeds is apparently important for the process of opening the cones. Seeds falling on the hot soil should completely lose their ability to germinate according to the results of our experiment.

The second question, which method is best for planting Sequoias, was resolved by the fact that cultivation in a complex substrate proven to be good for sensitive plants that have difficulty germinating (the "biological method") is not essential (Šrůtka 2005). On the contrary, in the biological method no fungicide is used, and it is expected that injecting the natural microflora of a coniferous forest into the substrate causes concurrence or predation regulating the proliferation of pathogens. Biological protection of plants with the aid of Polyversum is based on this principle (Procházková, Rulfová 2009).

The third question, whether to germinate not only stratified seeds but also non-stratified ones, was resolved positively by experiments. Germination of non-stratified seeds, always less numerous than that of stratified seeds, has not been improved at all by cultivation using the "biological method", which was the fourth issue.

The fifth issue was the ability of an individual Sequoia growing in conditions of the Czech Republic, far away from its ideal environment, to produce germinable seeds. The ecology of seeds harvested in natural habitats was described by Stark (1968). He stated that about 66% of seeds produced during the life of a tree are abnormal, or are not released from cones at all. Another 25% are destroyed on the ground, usually by rodents. As a result, the germination rate is about 20% in natural conditions. Epigeic germination is usually unsuccessful, and hypogeic germination is ideal. The depth at which seeds germinate must be small though, and from a depth of 2.4 to 3.6 cm only very few seedlings will sprout to the surface. Stark (1968) sowed seeds gathered from various locations in the natural environment under artificial conditions, and they were non-stratified seeds. They germinated best at temperatures between 10 and 20°C. If only successfully germinating seeds were counted (mortality was respected just as it was in our experiments), the germination rate amounted to 21.0 to 55.5%. Compared to this value, the best successful germination rate of only 1.4%, as found by us, is at least 15 times lower than what is natural for Sequoia trees.

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