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The effect of the *Sequoiadendron giganteum* (Lindl.) Buchholz cone crystals on germination

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Abstract: The cones of the giant sequoia contain red, water-soluble crystalline substances known as cone crystals. The inhibitory effect of this extracted material on Norway spruce, Scots pine and European larch germination was newly examined. Sown seed representative samples without and with added cone crystals were compared after their incubation in the same appropriate conditions. All these cases have brought compelling evidence that cone crystals totally inhibit germination. However, the chemically inhibited seeds being rinsed afterwards germinated very well. This switch effect caused by the cone crystals of the three above-mentioned conifers proved to be a nonspecific tool. Seeds of the giant sequoia, naturally affected by the cone crystals, were sown as rinsed and non-rinsed samples. Both the samples, grown on a wet peat substrate in appropriate conditions for 16 weeks, exhibited an equal, yet very low viability of 1.3% at the same time. This low number, fixed in seeds of the tree being far from the ecological optimum, does not allow any disputation with other authors.

Keywords: cone pigment; giant sequoia; inhibitory effect; tannin

The giant sequoia [*Sequoiadendron giganteum* (Lindl.)] Buchholz grows naturally in the Sierra Nevada Mountains of California. It was discovered in 1841 and introduced into Europe in 1853 (Pilát 1964). The two oldest trees of this species in the Czech Republic occur in the castle park in Ratměřice, they were planted around the year 1879 (Pospíšil 2017). The giant sequoia is cultivated only for decorative purposes in the Czech Republic. However, due to its large dimensions and high-quality wood, it has a great potential for commercial use. This article is aimed at enhancing the knowledge of the life strategy and ecology of this species.

The giant sequoia's cones are ovoid in shape and the average length is 5.08–8.89 cm while the average diameter is 3.81–5.72 cm. One cone produces about 200 seeds (Hartesveldt et al. 1975). The cone size and the number of seeds in one cone are highly variable (Beidleman 1950). The cones are not shed when the seeds are ripe. They may remain attached to the tree for many years, to be shed gradually at some later time, many of them through an external action such as wind, snow, the intervention of squirrels or beetles etc. All such cones remain green, and the tissues in the cone scales remain alive. Throughout this period, the seeds may be retained; in several cones that were more than twenty years old, a good quantity of seeds was still obtainable. As soon as the cones become detached, they dry out, and the seeds are freed within a few days (Buchholz 1938). The age of sequoia cones can be determined in a similar way like the age of a tree's trunk, i.e. by counting the annual growth rings in the cone peduncle or the stem (Buchholz 1938; Hartesveldt et al. 1975).

During the process of the seed extraction, a red, water-soluble crystalline substance falls out of the cones. Beidleman (1950) reported that the seeds

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ordinarily appeared to be compressed against the underside of each scale and were packed among flakes of reddish tannin, a crystalline preservative. Harvey et al. (1980) called this substance as 'cone pigment', Kritchevsky and Anderson (1955) used the term 'cone crystals'. The authors mentioned that the cones may contain 1.2–2.6% of this substance. The cone crystals contained 66% of water-soluble material and additional 4.2% soluble in ethyl ether. Pyrogallol, catechol, phloroglucinol, gallic acid, and protocatechuic acid appear to be among the degradation products of the cone solids. These compounds, with the possible exception of catechol, also occur in a free state in the cone solids. No sugars were detected among the hydrolysis products. Martin (1958) used the German term 'Zapfenpigment' for this substance and described it as a tannin glucoside $C_{21}H_{20}O_{10}$, which, when hydrolysed, yielded sugar, gallic acid and phlobaphene. The biological effect of this substance has not yet been completely detected. What is often broached is the subject of whether the substance is able to prevent the growth of fungal pathogens.

It has been proved that even the highest concentrations of this substance are not able to inhibit the growth of fungal cultures. However, it has also been found out that the seeds obtained from 14-year-old cones retain their ability to germinate, while cleaned seeds lose their ability to germinate very quickly when stored. This behaviour indicates that this substance preserves the seeds in the cones for many years and prevents their premature germination in the cones. After the cones open, the seeds fall out, the substance is washed away, and then the seeds can successfully germinate (Martin 1958). The inhibitory effect on giant sequoia seed germination has been proved in previous studies (Stark 1968; Harvey et al. 1980).

The aim of the experiments in this study was to confirm the inhibitory effect of the cone crystals on the germination of giant sequoia seeds and to explore the effects of the crystals on seeds of the other conifers. As giant sequoia germinates at a low rate (Prknová 2018), the first experiment was done with seeds of our main commercial species – Norway spruce (*Picea abies* [L.] Karst.), Scots pine (*Pinus sylvestris* L.) and European larch (*Larix decidua* Mill.). The second part of the experiment examined the effect of the cone crystals directly on giant sequoia seeds.

MATERIAL AND METHODS

The basic experiment with seeds of Norway spruce, Scots pine and European larch was done in this way: two screw-top jars, each with a capacity of 200 ml, were filled with 100 seeds of Norway spruce and 80 ml of water. Subsequently, 50 g of cone crystals extracted from giant sequoia cones were added to one of the jars. The cone crystals dissolved immediately. Both the jars were stored in a refrigerator.

After 40 hours in the refrigerator, the seeds were sown in the following steps:

– Two transparent plastic boxes with dimensions of 18 × 18 × 8 cm were filled with a 3 cm layer of the inorganic substrate Seramis (porous clay granules with water storage capacity up to 30%, Mars GmbH, Germany). A two-layer filter paper of 15 × 15 cm was placed on the surface of the Seramis substrate and both the filter paper and the Seramis substrate were moistened with water containing Previcur Energy fungicide (Bayer S.A.S., France) against mildew; the solution from the jar containing cone crystals was added to one of the boxes (the variant with cone crystals).
– On the filter paper were placed 100 seeds aligned in a 10 × 10 grid.
– A high concentration of cone crystals, which were moistened with water from a spray bottle, was poured over the seeds that had been soaked in the cone crystal solution.
– Both boxes were covered loosely with a transparent plastic lid (with slots on the sides) and were left in daylight at room temperature.
– The germination was monitored, and after one week the germination percentage was determined.

After the basic experiment was finished, the seeds from the variant with cone crystals were rinsed out in a sieve with running water and placed in a screw-top jar with a capacity of 500 ml and left in daylight at room temperature. The seeds were rinsed out twice each day and immediately returned to the jar which had been refilled with fresh water. After five days, the seeds were aligned on the filter paper in the same way as in the basic experiment, including the Previcur Energy fungicide treatment. The germination percentage was again determined after one week. The same steps were followed in experiments with Scots pine and European larch seeds. The methodological steps of the experiment with

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Table 1. The summary of steps taken in the experiment with the seeds of Norway spruce, Scots pine, and European larch

Jar 1	Jar 2
Norway spruce (Scots pine, Eur. larch) 100 seeds, 80 ml water 50 g cone crystals	Norway spruce (Scots pine, Eur. larch) 100 seeds, 80 ml water
Refrigerator 40 hours	
Transparent plastic box (18 × 18 × 8 cm) 3 cm inorganic substrate Seramis 2-layer filter paper (15 × 15 cm) Moistened with water + fungicide Solution from jar 1 100 seeds 10 × 10 grid on the filter paper High concentration of cone crystals + water (spray) Covered loosely (slots on the sides)	Transparent plastic box (18 × 18 × 8 cm) 3 cm inorganic substrate Seramis 2-layer filter paper (15 × 15 cm) Moistened with water + fungicide 100 seeds 10 × 10 grid on the filter paper Covered loosely (slots on the sides)
1 week	
% of the germination determined	
END of basic experiment	
Seeds rinsed out in a sieve with running water Placed in a screw-top jar (500 ml) Daylight, room temperature Rinsed out twice a day (returned to the jar, refilled with fresh water)	
5 days	
Seeds aligned to the filter paper (same way as basic experiment, incl. fungicide)	
1 week	
% of the germination determined	

seeds of Norway spruce, European larch and Scots pine are summarized in Table 1.

What followed was an experiment with seeds originating from an approximately 140-year-old tree of giant sequoia that is 44 m tall with a trunk measuring 170 cm in diameter at a breast height of 1.3 m, growing in a castle park in Ratměřice. The cones were picked on 9 February 2018. The average size of the cones of this individual tree (based on a sampling of 100 cones) was 5.35 cm in length and 4.24 cm in width. The detected weight of 1,000 seeds was 4.013 g. The seeds were extracted at the Truba breeding station. Two batches of seeds (2 × 200.65 g, which corresponds to 50,000 seeds) were weighed. The first batch of seeds was placed in a plastic sack and refrigerated. The second batch was rinsed out in a sieve and then soaked in water in a plastic bucket with a capacity of 10 l. The bucket was left in daylight at room temperature. Twice a day for one week, the seeds were rinsed and returned to the bucket which had been refilled with fresh water. After one week both seed variants were divided into five similar batches and sown in the following way: first the substrate was mixed – Baltic peat and silica sand (grains of 0.5–1.5 mm) at a ratio of 3 : 1 – and placed in perforated plastic containers measuring 60 × 40 × 11.6 cm lined with water-permeable geotextiles. Germination took place in a greenhouse at temperatures between 15°C and 22°C. Sowing was done in such a way that the seeds were covered with a 5 mm layer of silica sand with grains measuring 0.5 to 1.5 mm. Both of the sown variants were monitored for four months, and the number of seedlings was recorded every third day. The methodological steps of the method of the experiment with seeds of giant sequoia are summarized in Table 2.

RESULTS

This cone crystal experiment discovered the effect of cone crystals on the germination of Norway spruce, Scots pine, and European larch seeds; all cases showed compelling evidence that cone crystals totally inhibit germination. After one week,

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Table 2. The summary of steps taken in the experiment with the seeds of giant sequoia

Batch 1	Batch 2
Giant sequoia (200.65 g = ca 50,000 seeds) Plastic sack Refrigerated	Giant sequoia (200.65 g = ca 50,000 seeds) Rinsed out in a sieve Soaked in water in plastic bucket (10 l) Daylight, room temperature Twice a day seeds rinsed (returned to the bucket + refilled with fresh water)
1 week	
Divided into 5 similar batches Sown (substrate mixed with peat and sand (3 : 1) Placed in perforated plastic containers with water-permeable geotextiles Greenhouse (15–22°C) Covered with 5 mm silica sand (0.5–1.5 mm)	Divided into 5 similar batches Sown (substrate mixed with peat and sand (3 : 1) Placed in perforated plastic containers with water-permeable geotextiles Greenhouse (15–22°C) Covered with 5 mm silica sand (0.5–1.5 mm)
4 months	
Every 3rd day number of seedlings recorded	Every 3rd day number of seedlings recorded

the variant that had been soaked with cone crystals showed 0% germination (for Norway spruce see Fig. 1). The control variants without the cone crystals showed higher germination percentages: 87% – Norway spruce (Fig. 1), 38% – Scots pine, and 28% – European larch. After being rinsed and placed on filter paper without cone crystals, the seeds that had not initially germinated due to their contact with cone crystals, reached the following germination percentages: 92% – Norway spruce, 57% – Scots pine, and 35% – European larch.

The giant sequoia seeds that had not been rinsed (hereinafter referred to as variant I) and the giant sequoia seeds that had been rinsed (hereinafter referred to as variant II), started to germinate simultaneously 16 days after having been sown. In the case of variant I, only 8 seeds germinated, while in the case of variant II, 315 seeds germinated. Exactly one month after sowing, 105 seeds of variant I germinated, while 600 seeds of variant II germinated. Two months after sowing, 433 seeds of variant I germinated, while 683 seeds of variant II germinated. After 16 weeks, the number of seedlings of variant I levelled off at 649. In the case of variant II, the number of seedlings increased more rapidly but levelled off after 10 weeks at

(a) (b)

Fig. 1. Norway spruce seed variants: with cone crystals (a) and without cone crystals (b) one week after sowing

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Table 3. The number of giant sequoia seedlings: with cone crystals (Variant I) and with rinsed seeds (Variant II)

The day after sowing	16	30	40	50	60	70	80	90	100	110	120
Variant I	8	105	315	390	433	526	586	629	638	649	649
Variant II	315	600	652	669	683	684	684	684	684	684	684

684 seedlings (Table 3, Fig. 2). A comparison of both variants 10 weeks after sowing is depicted in Fig. 3.

DISCUSSION

Harvey et al. (1980) tested 3,000 seeds at 5 different concentrations of cone crystals (cone pigment in their terminology), i.e. 22%, 18%, 9%, 4.5%, and 2.2% by weight. The seeds showed a statistically significant ($P < 0.01$) delay and reduction in germination at the two highest concentrations of 22% and 18%. Stark (1968) also used 3,000 and 5 solutions of 10%, 25%, 50%, 75%, and 85% by weight. The cone crystals in a highly concentrated dosage (85%) retarded seed germination by one month. This concentration also produced smaller seedlings than did the other lower concentrations. Martin (1958) stated that the seed germination time increased with an increased concentration of cone crystals (Zapfenpigment in his terminology); he found that at concentrations of 30% or more the seeds failed to germinate at all. Thus, he reasoned that the cone crystals' role was to arrest the ger-

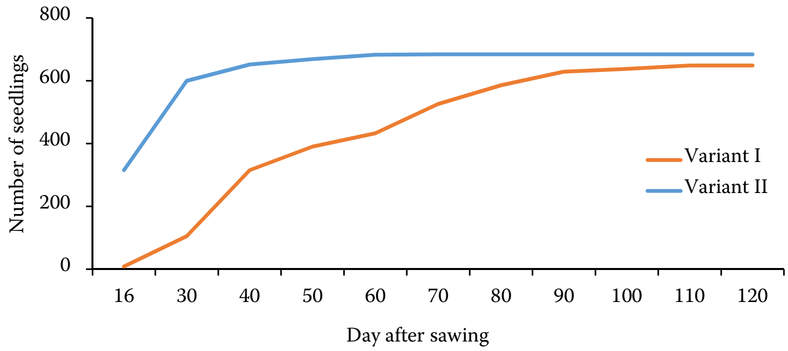

Fig. 2. Comparison of the number of giant sequoia seedlings: with cone crystals (Variant I) and with rinsed seeds (Variant II)

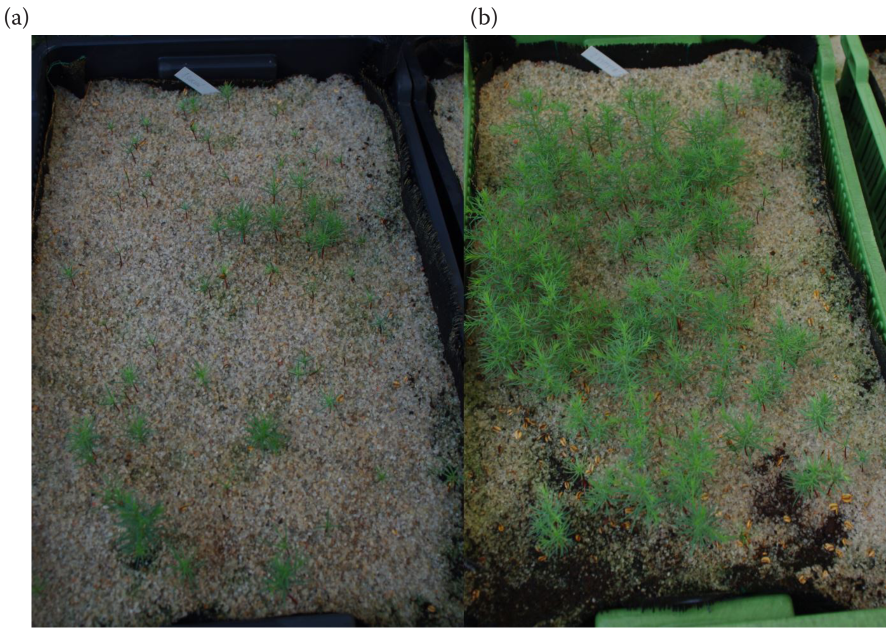

Fig. 3. Comparison of giant sequoia variants: with cone crystals (a) and with rinsed seeds (b) 10 weeks after sowing

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mination of the seeds within the cones through the establishment of a reverse osmotic gradient. He further reasoned that when the cones opened, the seeds were freed only when rainfall dissolved and washed away the cone crystals. He mentioned that the cone crystals were also able to maintain germinability, which could be useful for the long-term storage of seeds. He recommended saturating seeds in a solution of cone crystals for 1 hour and then letting them dry; in this way, full germinability could be maintained almost indefinitely. Fry and White (1930) mentioned that cone crystals also contributed to maintaining seed viability.

This experiment has proved that a high concentration of cone crystals completely inhibits the germination of Norway spruce, Scots pine, and European larch seeds. However, after the cone crystals were rinsed off, the seeds germinated very well. Three model samples prove the effect of the cone crystal extracted from giant sequoia cones. In the case of giant sequoia seeds sown on a peat substrate, a breakdown in the inhibitory effect of non-rinsed seeds was observed. The whole sample required up to 16 weeks to overcome the inhibitory effect, and the germination was highly asynchronous. Similarly to the rinsed seeds, the sample of non-rinsed seeds contained a share of 1.3% of seeds that germinated after 16 weeks. Thus, the nonspecific effect of cone crystals on germination has been proved.

Future research is recommended to reveal if cone crystals can affect the germination of deciduous tree species. Furthermore, it should be investigated if commercially produced tannins can have the same inhibitory effect on germination. It should also be explored if cone crystals maintain the viability of seeds of various woody species in long-term storage.

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