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Effect of gibberellic acid on germination capacity and emergence rate of Sycamore maple (*Acer pseudoplatanus* L.) seeds

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ABSTRACT: Seedlots of sycamore maple (*Acer pseudoplatanus* L.) originating from three different provenance regions were collected in autumn 2012. The seed quality, namely vitality and germination rate, was assessed according to Czech conventions. There were four replications within each seedlot, i.e. twelve treatments altogether for each experimental variant. The effect of gibberellic acid on germination and vitality was studied using three treatments including a control for germination capacity and three treatments including a control for emergence rate. The data shows that the gibberellic acid did not substitute for the stratification procedure as it did not improve the germination capacity of seeds with pericarp when compared with stratified seeds; however for seeds without pericarp the gibberellic acid improved the germination capacity to the level of stratified seeds. The data also shows the positive influence of gibberellic acid on emergence rate. All variants where acid gibberellic was applied have a statistically higher emergence rate than the control. The increase was about 50% higher than in the control, i.e. without the influence of gibberellic acid.

Keywords: sycamore maple; seed quality; vitality; stratification; pre-sowing treatment; seed humidity

Sycamore maple (*Acer pseudoplatanus* L.) trees are the most common maple species in European forests (Rusanen, Myking 2003). It is a native species to the Czech Republic growing on deep slopes covered by rock detritus (Pikula 2003). Sycamore is distributed through the whole country but it is more frequent in foothill and mountain areas (Jeník 1961). It is an important stabilizing species in mountain spruce forests growing at a higher altitude than beech (*Fagus sylvatica* L.), having a positive influence on the litter in forest soils (Kovář et al. 2013). This species is often the only broadleaved species in mountain coniferous forests (Sarvaš et al. 2007). The proportion of sycamore in Czech forests is about 1.3% and it is the most common maple species (Anonymous 2012). It is appreciated as a species which improves the soil quality and makes stands more stable against wind blow (Kremer 1995). Sycamore is planted by roadsides and also in parks (Slávik 2004).

Sycamore reproduction starts usually at the age of 40 years, but much earlier in some individuals (Suszka et al. 1996). Seed production is highly variable between years and can be quite different from one stand to another (Bjorkbom 1979). The sycamore fruit consists of an achene of 3–6 cm long (Coombes 1996). These are borne in pairs, the angle between the two achenes is between 60 to 90°. The achene comprises one seed which is of the size of a pea without endosperm, covered by a pericarp (Suzska et al. 1996). The pericarp starts growing before fertilization and ends with its ripening (Kupka 2005). This pericarp can be extremely hard, namely when seed humidity is low. The pericarp is the main cause of dormancy, although the

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mechanism is complicated (Booner 2008). Seeds usually ripen in September and they fall in October through the beginning of winter (Musil 2005). Their vitality is the highest right after ripening and then decreases, with a loss of germination capacity (Procházková 2010).

The quality of planting stock in forest nurseries is obviously dependent not only on high quality seed but also on proper pre-sowing treatments for dormant seeds including stratification (Bezděčková et al. 2012). Dormancy is the last period of sycamore seed ripening (Bewley, Black 1982) and it protects the seed against damage caused by germination at the wrong time of the year (Kolotelo 1998).

The sycamore seeds develop through two types of dormancy. The first one is a physiological (embryonic) dormancy which occurs in the tree crown before it falls down (Procházková 1992). The second phase of dormancy is so called morphological dormancy caused by the impervious pericarp which impedes the flushing out of inhibitors from the embryo (Webb, Wareing 1972a).

Maple seeds need a chilling temperature for successful germination. Common methods of pre-sowing preparation are stratification in a suitable medium, i.e. wet sand mixed with peat and temperature between 1–5°C (Procházková 1993). The recommended stratification length varies between 35 and 140 days (Vincent 1965; Schopmeyer 1974; Tóth, Garrett 1989; Suzska et al. 1996).

The genus Acer contains species producing both recalcitrant and orthodox types of seeds (Tylkowski 1984). But sycamore seeds are predominantly recalcitrant (Hong, Ellis 1990; Dickie et al. 1991; Greggains et al. 2000) and therefore the water content in seeds should not decrease below the level of 30–50% (Procházková 2010). Chilling temperature should be applied to swelled seeds, not to seeds with insufficient water content. Chilling results in the degradation of abscisic acid (ABA) and in an increase of the gibberellin level (Zasada et al. 1998). Stratification means not only overcoming dormancy but also better and quicker germination (Cafourek 1999). On the other hand, inappropriate chilling may cause germination to decrease. Gibberellic acid (GA3) could possibly substitute for chilling (Kolářová et al. 2010). Gibberellins are the part of phytohormones which have multifunctional effects on the regulation of ontogenesis. They influence germination, flowering, are involved in sex determination and regulate seed and fruit development (Procházka et al. 1998). Gibberellic acid is considered as a medium to overcome seed dormancy of many species (Chen, Chang 1972).

Gibberellins were discovered during scientific studies on diseases of rice caused by the fungus *Gibberella fujikuroi*. The disease symptoms were excessive stem prolongation without sufficient stabilizing tissue. Japanese scientists isolated gibberellin A and B from this fungus in the 1930s. The active substance was isolated and identified in the 1950s and named gibberellic acid (GA3). Gibberellic acid was later identified in other plants. The effects of GA3 were studied on different plants and applications (Šetlík et al. 1998). Gibberellins support seed germination between many other effects by alpha-amylase which breaks down starch. Released sugars support the embryo growth until it becomes autotrophic (Procházka et al. 1998).

The positive influence of gibberellins on the germination of many non-dormant seeds has been proved many times (Chen 1972, 1973; Bewley; Black 1982; Procházka, Šebánek 1997; Baskin, Baskin 2001; Koyncu 2005). The influence of gibberellins on the germination of dormant seeds was also studied in *Acer platanoides* (Pawlowski 2009), *Acer saccharinum* (Simmonds, Dumbroff 1974; Marshall et al. 2000) and *Prunus avium* (Cetinbas et al. 2006) and some other species.

The aim of this paper is to evaluate the influence of gibberellic acid on overcoming physiological dormancy and its contribution to the improvement of germination capacity and emergence rate of sycamore seeds.

MATERIAL AND METHODS

Three seedlots were used originating from different provenance regions (number 16 – Bohemian-Moravian hills, 29 – Low Jesenik Mountains and 33 – Eastern part of Moravian hills). Seeds were collected in autumn 2012 and stored in closed plastic bags to keep the humidity constant. The seed quality was evaluated at the beginning of experiments according to the Czech standard at the laboratory of the Forest and Game Management Research Institute, Kunovice. Details of the seed quality for each seedlot are given in Table 1.

The collected seedlots had a lower germination rate than the Czech standard supposes but that is quite a common situation for this species. There were not any significant differences between the seedlot qualities and therefore all seeds were bulked and analysed together (Table 1). The experimental design was prepared for three treatments (including a control) for germination capacity and three treatments (including a control) for emergence rates. There were four replications within each seedlot,

Table 1. Basic characteristics of seedlots

Seedlot	Natural forest region	Altitude zone (m a.s.l.)	Moisture content (%)		1,000 seed weight (g)	Viability (%)	Standard germination rate (%)	Germination rate without stratification (%) after	
			whole samara	seed				21 days	42 days
1	29	500–600	28.8	32.6	143	86	38	0	6
2	33	500–600	34.7	39.1	141	93	43	0	10
3	16	500–600	36.4	39.5	155	97	39	0	17
Czech Standard	–	–	–	–	95	80	80	–	–

seed collection in November 2012, test started on the date 20.12.2012

i.e. twelve replications altogether for each experimental variant. All seed boxes were put in an incubator in a randomised complete block design. There were one hundred seeds in each box. The germination treatments are given in Table 2.

As sycamore dormancy is caused by the pericarp, the germination rates were evaluated in two variants: seeds with pericarp (GI) and seeds without pericarp (GII). The seed hydration was performed before the operation for 24 h. As a control, germination was tested for sycamore seeds without stratification. Gibberellic acid was prepared according to the recommended concentration (Suzska et al. 1996; Bezděčková et al. 2012), i.e. 200 mg of GA3 powder in 1,000 ml of distilled water.

The germination capacity was tested in plastic boxes with cotton wool soaked in gibberellic acid. The boxes were sealed and placed in a dark, climatically controlled environment at a temperature of 20°C. The GI treatment was unstratified seeds with a pericarp, the variant GII was unstratified seeds without a pericarp (Table 2). The germination capacity of both variants was compared with the germination capacity of unstratified seeds under standard conditions for assessing the relative effect on germination capacity.

Assessment was performed every 7th day and germinated seeds with a twice longer shoot than the seed were classified as germinated and removed. The germination capacity test was ended after 42 days from treatment. Those seeds that had not germinated were examined to recognize which were empty, dead or hard seeds at the end of the experiment.

The emergence rate was also tested in plastic boxes but this time the sycamore seeds were placed in peat mixed with sand (2:1 ratio). The temperature was 20°C during the whole experiment and the seeds were watered whenever needed. The effect of gibberellic acid was tested with 3 treatments including a control. The first treatment involved soaking the seeds in gibberellic acid for one day. The second treatment involved soaking the seeds in pure water as a pre-sowing treatment and then watering them with a gibberellic acid solution (Table 3). Checking for germinated seeds was performed again every 7th day.

Statistical analysis was performed by the STATISTICA v. 11 software (StatSoft Inc., Tulsa, USA) using multifactorial ANOVA to evaluate the importance of seedlot origin, gibberellic acid and treatment time. The normality of frequency distribution was checked by the Shapiro-Wilks test as well as their homogeneity. The significance of differences between the variants was evaluated by post-hoc Tukey and Scheffé HSD test.

Table 2. Variants for evaluation of the germination capacity of unstratified seeds

Variant label	Germination of unstratified seeds	Pre sowing treatment – seeds were soaked in H_2O for	Variant description – seeds	Time length (days)
GI	with pericarp	24 h	with pericarp on cotton wool soaked in gibberellic acid (no light, 20°C)	42
GII	without pericarp	24 h, pericarp cut out	without pericarp on cotton wool soaked by gibberellic acid (no light, 20°C)	42
Gco	with pericarp (control treatment)	24 h	on cotton wool soaked in H_2O (no light, 20°C)	21/42

GI – germination of seeds with pericarp (effect of GA3), GII – germination of seeds without pericarp (effect of GA3), Gco – control variant (no effect of GA3), GA3 – gibberellic acid

Table 3. Treatments for evaluation of emergence rate

Variant	Variant label – emergence	Pre sawing treatment – seeds were soaked in	Variant description – seeds were placed in substrate at 20°C and watered by	Variant length (days)
E I	seeds soaked in GA3 for 24 h, watered GA3	GA3 at 5°C for 24 h	GA3	
E II	seeds soaked H_2O for 24 h, watered GA3	H_2O at 5°C for 24 h	GA3	63
Eco	control variant (no GA3)	H_2O at 5°C for 24 h	H_2O	

GA3 – gibberellic acid

RESULTS AND DISCUSSION

Prerequisites for high germination capacity and emergence rate are good seed quality and appropriate pre-sowing treatment. These also apply to sycamore seeds which have a dormant embryo (Nikolaeva 1969; Webb, Dumbroff 1969; Pinfield et al. 1974) and two types of dormancy: (*i*) physiological and (*ii*) morphological (Webb, Wareing 1972b; Prochazková 1992). As sycamore seeds are recalcitrant seeds, internal humidity needs to be maintained (Hong, Ellis 1990; Dickie et al. 1991; Greggains et al. 2000).

The germination capacity of sycamore seeds varies between 38 and 43% according to the standard test (the temperature of 20°C). The vitality of sycamore seeds (tetrazolium stain) was 86–97%. The differences between these standard tests which are considered as equal in the Czech Standard are quite common for this species (Procházková 2005).

The gibberellic acid influence on germination capacity was evaluated by comparison of treatment GI (seeds on cotton wool soaked with gibberellic acid with no stratification but with the pericarp retained) with the standard germination capacity [after 60 days of stratification see Table 1 "Standard germination rate (%)"]. The germination capacity of variant GI was significantly lower (on a probability level $P = 0.05$ for $n = 144$) not only within the standard time period (21 days) but also after 42 days. The results are similar to those seeds germinating in standard conditions (in wet cotton wool without gibberellic acid) without a stratification period (Fig. 1). The data suggested that the gibberellic acid did not substitute for the stratification procedure as it did not improve the germination capacity of seeds with their pericarp when compared with stratified seeds (Fig. 1). Similar results were published by Pinfiel and Stobart (1972), or Webb and Wareing (1972a) who did not find any influence when applying exogenous gibberellic acid to sycamore seeds.

Different results were obtained when the same procedure was applied to sycamore seeds without pericarp (variant GII). The germination capacity of seeds without stratification and without pericarp reached the same level within the standard time as the sample treated according to the standard. The number of germinated seeds was at about the same level as the germination capacity achieved after 60 days of stratification.

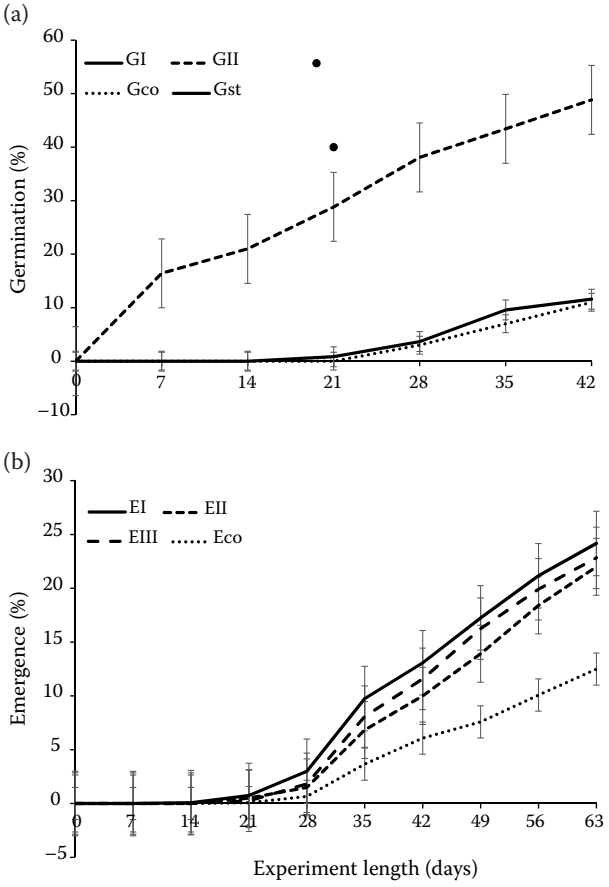

Fig. 1. Sycamore seeds: germination capacity influenced by gibberellic acid (a), emergence rate treated with gibberellic acid (b)

GI – germination of seeds with pericarp (effect of GA3), GII – germination of seeds without pericarp (effect of GA3), Gco – control variant (no effect of GA3), Gst – germination according to Czech Standard CSN 48 1211 (stratification, no effect of GA3); EI – emergence, seeds soaked in GA3 for 24 h, watered GA3, EII – emergence, seeds soaked in H_2O for 24 h, watered GA3, Eco – emergence, control variant – no effect of GA3); GA3 – gibberellic acid; bars – standard deviations

The data showed that gibberellic acid had an important influence on the germination capacity of unstratified seeds without their pericarp by substituting for the stratification process. Similar results were published by Procházková (1993). It seems that the pericarp is impermeable for gibberellic acid and therefore its influence could be limited or nullified.

The influence of gibberellic acid on emergence rate was evaluated across two treatments and compared with a control (no gibberellic acid added).

All variants where gibberellic acid was applied have a statistically higher (on a probability level $P = 0.05$) emergence rate than the control (Fig. 2). The emergence rate of seeds treated with gibberellic acid was about 50% higher than in the control, i.e. without the influence of gibberellic acid. The seed emergence started earlier than in the control variant and kept its lead all the time. However, when compared with the emergence rate of seeds treated according to the standard (stratification and standard conditions for emergence test), it was lower.

CONCLUSIONS

Sycamore maple is the most common maple in European forests but the knowledge of pre-sowing treatment of its seeds has been so far limited. Sycamore fruit is a swinging achene 3–6 cm long (Coombes 1996). The achene comprises one seed which is of the size of a pea without endosperm, covered by pericarp (Suzska et al. 1996). The pericarp could be extremely hard, mainly when seed humidity decreases. The pericarp is the main cause of dormancy, even the phenomenon is more complicated (Booner 2008). Seeds are usually ripe in September and they fall down in October and at the beginning of winter (Musil 2005). Their vitality is the highest right after ripening and then it is going down losing their germination capacity (Procházková 2010). The role and necessity of stratification were evaluated during experiments where the role of pericarp and gibberellic acid was evaluated.

The gibberellic acid influence on germination capacity was evaluated in variants with or without pericarp. The seeds were soaked in gibberellic acid on cotton wool. There was no stratification of these seeds and they were compared with the control where no gibberellic acid was added. The results are distinctly different for seeds with pericarp and those without pericarp. The data shows that the gibberellic acid did not substitute the stratification procedure for the seeds with pericarp as it did not improve the germination capacity (Table 1 – standard germination rate). The seeds without pericarp soaked in gibberellic acid on cotton wool have similar results to those seeds germinating in standard conditions (Table 1 – standard germination rate). Our data suggests there could be an alternative procedure for assessing germination rate which would be to skip the stratification and soak the seeds without pericarp in gibberellic acid on cotton wool.

Partly different results were obtained in seeds tested for emergence rate. A positive influence of gibberellic acid has been proved for all variants where the acid was applied. The emergence rate of seeds treated with gibberellin acid was about 50% higher than in the control, i.e. without the influence of gibberellic acid (Fig. 2).

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