JOURNAL OF FOREST SCIENCE, *60*, 2014 (5): 212–217

Influence of sycamore seed stratification length on their germination capacity

J. Stejskalová, I. Kupka, O. Nováková

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

ABSTRACT: Sycamore (*Acer pseudoplatanus L.*) seeds are frequently used in forest nurseries and therefore the knowledge of their proper treatment is desirable. Seed stratification recommended by the Czech standard is two months but there are not many studies which review the appropriate length in Central European conditions. The paper evaluates the influence of seed stratification length on their germination capacity. The seed samples were treated in different ways to evaluate their reaction to these different handling conditions. The data proved a significant increase of germination after 42 days of stratification. The data also suggests that the better stratification medium is a substrate than wet filter paper.

Keywords: sycamore; seed handling; stratification; germination; vitality

Sycamore *(Acer pseudoplatanus L.)* is the most wide spread maple in Europe (Rusanen, Myking 2003) and the same applies to the Czech Republic. Sycamore trees have been found from uplands to the mountains, they are the most common at the elevation of 600–900 m a.s.l. (Jeník 1961).

Sycamore is a shade tolerant species, however, in maturity it could grow also under full sunshine conditions. Good soil conditions with good nutrient and water supply are preconditioned for good growth and therefore it belongs to a high demanding species (Slávik 2004). The species is also sensitive to hard frost (Musil 2005).

The actual maple share in Czech forests according to the report on Czech forests and forestry (Anonymous 2011) is 1.3%, while originally it was only 0.7% and it is expected to increase to 1.5%.

Sycamore seeds have two-wing achenes containing a single seed without endosperm (Carl, Snow 1971). When fully matured, the seed has dry wrinkled features (Palátová 2008). They are fully matured in September and fall down from October to November (Musil 2005).

The mast year usually occurs every third year (Suzska et al. 1996). There are large differences in seed production among stands and even within one stand among trees (Bjorkbom 1979).

The pericarp covers the embryo with the testa. Storage tissue supports the embryo growth after fertilization. The testa is developed before fertilization and after its maturation period the seed ends its evolution (Kupka 2005). The existence of the testa is the main source of dormancy (Booner 2008).

Seed vitality is the highest after the maturation period and then its vitality is dropping down. Aging leads to a decrease in the germination capacity. Aging is manifested by colour changes. The seeds are intolerant to unfavourable storage conditions that can cause the worse germination of seedlings (Procházková 2010). The seed size seemed to be clearly correlated with germination within the species (Ahirwar 2012). This fact was confirmed by many authors (Ledgard, Cath 1983; Murali 1997) who found out that the seed weight is positively correlated with the viability of seeds.

Dormancy is the last phase of the seed maturing process. Dormancy is an important quality for the survival of the species as it shifts the germination in time and space and influences the geographical distribution of the species (Bewley, Black 1982).

Supported by Czech University of Life Sciences in Prague, Project No. 20124304.

Sycamore seeds have two types of dormancy: (*i*) embryonic (physiological) and (*ii*) morphological (caused by the testa). Embryonic dormancy started even in the tree crown. The seeds at the end of their maturation do not germinate even after removal of the testa (the testa removal takes away morphological dormancy) while mature seeds after the testa removal germinate easily at the temperature of 20°C (Procházková 1993). The testa dormancy of mature seeds is caused by an impermeable seed coat which prevents the washout of inhibitors from the embryo (Webb, Wareing 1972).

To overcome dormancy, the seeds should be wet and stratified. The stratification of sycamore seeds is usually done in a wet medium – e.g. sand (Procházková 1993) at a temperature of 1–5°C for 40, 60 or 120 days (Vincent 1965; Schopmeyer 1974; Tóth, Garrett 1989). Suzska et al. (1996) proposed the seed stratification from 56 to 84 days. The stratification length is unclear for many species as well (Martiník, Palátová 2012).

The recommended temperature for stratification of sycamore seeds is 1–5°C. The proper stratification period could differ for seeds from the same tree according to the crop year (Procházková 1993). Sycamore seed stratification for 2 months is required by our standard but so far there have not been many results confirming this prerequisite (Procházková 1992). Suzska et al. (1996) recommended a stratification period from 8 to 12 weeks, proposing the period could be even longer. Cold during stratification must act on wet seeds, not on dry seeds. During stratification there is a gradual degradation of abscisic acid (ABA) and an increase in levels of gibberellins (Zasada, Strong 2008). The seed quality is influenced by the origin of the parent tree and the better category of reproductive material means usually better seed quality (Procházková, Bezděčková 2007).

The research was initiated in the framework of CIGA project "The influence of brassinosteroids on seed germination rate and performance of selected forest tree species" as a parallel process to evaluate the germination without the steroid influence.

The purpose of this paper is to evaluate the influence of the sycamore seed stratification length on germination capacity. The second goal of the paper is to evaluate the influence of stratification medium on the germination level.

MATERIAL AND METHODS

Seed material was gathered from three parent trees of the family which belongs to the category Qualified and one seed unit from a forest stand (Source-identified category). The category of reproductive materials expresses the quality and the origin of the reproductive material. Council Directive No. 1999/105/EC in Article 2 gives the definitions of four categories of reproductive material to which these two categories belong, i.e. Source-identified and Qualified categories. The samples were labelled Q1, Q2, Q3 for Qualified category and one seed unit from a forest stand (Source-identified category) was labelled as I1. All seed units were collected at the same time. The origins are identical (provenance zone number 16, vegetation zone number 4). All trees were dominant trees (tree class 2) with very good quality of crown and stem.

Collected seeds were stored at a low temperature (1–4°C) in airtight plastic bags before the tests started. Four samples (100 seeds) were prepared from each of these four seed units for the tests. The basic information and overview of the used seed material are given in Table 1.

Quality variation of seed is well known and it could strongly influence results and quality evaluation (Procházková 1992). Therefore the first step was to do basic quality tests to evaluate the seed quality. The quality tests were as follows:
– viability (immersing the seeds in tetrazolium staining solution and their colour assessment),
– weight of 1,000 seeds,
– moisture content.

The tests were executed according to the Czech standard (Anonymous 2006) and ISTA Working Sheets on Tetrazolium Testing (Anonymous 2003).

The design of the investigation was to evaluate three variants of stratification length for two different germination periods as both time frameworks have not yet been supported by large research and data confirming the time period for proper seed treatments are rather poor. The details are given in Table 2.

Table 1. Basic description of the source type of seeds used for the tests

Seed unit	Category	Source type	Age	DBH (cm)	Height (m)
Q1	qualified	parents of family	90	87	26
Q2	qualified	parents of family	90	73	26
Q3	qualified	parents of family	90	79	24
I1	source-identified	stand	90	80	23

seed collection date 11. 11. 2011, test started on the date 29. 11. 2011

Table 2. The design of different treatments of sycamore seeds (in days)

Label	Stratification length	Germination length
S21G21	21	21
S21G42	21	42
S42G21	42	21
S42G42	42	42
S63G21	63	21
S63G42	63	42

S, G – stratification and germination period respectively in the labels

The series of stratification periods starting from 21 days and their multiples was used in order to obtain a time series which gives a possibility to evaluate the proper stratification length. The longer stratification period was not possible as sycamore seeds started geminating spontaneously after 63 days of stratification.

The most common procedure recommended by the Czech standard (ČSN 48 1211) is two months of stratification and 21 days of germination test which corresponds to the samples labelled S63G21.

Tests were done in a certified laboratory of the Forest and Game Management Research Institute, Kunovice research station. Each treatment has 4 samples of 100 seeds (replications). Stratification was carried out in a wet mixture of sand and peat (1:1), substrate moisture was at least 30%, temperature between 1°C to 5°C. The samples were moved to the temperature of 20°C for the germination test when the given stratification period was over. The germination test lasted for a given number of days (Table 2). The criterion for germinated seeds was the germ length longer than the seed size. The germinated, fresh but not germinated, and dead seeds were recorded when the germination tests were completed.

The germination rate on wet paper could be different from that in wet substrate and therefore the germination on wet filter paper was carried out as well. The procedure was the same as the Czech standard proposes, i.e. stratification for 2 months (63 days) and 21 days of germination where the medium was wet filter paper.

Data were statistically tested using the STATISTICA v. 9.1 software (SPSS, Tulsa, USA). The equality of variances was tested by Levene's test. After the data confirmed their equality, the differences were tested by multi-factorial ANOVA. The significance of differences on the level $P = 0.05$ was evaluated by Tukey's test.

RESULTS AND DISCUSSION

The basic seed quality evaluation of sycamore seeds was the first step of the procedure. The data are given in Table 3.

The moisture is not significantly different between the samples (seed units), but there are statistically significant differences in weight between all the units of qualified category, i.e. Q1, Q2, Q3. It could be expected that the seed moisture is a driving variable for the weight differences. It should be pointed out that the seed collection was executed on the same day and storage conditions were also identical. It shows a high variation in the weight of sycamore seeds coming from the same region not influenced by the time of seed collection or storage conditions.

The fourth tested unit I1 (source-identified category) has significantly heavier seeds than the value given in the Czech standard. It shows that even the source-identified category, which is the lowest category in respect of the genetic source quality which indirectly corresponds with seed quality, could have high weight which is usually connected with higher vitality. Vitality (expressed by evaluated viability) of the investigated samples was not different from the Czech standard and the samples were not significantly different from each other except of the unit Q3 which has the vitality value slightly below the Czech standard.

Stratification is the final period of seed ripening for dormant seeds. However stratification – namely its cold phase – is a complicated and not fully understood process. The germination tests after different stratification length reflect the influence of the pre-treatment on germination capacity. The results are given in Table 4.

Results clearly show that the length of stratification period strongly influences the germination capacity. The "standard" level of germination

Table 3. Basic seed quality characteristics of sycamore (*Acer pseudoplatanus L.*) seeds and their reference values given in the Czech standard

Seed unit	Moisture content (%)	1,000 seed weight (g)	Viability (%)
Czech standard	–	95	80
Q1	36.7[a]	154[b]	87[a]
Q2	40.5[a]	215[c]	95[a]
Q3	32.9[a]	79[a]	73[a]
I1	41.7[a]	221[c]	87[a]

same letter indicates non-significant differences on the probability level 0.95 between the seed unit values

was reached after 2 months of stratification (63 days) while in the shorter periods the germination capacity was significantly lower (about less than 20%). The data confirm clearly that the stratification period should be longer than 42 days. The data suggest that the critical length of stratification period is very much close to 63 days (Table 4).

Table 4. Germination test results of seed lots

Design of treatment	Germination rate (%)			
	Q1	Q2	Q3	I1
S21G21	1	1	0	1
S21G42	8	20	9	14
S42G21	41	35	20	20
S42G42	66	77	57	63
S63G21	85	88	84	80
S63G42	87	93	88	85

S, G – stratification and germination period respectively in the labels

The germination capacity of seeds which were not stratified long enough (less than 63 days) is however better if they have a longer period for germination, in other words the germination is slower under these conditions and did not reach the expected germination capacity level within the standard time framework (compare data on the treatment labelled S42G21 and S42G42 where the germination period of 42 days increased the germination by 37% on average).

However, when the stratification is done properly for a recommended length, then the germination is not significantly different for the period of 21 or 42 days (compare data on the treatment labelled S63G21 and S63G42 where the germination period of 42 days did not increase the germination significantly).

The development of germination capacity is illustrated in Fig. 1, showing a large gap between the germination for the period of 21 days (G21) and especially for 42 days (G42) for the short stratification length (between 21 and 42 days). The difference is much smaller for the stratification length of 60 days and longer. Similar results were described by many authors (Webb, Wareing 1972; Schopmeyer 1974; Tóth, Garrett 1989; Suzska et al. 1996).

The insufficient stratification length is substituted by a longer germination period in the case of 42-day germination period (G42), which is not the case of 21-day germination period (G21). A longer stratification period (about 60 days and more) dramatically decreases the gap between these different germination capacities confirming that the stratification length should be lying within this period.

Suzska et al. (1996) recommended for fresh sycamore seeds the stratification period of 8 to 12 weeks, i.e. 56 to 84 days. Our data falls within the declared period.

Another interesting topic was the influence of stratification medium on the germination potential. Two different media (wet mixture of peat and sand and wet filter paper) were used for the test. The influence of stratification medium on the germination level is shown in Fig. 2.

There are statistically highly significant differences between these different stratification media (on the significance level $P = 0.01$) for all seed units and categories. The germination rate of sycamore seeds on wet filter paper is by 38.6% lower on average than in substrate. A possible explanation could be the poorer contact of seeds with the wet medium (filter paper), which slows down the internal processes in seeds. However, this hypothesis needs more investigations based on larger data sets than it was done in this study.

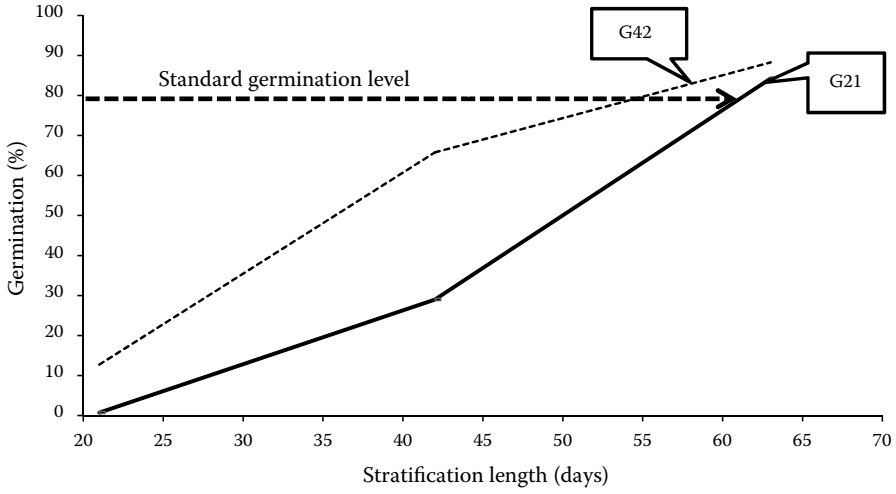

Fig. 1. The development of average germination capacity within different stratification periods for two germination lengths

G21 – germination for 21 days, G42 – germination for 42 days) and standard germination capacity level

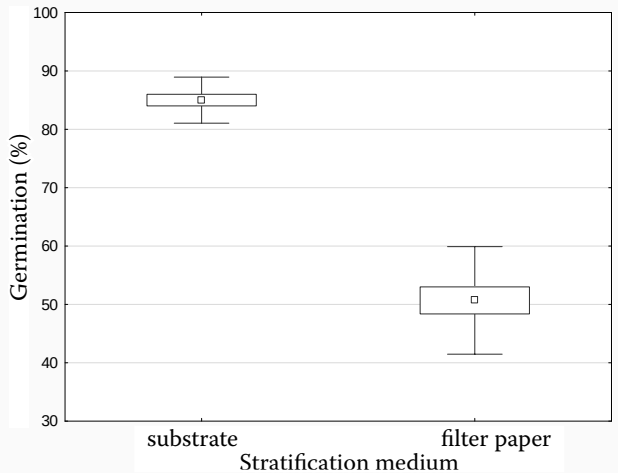

Fig. 2. The influence of stratification medium on germination rate under standard conditions (germination capacity of sycamore seeds stratified for 63 days at 1–5°C and then germinated at 20° C on sand-peat substrate or filter paper)

CONCLUSIONS

Our data proved that the stratification is an indispensable process for the good germination rate. The germination rate has been low or nearly zero without stratification, while seed stratification increased (four or even five times) the germination capacity of the seed according to the data in Table 4. The right time framework for the stratification of sycamore seeds is about 60 days.

When the stratification is done properly, the germination length of 21 days should be enough. The data also suggest that the substrate (wet sand and peat) seems to be a better stratification medium than the wet filter paper giving more stable and reliable results of germination rate.

References

Ahirwar J.R. (2012): Effect of seed size and weight on seed germination of *Alangium lamarckii*; Akola, India. Research Journal of Recent Sciences, ***1****:* 320–322.

Anonymous (2011): Zpráva o stavu lesa a lesního hospodářství České republiky v roce 2010. [Report on the State of Forest and Forest Sector of the Czech Republic for the year 2010.] Praha, Ministerstvo zemědělství ČR: 130.

Anonymous (2006): ČSN 48 1211 Lesní semenářství: Sběr, jakost a zkoušky jakosti plodů a semen lesních dřevin. [Czech Standard 48 1211 Forest Seed Management: Collection, Quality and Methods for Testing Forest Tree Seeds.] Praha, Český normalizační institut: 60.

Anonymous (2003): ISTA Working Sheets on Tetrazolium Testing. Volume II: Tree and Shrub Species. Switzerland, Basserdorf, International Seed Testing Association: 153.

Bewley J.D., Black M. (1982): Physiology and Biochemistry of Seeds in Relation to Germination, Viability, Dormancy and Environmental Control. Berlin, Springer: 375.

Bjorkbom J.C. (1979): Seed Production and Advance Regeneration in Allegheny Hardwood Forests. Research Paper No. NE-435, Broomall, U.S. Department of Agriculture, Northeastern Forest Experiment Station: 10.

Booner F.T. (2008): Seed Biology. In: Bonner F.T., Karrfalt R.P. (eds): The Woody Plant Seed Manual. Agriculture Handbook No. 727. Washington, DC. U.S. Department of Agriculture, Forest Service: 12–33.

Carl C.M., Snow A.G. (1971): Maturation of Sugar Maple Seed. USDA Forest Service research paper NE-217. Upper Darby, PA, U.S. Department of Agriculture, Northeastern Forest Experiment Station: 8.

Jeník J. (1961): Alpinská vegetace Krkonoš, Králického Sněžníku a Hrubého Jeseníku. [Alpine Vegetation in Krkonoše Mountains, Kralický Snežník Mountain and Hrubý Jeseník Mountain.] Praha, Academia: 407.

Kupka I. (2005): Základy pěstování lesa. [Basics of Silviculture.] Praha, Česká zemědělská univerzita v Praze: 174.

Ledgard N.J., Cath P.W. (1983): Seeds of New Zealand Nothofagus species: studies of seed weight, viability, shape and effect of varying stratification periods. New Zealand Journal of Forestry, ***28***: 150–262.

Martiník A., Palátová E. (2012): Je předosevní příprava osiva douglasky tisolisté nezbytná? [Is the pre-sowing treatment of Douglas fir seed material necessary?] Zprávy lesnického výzkumu, ***57***: 47–55.

Murali K.S. (1997): Patterns of seed size, germination and seed viability of tropical tree species in southern India. Biotropica, ***29***: 271–277.

Musil I. (2005): Lesnická dendrologie, díl 2 Listnaté dřeviny [Forest Dendrology, Volume 2 Broadleaved Tree Species.] Praha, Česká zemědělská univerzita v Praze: 216.

Palátová E. (2008): Zakládání lesa I.: lesní semenářství. [Reforestation I.: Forest Seed Management.] Brno, Mendelova zemědělská a lesnická univerzita v Brně: 120.

Procházková Z. (1992): Javor klen (*Acer pseudoplatanus*): Sběr, skladování a předosevní příprava. [Sycamore maple: collection, storage and seed pretreatment.] Zprávy lesnického výzkumu, ***3***: 22–29.

Procházková Z. (1993): Skladování a předosevní příprava semen a plodů listnatých dřevin (javoru klenu, mléče a habru). [Storage and seed pretreatment of broadleaves species (Sycamore maple, Norway maple and Hornbeam).] Zprávy lesnického výzkumu, ***1***: 25–33.

Procházková Z. (2010): Způsoby získávání (sběru), přepravy a skladování osiva lesních dřevin. [Collection, transportation and storage of forest seeds.] In: Foltánek V. (ed.): Inovace kvalifikačních znalostí v oboru lesního školkařství. Soubor tématických přednášek přednesených v průběhu vzdělávacího cyklu určeného pro technické pracovníky v lesním školkařství a financovaného z pro-

jektu Programu rozvoje venkova v r. 2010. Brno, Mendelova zemědělská a lesnická univerzita v Brně: 126–129.

Procházková Z., Bezděčková L. (2007): Porovnání kvality semene ze semenných sadů a porostů. [Comparison of seed quality from seed orchard and stands.] In: Sarvaš M., Sušková M. (eds): Aktuálné problémy lesního školkárstva, semenárstva a umelej obnovy lesa 2007. Zborník referátov z medzinárodného seminára. Liptovský Ján, 27.–28. March 2007. Zvolen, NLC: 44–52.

Rusanen M., Myking T. (2003): EUFORGEN Technical Guidelines for Genetic Conservation and Use for Sycamore (*Acer pseudoplatanus*). Rome, International Plant Genetic Resources Institute: 6.

Schopmeyer C.S. (1974): Seeds of Woody Plants in the United States. Agricultural Handbook No. 450. Washington, DC, Forest Service, U.S. Department of Agriculture: 883.

Slávik M. (2004): Lesnická dendrologie pro bakalářské studium HSSL. [Forest Dendrology for Bachelor Degree.] Praha, Česká zemědělská univerzita v Praze: 80.

Suzska B., Muller C., Bonnet M., Simbert M. (1996): Seeds of Forest Broadleaves: from Harvest to Sowing. Paris, INRA Editions: 334.

Úradníček L., Maděra P. (2001): Dřeviny České republiky. [Tree Species of the Czech Republic.] Písek, Matice lesnická: 333.

Toth J., Garrett P.W. (1989): Optimum temperatures for stratification of several maple species. Tree Plant Notes, ***40***: 9–12.

Vincent G. (1965): Lesní semenářství. [Forest Seed Management.] Praha, SZN: 329.

Webb D.P., Wareing P.F. (1972): Seed dormancy in *Acer*. Endogenous germination inhibitors and dormancy in *Acer pseudoplatanus* L. Planta, ***104***: 115–125.

Zasada J.C., Strong T.F. (2008): Acer L. In: Bonner, F.T., Karrfalt R.P. (eds): The Woody Plant Seed Manual. Agriculture Handbook No. 727. Washington, DC., U.S. Department of Agriculture, Forest Service: 204–216.

Received for publication February 24, 2014
Accepted after corrections May 12, 2014

Corresponding author:

Prof. Ing. Ivo Kupka, CSc., Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Department of Silviculture, Kamýcká 129, 156 21 Praha 6; e-mail: kupka@fld.czu.cz