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SEED QUALITIES AND SEED DORMANCY BREAKING OF SYCAMORE MAPLE (*ACER PSEUDOPLATANUS* L.) SEEDS

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Abstract

Mature seeds of Sycamore maple have a physiological dormancy. Different studies suggest fall sowing or cold stratification for a period varying from 3 weeks to 3 months. The aim of the study is to find optimal way for dormancy breaking of sycamore seeds in South-East Europe conditions. Variants of fall sowing, spring sowing of stored in dry substratum (untreated) seeds and sowing after 1, 2, and 3 months cold stratification were tested. The results showed that during a 2 and 3 months cold stratification the seeds germinate in substratum (relatively 51.5 % and 71.8) and they become useless for sowing. Only 7.0 % of seeds germinate during 1 month cold stratification period in the substrate. The germination after fall sowing is 68.8 %. After spring sowing of untreated seeds and 1 month cold stratificated seeds the germination is relatively 82.3 % and 82.75 %. The height of seedlings was smaller after fall sowing – average 9.76 cm. The seedlings produced after spring sowing of untreated seeds had average height 12.3 cm. The seedlings of seeds, which were cold stratified for 1, 2 or 3 months prior to spring sowing, exhibited the best growth in height (18.5–19.5 cm). The results bring to some conclusions. It is not recommended to store Sycamore maple seeds till spring in wet substrate because it has characteristics of cold stratification and lead to their early germination. The best way for seed dormancy breaking is 1 month cold stratification at 4 °C. The germination dynamics show that stratification have to begin 45 days before the date of last spring frost and the sowing have to be done 15 days before the determinate date. Second variant is spring sowing of seeds stored in dry substrate. In this case germination begins 25 days after the sowing and there is no danger of frost caused damages.

Key words: cold stratification, fall and spring sowing.

Introduction

In the recent years, there is a growing interest in broadleaves for afforestation in Bulgaria (EFA 2012). On the other hand, the conservation of biodiversity requires attention to the native species (Anonymous 2011). In this regard there is a reserve in usage of the species from genus *Acer*. It is considered that Sycamore (*Acer pseudoplatanus* L.) is one of the most valuable for the forestry (Pandeva 2004). It has relatively limited distribution in the country. It is found mostly in the mixed deciduous forests, separately, in small or bigger groups in all mountains, on fresh habitats in altitude up to 1400 m (Milev et al. 2004). It is spread over 2511 ha, which is 0.07 %

of the forest territory and 0.11 % of the deciduous forest territory (Dobrinov et al. 1982).

The information about Sycamore in the scientific literature in Bulgaria and other European countries is limited. Collection of reproductive materials and production of seedlings for afforestation in Bulgaria are not on the needed level from selection prospective (Pandeva 2004). The Sycamore seeds in Bulgaria are collected from the stands included in the National Register of Forest Seed Sources (National Register ... 2015), but the breeding work with it is limited to species level.

For the forest seed production specific characteristics of the Sycamore seeds are dynamic changes and high level of the moisture content which lead to perishability, as well as the physiological dormancy at the stage of ripping of fruits (Hong and Ellis 1990). In various researches, the authors recommend different periods of sowing and conditions of storage and stratification for successful germination of the Sycamore seeds.

It needs to be considered, that the optimal period of stratification depends on a lot of factors – the species, the origin, meteorological conditions in the ripping stage, physiological condition in the stage of collecting, period and conditions of storage (Milev et al. 2004).

Sycamore produces seeds every year, starting between 11th (Milev et al. 2004) and 25th year of age (Suszka et al. 1996, Joyce 1998) for the solitary growing individuals. In stands it starts around 25th (Milev et al. 2004) or around 30th year of age (Suszka et al. 1996, Joyce 1998). Harvests are regular, but abundant ones are in 1 or 2 years (Milev et al. 2004).

Collection of the seeds takes place in September (Krüssmann 1997), October or November. The yield of samaras is 80–90 % (Milev et al. 2004). The moisture content at this stage is 42–58 % (Joyce 1998, Milev et al. 2004, Iliev et al. 2015). Stejskalová et al. (2014) reported lower level of moisture content from 32.9 to 41.7 %. Suszka et al. (1996) differs the information for intact fruits (24–32 %), seeds (30–41 %) and pericarp (14–16 %). According to Milev et al. (2004) and Iliev et al. (2015) when the moisture level drops to 30 % the seeds die, Suszka et al. (1996) on the other hand reported lower minimum of 24 % moisture content.

The weight of a thousand seeds is approximately 85–95 g (Stilinović 1985) and varies from 70 to 221 g (Joyce 1998, Stejskalová et al. 2014), and in 1 kg there are between 6000 and 14,000 samaras (Milev et al. 2004).

Viability of the seeds varies from 50 % (Milev et al. 2004, Iliev et al. 2015) to 70–80 % and maintains for two years (Michev 1953, Stilinović 1985, Milev et al. 2004, Iliev et al. 2015). According to Suszka et al. (1996) the germination reaches 95 % in some cases.

There is unanimous opinion that fully ripened seeds fall in a physiological dormancy (Dirr and Heuser 1987, Krüssmann 1997, Joyce 1998, Milev et al. 2004, Hartmann 2010, Bertels 2012, Stejskalová et al. 2014, Iliev et al. 2015). Some authors assume presence of chemical compounds in the testa that inhibit germination (Thomas et al. 1973, Hong and Ellis 1990, Milev et al. 2004, Iliev et al. 2015).

In the literature recommendations to harvest seeds during the stage of physiological maturity is relatively rare (Bertels 2012). It is mostly recommended seeds to be sown in autumn (Michev 1953, Nesto-

rovich et al. 1967, Dirr and Heuser 1987, Joyce 1998), as well as soaking the seeds one week before that (Hartmann 2010). Joyce (1998), however, takes note that very often the seeds germinate very early and the seedlings are damaged by the frosts.

Probably that is the reason most of the authors to recommend spring sowing of preliminary treated seeds.

For short storage from autumn to spring, there is no need for lowering the moisture content of the seeds, but for 2–3 years storage the moisture needs to be 24–32 % (for intact fruits) or 30–42 % (for seeds), and the temperature between -3 and -5 °C (Suszka et al. 1996). There are some opinions that Sycamore seeds can be stored up to 6–7 years, without losing their viability (Joyce 1998).

For the autumn ripping maples Hartmann (2010) gives a summarized recommendation for cold stratification at 4 °C for 60–120 days before spring sowing. For fruits with low moisture content (under 12 %) of the pericarp Bertels (2012) suggests even worm-cold stratification with one month warm stage at 20ºC or the alternative of 48 hours soaking at water at 40 ºC.

The most common suggestion is cold stratification for 3 to 8 weeks (Krechetova et al. 1978, Krüssmann 1997, Joyce 1998, Milev et al. 2004, Stejskalová et al. 2014, Iliev et al. 2015) or 2–3 months (Michev 1953, Nestorovich et al. 1967, Thomas et al. 1973, Dirr and Heuser 1987).

The stratification can take place in moist sand (Krüssmann 1997) or 50:50 moist peat and sand (Suszka et al. 1996, Stejskalová et al. 2014). It has been reported for stratification without substrate in open plastic bags for seeds preliminary soaked for 24 hours (Suszka et al. 1996, Joyce 1998).

The optimal temperatures for stratification are from 0 to 5°C (Michev 1953, Krechetova et al. 1978, Thomas et al. 1973, Suszka et al. 1996, Joyce 1998, Hartman et al. 2010, Bertels 2012, Stejskalová et al. 2014) with optimum moisture levels for stratification 44–50 % for intact fruits or 50–58 % for seeds (Suszka et al. 1996).

It needs to be periodically checked for early germination during the stratification right after the 20th day (Milev et al. 2004, Iliev et al. 2015). It is known that when 5–10 % of the seeds germinate and the sowing is not possible at the time, the seeds can be stored at 3°C up to 3 months without losing viability (Joyce 1998). The alternative is storage in snow up to 45 days (Krechetova et al. 1978).

For practical reasons the samaras are removed before sowing and the fruits become oval and free flowing (Milev et al. 2004, Bertels 2012).

A rate of 32.5 to 49.0 g/m^2 is recommended when sowing in furrow and 80–100 g/m^2 when sowing freely (Suszka et al. 1996). In Bulgaria there is practice for furrow sowing with rate of 4.4 g/m (Iliev et al. 2015).

The germination starts 2–4 weeks after sowing (Krüssmann 1997).

The production rate for furrow sowing is 100, and for free sowing – 300–500 seedlings per 1 m^2 (Suszka et al. 1996, Krüssmann 1997), or mean number of 25–30 one year old seedlings or 20 numbers of 2-years old seedlings per meter (Iliev et al. 2015).

The aim of the present study is to test the germination qualities and to determine the optimum method for dormancy breaking of the Sycamore seeds, in the South-East European region.

Material and Methods

Individual Sycamore tree in park 'Vrana' area near Sofia was used for a source of reproductive materials. The fruits were collected on 7 October 2013. The fruits were stored in conditions which keep their initial moisture level, in mixture with vermiculite in 1:3 ratio, at 4°C in polyethylene package. Not fully developed, damaged by insects and empty seeds were preliminary removed by hand from the mixture.

The sowing qualities of the seeds were tested according to requirements of ISTA (1996). The weight of 1000 seeds and the relative moisture were determined in the laboratory of 'Forest plantations' at the University of Forestry in Sofia.

The viability of the seeds was tested by tetrazolium method in Forest Seed Control Station – Sofia and by excised embryo test in the laboratory of 'Forest plantations' at the University of Forestry in Sofia, where the result was evaluated on the 10th day of incubation. Furthermore, germination of the untreated seeds was tested in germination device, where the evaluation was made on the 30th day (germination on Jacobsen apparatus).

The seeds, stored in vermiculite were moved prior to spring sowing to mixture of pearl-stone (1:3) for one, two and three months of cold stratification at 4°C and 80 % moisture.

The sowing was made on 1 March 2014 in 'Sequoia' ornamental nursery. The seeds were sown in open air, in plastic plates for seedlings production with parameters of the plug 5×5×18 cm. and volume of 240 ml. The substrate was microfibril sphagnum peat with parameters of 0.5 mm particles; 20 kg/m³ volume density; pH in water was 5.5–6.5; electrical conductivity 35–45 mS/m ±25 %; enriched with fertilizer NPK (14:10:18) 1.5 kg/m². The depth of the sowing was 2 cm.

To determine the type and deep of the seed dormancy the following variants ware tested:

1. autumn sowing – 13.10.2013;
2. spring sowing of dry stored (not stratified) seeds;
3. spring sowing after 1 month cold stratification;
4. spring sowing after 2 months cold stratification;
5. spring sowing after 3 months cold stratification.

All variants were placed in 4 replications of 100 seeds. The seeds were irrigated daily through automatic irrigation system.

Germination was evaluated daily to the 45th day, when it finished.

The mean germination and the energy of germination were evaluated up to the 15th day. For calculation of the average seed dormancy, the following formula was used:

$$S_d = \frac{a_1 \cdot n_1 + a_2 \cdot n_2 + ... + a_i \cdot n_i}{\sum n_i}, \text{ in days,}$$

where: a_i – day of evaluation; n_i – sum of germinated seeds at the a_i-day of evaluation.

As additional criteria for the efficiency of the different methods for dormancy breaking, the height of the seedlings was measured after the end of vegetation period.

All results were analyzed by analysis of variance (ANOVA) and post hoc LSD test using SPSS 10.0 (SPSS for windows 1999). Percentage values were transformed using arcsine square root ($\sqrt{p}$) (Compton 1994) to normalize error distribution prior variance analysis.

Results and Discussion

The preliminary evaluation of the sowing materials showed that 72.3 % of them are morphologically fully developed (Table 1). With not fully developed embryo or damaged embryo were 27.7 % of the seeds. There is statistically proved difference between the weight and the moisture level of the two categories (Table 1). The moisture content of the fruits with normally developed embryos is 51.5 %, and the weight of 1000 fruits is 246.41 g. The same parameters of fruits, with the not fully developed embryos, are 42.42 % moisture content and 79.95 g weight of 1000 fruits. With the removal of the wings the mean weight changes significantly to 223.54 g.

The results show higher weight of 1000 fruits compared to the data reported by Stilinović (1985), Joyce (1998), Milev et al. (2004), Stejskalová et al. (2014), which is due to the preliminary separation of the fruits with not fully developed embryos. This method gives a better opportunity for forming quality lots and equally spread germination and equal distribution of the seedlings in the nurseries.

The moisture content of the intact fruits is 51.50 %. This result is in agreement with Joyce (1998), Milev et al. (2004), Iliev et al. (2015) and is higher than the data reported by Suszka et al. (1996) and Stejskalová et al. (2014). The fruits with not fully developed embryos are with statistically different moister content, with mean value of 42.42 %.

The results for the viability (Table 2) from the tetrazole method and the excised embryo test are statistically identical – 90.0 % and 92.0 %, respectively, which refers to first quality in the Bulgarian National Standard (BNS) 208:1999.

The extremely low percentage of germinated seed tested in germination device (6.0 %) proofs the presence of physiologi-

Table 1. Physical parameters and quality of the fruits.

Parameter	Normally developed embryo	Not fully developed embryo	De-winged fruits
Morphological development, %	72.34 ±2.42a	27.66 ±2.42b	–
Weight of 1000 fruits (samaras), g	246.41 ±6.61a	79.95 ±3.74b	223.54 ±6.31c
Relative moisture content, %	51.50 ±0.64a	42.42 ±1.39b	–

Note: The means and standard error within a column followed by tvvhe same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

Table 2. Viability of the seeds tested by different method.

Method	Viability, %
Tetrazole method	92.00 ±1.35a
Excised embryo test	90.00 ±3.46a
Germination	6.00 ±1.15b

Note: The means and standard error followed by the same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

cal dormancy in the seeds and the need of breaking it based on the right time of sowing and the appropriate period of cold stratification.

It needs to be considered that the values of viability presented are for seed lots that have been separated preliminary from the not developed seeds. If we consider that the percentage of fully developed seeds was 72.34 % then the real percentage of viability is 65.1–66.6 %. Nevertheless those results confirm the high germination ability of the Sycamore seeds reported by Stilinović (1985), Suszka (1996), Milev et al. (2004).

The test on different periods of stratification showed that for one month of cold stratification 7.0 % of the seeds germinate in the medium. From that point of view it is reasonably recommended by Milev et al. (2004), checking for germination after the 20th day of stratification. This reaction is a landmark that the lot is ready, the dormancy is already broken and in good conditions after sowing, a high percentage of germination can be expected. Confirming that is the high percentage of germination during the 2 months stratification (51.5 %) and the 3 months stratification (71.7 %) (Table 3).

Those high percentages of germination in the second and third month are an indicator that the stratification has to last no more than one month, because the germinated seeds are not suitable for sowing. Identical results have been reported by Stejskalová et al. (2014). Bertels (2012) recommends the already germinated seeds during stratification to be frozen until coming of a good period for sowing, but this method can be used for limited number of species and Sycamore is not one of them.

The germination started earliest (1 March) in the seeds sown in autumn. Stratified seeds start to germinate on 11th day after sowing, while at the 25th day of the sowing start to germinate unstratified seeds sown in spring (control variant) (Table 4).

The statistically proved lowest germination was registered in the lot that was sown in autumn – 68.75 % (Table 4). It can be considered that exposure of natural conditions during winter leads to loss of some of the initial germination ability, probably due to frosts.

When the seeds were stratified one month or not stratified the germination percentages were statistically identical – 82.75 and 82.25 %, respectively. It means that the temperature was still low after the sowing on 1 March and provided the necessary conditions to break the non-deep seed dormancy.

From the seeds not germinated during stratification 24.25 % germinate from the 2 months stratified and 15.25 % germinate from the 3 months stratified. Together with the germination during stratification the percentage is very high – 75.75 % and 87.0 for the 2 and 3 months stratified respectively.

Considering the germination dynamic we find out that the process continues 41 days for the autumn sown seeds and a little bit longer (45 days) for the control lot sown in spring and the lot stratified for one month and sown in

Table 3. Germination in the medium during the stratification.

Period of stratification, months	1	2	3
Germination, %	7.00 ±1.35a	51.50 ±4.21b	71.75 ±2.84c

Note: The means and standard error followed by the same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

Table 4. Germination of seeds depending on different methods of preparation and season of sowing.

Method	Germination, %	Beginning of germination, date	End of germination, date	Period, days	Eg, %	ASD, days
Autumn sowing	68.75 ± 3.75c	01.03	10.04	41	40.75	10.2
Spring sowing control	82.75 ± 2.32d	25.03	09.05	45	0.00	12.7
Cold stratification 1 month	82.25 ± 0.63d	11.03	24.04	44	1.00	13.1
Cold stratification 2 months	24.25 ±1.89b	11.03	10.04	31	0.25	10.5
Cold stratification 3 months	15.25 ± 0.75a	11.03	10.04	25	1.50	8.5

Notes: Eg – energy of germination; ASD – average seed dormancy. The means and standard error within a column followed by the same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

spring (44 days). The longer stratification shortens the period of germination to 25–31 days.

The energy of germination was highest with the autumn sown seeds (40.75 %) and very low for spring sown seeds – from 0.0 % (spring control lot) to 1.5 % (spring lot after 3 months stratification).

The average seed dormancy of the autumn sown seeds was 10.2 days. For the spring control lot it was prolonged to 12.7 days, and for the spring lot stratified for 1 month it was 13.1 days.

The parameters of physiological qualities of the seeds recorded for the different methods of preparation are the main criteria for the efficiency of those methods. A secondary criterion is how those methods influence the growing of the seedlings (Table 5). The results show formation of three statistically different groups. With slowest growth are the seedlings from seeds sown in autumn (mean height 9.76 cm). The seeds stratified for 1, 2 and 3 months produce the highest seedlings with height from 18.46 ± 5.71 to 19.40 ± 5.49 cm (Table 5).

Statistical parameters that proof the significance (significance level p = 0.000) of the different methods for dormancy breaking on the seedlings growth during first vegetation period, are shown in Table 6.

Table 5. Influence of the different seed preparation methods on the seedlings height.

Method	Height of the seedlings, cm
Autumn sowing	9.76 ± 1.84a
Spring sowing control	12.27 ± 2.48b
Cold stratification 1 month	19.24 ± 4.68c
Cold stratification 2 months	19.40 ± 5.49c
Cold stratification 3 months	18.46 ± 5.71c

Note: The means and standard error followed by the same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

In general our results show the opportunity which Suszka (1996) mentioned,

Table 6. Significance of the different preparation methods on the seedlings height.

Factor	*df*	*F*	Sig
Method of preparation	4	346.425	0.000

Fig. 1. Seedling with 3 cotyledons.

that the Sycamore seeds can be successfully sown in spring without stratification. This is possible because their high moisture content keeps their physiological activity during storage in dry conditions and the moderate physiological dormancy can be broken from the cold temperature influence, during this period of storage.

In addition we observed some anomalies in germination. Seedlings with 3 cotyledons appeared with small frequency 0.091 % (Fig. 1). Similar information exists for some other tree species as Black locust (Iliev and Chavdarov 1996) and Honey locust (Iliev et al. 2015).

Conclusion

The usual practice in Bulgaria of sowing the Sycamore seeds in autumn is not the most appropriate method of work with the species.

It is not recommended to store Sycamore seeds in high moisture conditions during winter, because it acts as a cold stratification and germination starts before the right period for spring sowing. Storage at dry conditions at temperature of 4 °C, for keeping the initial moisture content of the seeds, is appropriate.

The seeds are characterized with non-*deep* physiological dormancy. The best method to break it is cold stratification at 4 °C for one month, followed by spring sowing.

Stratification needs to start 45 days before the average date of the last spring frosts and sowing needs to take place 15 days before that date.

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