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Effects of dormancy breaking in stored seeds on germinability and seedling emergence of *Tilia platyphyllos*

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Abstract: Fruits of large-leaved lime dried to 10% may be stored for 16 years in sealed containers at –3°C without loosing seed viability. Dormancy of seeds, extracted from hard fruit coats, may be released after chemical scarification in concentrated sulphuric acid for 10 minutes, followed-by stratification without any medium (chilling) at the temperature of 3°C, for 20–24 weeks, i.e. until the first seeds start to germinate. After such pretreatment, during the germination test conducted at alternating temperatures 3~15°C (16 + 8 hours/day) seeds germinate near 90% in several weeks. For seedling production scarified and stratified seeds should be sown in early spring into trays under a plastic tunnel which ensures a high percentage of seedlings emergence. Sowing of the pretreated seeds in spring in a open nursery gives poor results.

Additional key words: scarification, stratification, seedling emergence

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Introduction

The large-leaved lime (*Tilia platyphyllos* Scop.) is often planted in urban habitats and in open landscapes. The northern limit of its natural range crosses Poland at the latitude of about 52°C. Despite this fact, this species is often grown also at higher latitudes, thanks to its adaptability. The main factor limiting its utilization is the difficulty in propagating it from seeds.

Ripe seeds of hard-fruited *Tilia* species, including *T. platyphyllos*, are deeply dormant. Under natural conditions, dormancy release is observed after 2 or rarely 3–4 winters. This is due mainly to the hard pericarp, the impermeable seed coat and embryo dormancy conditioned by inhibitors present in the endosperm. Lime seeds are thus characterised by a deep combined endogenous dormancy (Nikolaeva 1968).

The seed coat significantly limits the leaching of inhibitors and reduces the access of oxygen to the embryo, both mechanically and chemically as a result of oxygen binding by phenols present in the seed coat (Nagy and Szalai 1973, Szalai and Nagy 1974, Nagy 1976).

The difficulty in breaking the dormancy of lime seeds results to a large extent from the variation in seed coat hardness, which depends on many factors, such as provenance, date of collection, and storage conditions (including temperature and seed moisture content). In seeds of various lime species, seed coat hardness varies both between and within seed lots from individual trees.

For practical reasons it is important to be able to prepare seeds for sowing in a short time, i.e. to break their dormancy more quickly than naturally. Moreover, the prepared seeds should be characterised by a high viability and germinate uniformly in a short time.

To achieve this, unripe fruits may be collected when the embryo is already fully developed and the green pericarp starts to turn pale yellow while the seed coat is still soft and permeable. However, seeds collected so early are often characterised by low germinability and decay easily during stratification under natural conditions.

The previously developed protocols of sowing pretreatment do not ensure high germinability and emergence rate in any lime species. A great variation between and within species is observed in respect of permeability of the pericarp and the seed coat, and consequently also in respect of stratification requirements (Rowe and Blazich 2000).

The aim of this study was to determine conditions for dormancy breaking in ripe seeds of *T. platyphyllos* and to analyse the influence of storage conditions on germinability and emergence rate in the open nursery and in trays kept in a polyethylene tunnel.

Materials and methods

Ripe fruits were collected separately from 3 trees, about 8 m high, grown in the vicinity of the Środa Wielkopolska road, on 12th November 1999, after the first autumn frosts and leaf fall. Fruits with bracts were shaken off from branches with a long stick onto large cloths. Some fruits, after separation from bracts, were dried at room temperature to 10–12% moisture content (in fresh weight), and next closed in air-tight containers and stored at –3°C. From other fruits, seeds were extracted (by a method submitted to the Polish Patent Office, application no. P–365775). The extracted seeds (moisture content ca 12%) from each tree were packed into 4 sealed bottles and stored at –3°C and –10°C for 1, 2, 3 or 4 years.

After storage, seeds were subjected to chemical scarification in concentrated sulphuric acid for ten minutes, next rinsed, and soaked in water for 20 hours. Seeds stored in fruits were extracted from pericarps before scarification. After scarification, the seeds were stratified without substrate, according to a method (Tylkowski 1994, 1998) used for seeds of the small-leafed lime (*Tilia cordata* Mill.). Stratification lasted for 20 or 24 weeks. For the first 5 weeks, seeds were soaked in water for 1 hour every week. In the later period of stratification, the seeds were soaked in water every 2 weeks. After soaking, the drained seeds were left in a partly covered container at 3°C until the next soaking.

After stratification, 4 replicates of 50 seeds each were subjected to a laboratory germination test in a mixture of peat (pH 5.5–6.5) and quartz sand (1:1, v/v), at cyclically changing temperature (3~15°C, cycle 16 h + 8 h). The seeds were also sown at a depth of 1 cm in trays made of hard plastic (type HIKO V-150, BCC, Sweden) filled with a mixture of peat (pH 5.5–6.5) and perlite (2:1, v/v), with the addition of the fertilizer Osmocote (Scotts International BV, The Netherlands), which controlled release lasts for 5–6 months (Standard 5–6M). The trays with sown seeds were placed in spring in a shaded polyethylene tent. They were watered automatically twice a day, from an installation switched on by a controlling clock.

Only once, in 2002, the seeds after sowing pretreatment were sown also in an open forest nursery, in 4 replicates of 50 seeds each, in 2-cm-deep rows. After sowing in early April, seeds were covered with sandy soil and a 3-cm-thick layer of ground pine bark. When necessary, they were watered manually.

Additionally, some fruits when fully mature (moisture content of 11–12%) collected from one tree in Śrem, in November 1983 were stored till 1999 (i.e. for 16 years) in a tightly sealed bottle at –3°C. After storage, seeds were extracted from the fruits and subjected to chemical scarification followed by stratification without substrate at 3°C. After 26 weeks of stratification (onset of germination), the seeds were subjected to a germination test at cyclically changing temperature (3~15°C or 3~20°C, 16 h + 8 h).

We regarded seeds as germinated if the radicle was at least 5 mm long. Seedling height in containers and in the nursery was measured in autumn, after leaf fall.

Results of laboratory seed germination and seedling emergence tests were transformed to *arc* sin $\sqrt{\%}$ and subjected to the ANOVA analysis of variance using the Statistica (1997) programme and tested with the use of the Tukey's test at the confidence level of P=0.05.

Results

As it was shown in the analysis of variance (Tables 1 and 2), germination and emergence differed statistically significantly. It was found that a factor having a considerable effect on the variation in germinability of seeds was the effect of the provenance, i.e. maternal tree (Fig. 1). Generally seeds from tree no 3 had the highest germination and emergence rates (95.8%), while the lowest rates were found for tree no. 1 (90.1%).

No effect of the method of seed storage after collection (Fig. 2) was found on germinability under laboratory conditions. In contrast, this effect was significant in case of seed emergence after sowing in a polyethylene tent (Fig. 2). It was found that seeds removed from pericarps and stored at –3°C had the highest statistically significant emergence rate (84.7%). Seeds stored in fruits at –3°C (81.1%) emerged at a significantly lower rate, while the lowest percentage was found when seeds were stored at –10°C after being removed from pericarps (78.4%).

Significant differences were found both in germinability of seeds in individual years and emergence in a polyethylene tent (Fig. 3). Although the difference in germinability in 2002 and 2003 was slight (90.3% and

Table 1. Analysis of variance of seed germination results

STAT. ANOVA	Effects					
Effect	df effect	MS effect	df Error	MS Error	F	p
Length of storage (1)	3	204.9941	108	32.89255	6.23223	0.0006
Provenance (2)	2	511.9373	108	32.89255	15.56393	0.0000
Method of storage (3)	2	63.5272	108	32.89255	1.93135	0.1499
1×2	6	209.0257	108	32.89255	6.35480	0.0000
1×3	6	169.9050	108	32.89255	5.16545	0.0001
2×3	4	215.6313	108	32.89255	6.55563	0.0001
1×2×3	12	145.0510	108	32.89255	4.40984	0.0000

Table 2. Analysis of variance of seedling emergence

STAT. ANOVA	Effects					
Effect	df effect	MS effect	df Error	MS Error	F	p
Length of storage (1)	3	1613.025	108	18.46317	87.36448	0.0000
Provenance (2)	2	1272.149	108	18.46317	68.90202	0.0000
Method of storage (3)	2	257.909	108	18.46317	13.96884	0.0000
1×2	6	210.859	108	18.46317	11.42051	0.0000
1×3	6	172.050	108	18.46317	9.31853	0.0000
2×3	4	198.054	108	18.46317	10.72700	0.0000
1×2×3	12	145.761	108	18.46317	7.89472	0.0000

95.9%, respectively), from the statistical point of view it is difficult to explain. In case of emergence in a polyethylene tent the highest percentage was found in 2001 (90.8%), a statistically lower emergence rate was observed in 2004 (86.8%) and statistically the lowest in 2002 and 2003, i.e. 73.8 and 74.3%, respectively.

After scarification, seeds needed 20–24 weeks (i.e. about 5 months) to start germinating during cold stratification (at 3°C) without substrate.

After storage for several months (both at –3°C and at –10°C) and stratification without substrate, seeds of 3 trees showed high germinability under laboratory conditions at cyclically changing temperature 3~15°C (Table 3). Among the 36 results listed in Table 3, as many as 20 are denoted with the letter 'a', which means that they form one homogenous group not differing statistically (values 79–95%).

The highest germination rate was observed in seeds extracted from fruits and stored at –3°C. Similarly high rates were recorded for trees 2 and 3 if they were stored in fruits, whereas seeds of tree 1 germinated significantly worse after such storage. Seeds extracted from fruits of trees 1 and 2, stored at –10°C, germinated equally well as after storage at –3°C, while seeds of tree 3 were characterized at –10°C by a lower germination rate (Table 3).

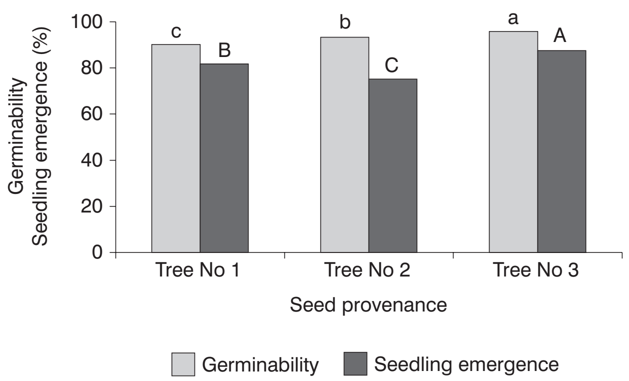

Fig. 1. Effect of seed provenance (trees 1–3) on germinability and seedling emergence. Results denoted with identical letters do not differ significantly at P=0.05. Germinability and seedling emergence were tested separately

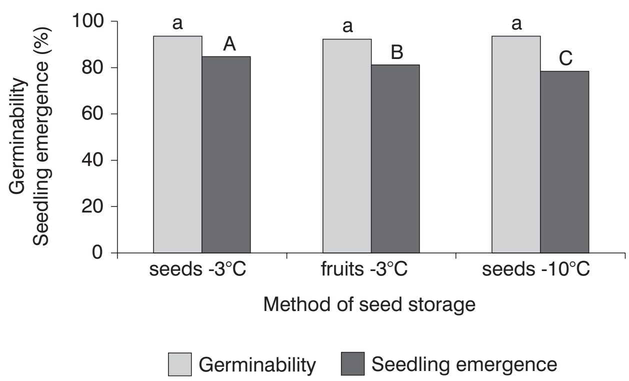

Fig. 2. Effect of method of seed storage (seeds at –3°C, fruits at –3°C and seeds at –10°C) on germinability and seedling emergence. Results denoted with identical letters do not differ significantly at P=0.05. Germinability and seedling emergence were tested separately

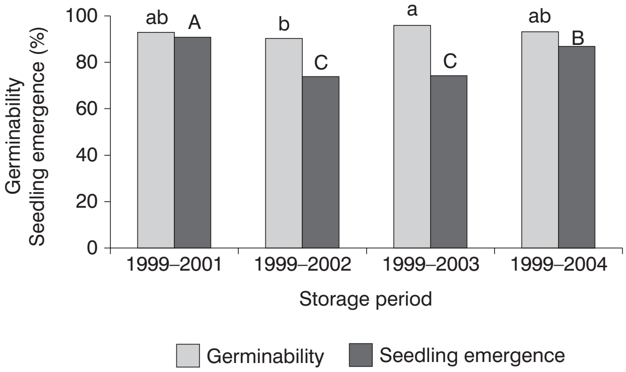

Fig. 3. Effect of length of seed storage period on germinability and seedling emergence. Results denoted with identical letters do not differ significantly at P=0.05. Germinability and seedling emergence were tested separately

A similarly high percentage of seedlings emerged in trays placed in a plastic tunnel (Table 4). A homogenous group with the lowest values not differing statistically is formed by one 2 values (42 and 60%), but the latter does not differ significantly from 9 other values. The group of the highest homogenous values denoted with the letter "l" consists of 17 variants, out of which as many as 9 are assigned to tree no. 3.

It needs to be mentioned here that at the end of the first vegetation season in the period 2001–2004 seedling height in the plastic tunnel ranged from 12 to 18.5 cm.

During a single test carried out in 2002 in the open nursery the seedling emergence rate was much lower than under the plastic tunnel, ranging from 0% to 11.5%, and mean seedling height reached only 8.8–10.2 cm (Table 5).

The other seeds stored in fruits for 16 years at –3°C also showed high germinability at temperature 3~15°C or 3~20°C (93% and 95%, respectively) after stratification without substrate (Fig. 4).

Discussion

The annual fruit yield of *T. platyphyllos* varies greatly and sometimes is very low for 3–4 successive years, so it seems advisable to accumulate seed reserves. Large amounts of fruits with high-quality seeds can be easily collected soon after leaf fall in the years of high yields. After the first autumn frosts, leaves fall but fruits remain on the tree for another few weeks, so fruits can then be shaken off the twigs onto large sheets of fabric or plastic spread under the tree. Frosts also facilitate the separation of bracts with fruits from twigs, so the date of collection should not be delayed then because fruits can be lost in case of strong winds.

Results of this study show that seeds of *T. platyphyllos*, collected when fruits are ripe, preserve high germinability for 16 years if after collection they are dried to 10–12% of moisture content and stored in tightly closed containers at –3°C. Results of the previous experiment indicate (Fig. 2) that this temperature of storage is significantly better than –10°C. Good results can be obtained if seeds are extracted from fruits before storage under identical conditions. This is beneficial because seeds are much smaller than fruits, so they require less space for storage. Moreover, it is easier to plan the timing of sowing pretreatment if seeds are extracted before storage.

Stratification should be started 20–24 weeks before the planned sowing date when about 5% of seeds germinate. Thus, to prepare seeds for sowing in mid-March or early April, stratification should be started in late September or in October.

Table 3. *Tilia platyphyllos*. Seed germinability at 3~15°C in 2001–2004, after collection in 1999 and storage at –3°C or –10°C of extracted seeds or after storage of fruits at –3°C. Results with identical letters do not differ significantly at P=0.05 (Tukey's test)

Period of storage	Storage temperature of seeds (S) and fruits (F)		Seed germinability (%)		
			Tree 1	Tree 2	Tree 3
1999–2001	–3°C	S	86.0 abcd	98.0 cde	93.0 abcde
		F	95.0 abcde	94.5 abcde	97.5 cde
	–10°C	S	81.0 ab	98.5 e	94.0 abcde
1999–2002	–3°C	S	90.0 abcde	81.0 ab	93.5 abcde
		F	81.0 ab	92.5 abcde	95.5 bcde
	–10°C	S	85.5 abc	97.5 cde	97.5de
1999–2003	–3°C	S	99.5 e	97.5 cde	93.5 abcde
		F	94.5 abcde	91.5 abcde	98.5 de
	–10°C	S	95.0 abcde	95.0 abcde	99.0 e
1999–2004	–3°C	S	98.0 e	96.0 bcde	97.5 cde
		F	79.0 a	91.0 abcde	97.5 cde
	–10°C	S	96.5 cde	87.5 abcd	97.5 cde

Table 4. *Tilia platyphyllos*. Seedling emergence in 2001–2004, after collection in 1999 and storage of extracted seeds at –3°C or –10°C or after storage in fruits at –3°C. Results with identical letters do not differ significantly at P=0.05 (Tukey's test)

Period of storage	Storage temperature of seeds (S) and fruits (F)		Seeding emergence (%)		
			Tree 1	Tree 2	Tree 3
1999–2001	–3°C	S	82.0 cdefghi	93.5 ijkl	95.0 jkl
		F	91.0 ghijkl	89.5 ghijkl	96.5 l
	–10°C	S	88.0 fghijkl	90.5 ghijkl	93.0 ijkl
1999–2002	–3°C	S	83.5 efghijk	68.5 bcde	88.0 ghijkl
		F	81.5 cdefghi	67.0 bcde	91.5 ijkl
	–10°C	S	77.5 bcdefgh	69.0 bcde	41.5 a
1999–2003	–3°C	S	82.5 defghij	58.5 ab	91.5 hijkl
		F	65.5 bcd	65.0 bcd	82.5 defghij
	–10°C	S	64.5 bc	72.5 bcdef	82.5 defghij
1999–2004	–3°C	S	91.5 ghijkl	83.0 fghijkl	96.0 l
		F	80.5 cdefghi	73.0 bcdef	95.0 kl
	–10°C	S	89.0 ghijkl	77.0 bcdefg	95.5 kl

Table 5. *Tilia platyphyllos*. Seedling emergence (in %) and mean seedling height (cm, in italics) in the nursery in 2002, after storage of extracted seeds (S) or after storage of fruits (F) collected in 1999

Storage temperature of seeds (S) and fruits (F)		Emergence rate in nursery (%) and mean seedling height		
		Tree 1	Tree 2	Tree 3
–3°C	S	n.d.	2.0 9.0 cm	2.5 10.2 cm
	F	11.5 9.5 cm	4.5 9.2 cm	2.5 8.8 cm
–10°C	S	n.d.	0.0	0.0

n.d. = no data

Seeds should be sown in containers kept in a polyethylene tunnel or scattered by hand in a cold frame, as only under such conditions the emergence rate is high. However, it can be expected that small differences in emergence rates between years will result probably from different weather conditions in individual years. Such a factor could have been e.g. temperature and its influence on the induction of secondary dormancy in seeds.

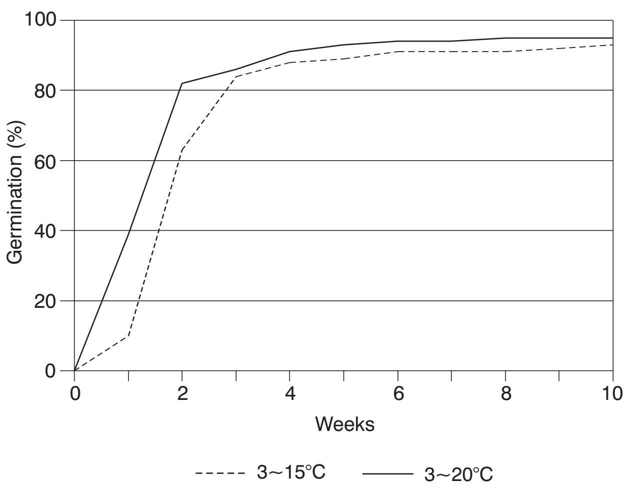

Fig. 4. Course of seed germination in cyclically changing temperature (3~15°C or 3~20°C) after storage of fruits for 16 years at -3°C, followed by extraction of seeds, chemical scarification and stratification at 3°C

Very good seedling growth is observed in a substrate composed of acid peat with perlite (2:1), supplemented with the slow-release fertilizer Osmocote. Sowing in an open nursery has an unfavourable effect on both emergence rate and mean seedling height. Young seedlings are also very sensitive to late spring frosts, so they must be protected against them.

The sowing pretreatment tested in this study was more effective than methods recommended earlier. Dirr and Heuser (1987) stratified fruits of *T. platyphyllos* for 3–5 months at high temperature, followed by 3 months at low temperature, but seed germinability was low then. Flemer (1980) applied cold stratification by placing fruits in a wooden crate filled with sand and buried outdoors. The crate with fruits was dug out the next autumn and the fruits were sown immediately into the soil, but the emergence rate in spring was highly variable. Magherini and Nin (1994) recorded a satisfactory germinability when seeds of *T. platyphyllos* were extracted manually from the hard pericarps, scarified in sulphuric acid, and next refrigerated without desiccation.

In conclusion, our results solve many problems with generative propagation of *T. platyphyllos*. Preliminary tests indicate that this method is suitable also for other hard-fruited *Tilia* species, such as the silver linden (*T. tomentosa* Moench) and the Caucasus linden (*T. caucasica* Rupr.). This will make it possible to avoid the unfavourable vegetative propagation by cloning (e.g. rooting of cuttings).

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