

INFLUENCE OF THE TERM OF SEEDS HARVESTING ON THE PHENOLOGICAL BEHAVIORS OF THE SEEDLINGS OF LINDEN (*TILIA* SSP.)

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Abstract

The main goal of the present study was to establish the differences between separate species of linden on the duration of several phenological stages of seedling, depends on the term of seeds harvesting. The experiments were carried out with seeds of three different species of linden *Tilia platyphyllos* Scop., *Tilia cordata* Mill. and *Tilia tomentosa* Moench. The seeds harvested at 30, 45, 60, 75 and 90 days after anthesis from mother tree from the region of Plovdiv Bulgaria. The seeds were sown immediately after harvest on the open air bed. The phenophases of flowering and seed formation of the mother tree were observed. The main phenophases of the seedling development to third true leaf were studied. In large-leaved lime (*Tilia platyphyllos* Scop.) the earliest flowering and seed maturity were established. In small-leaved lime (*Tilia cordata* Mill.) the seeds have sprouted at the earliest, while in the seedlings of silver linden (*Tilia tomentosa* Moench.) the formation of the cotyledons, first and third true leaves were at the earliest.

Key words: seeds, flowering, species, sprouting, phenological observation

INTRODUCTION

The genus *Tilia* includes about 30 species originating in the temperate regions of Europe, North America, and Asia. According to Chalupa (1999), the main species are *Tilia cordata* Mill. (small-leaved lime), *Tilia. platyphyllos* Scop. (large-leaved lime) and *Tilia americana* L. (basswood). Farar (1995) mentions that in American cities are most commonly used the *Tilia americana*, and in European ones - *Tilia tomentosa* Moench. In Europe, naturally spread linden species are *Tilia dasystyla* Stev., *Tilia tomentosa* Moench., *Tilia cordata* Mill. and *Tilia platyphyllos* Scop. (Radoglou et al., 2009). The last two species occur on the whole continent, with the exception of the Scandinavian countries. The species *Tilia tomentosa* Moench., however, is mainly spread in Bulgaria, Romania, Greece and the countries of the former Yugoslavia.

The small-leaved linden is characterized by very good adaptive ability. In most cases, this is due to the increased development of the root system, especially at an early age, as a result of the long-lasting evolutionary development (Shaikhatov and Seydarfarov, 2013). In this species, the limiting factor for its development and mostly for the guaranteed and good seed development is the temperature (De Jaegere et al., 2016). However, it is necessary to clarify the possibilities and ways of its more successful reproduction for its wider dissemination

The spreading of linden depends on a several geographical, ecological and climatic factors. According to Evarte-Bundere (2014), the main ones are the average January temperatures, the sum of the negative temperatures, the yearly rainfall, the length of the vegetation period, the altitude, the distance to the sea or large water basins. Seydadarov (2012) points out that the water content of the leaves, along with climatic conditions, especially in older trees, is essential for their development.

The subject of a study by Varaksin and Kladko (2010) are the changes in the phenological development of lime, depending on the location of the trees. The most prolonged vegetation of the small-leaved lime has been observed when the trees were planted nearby to the areas to highways and with increased urban pollution. Under these conditions, this species first entered vegetative development at the earliest, but at the same time ripening was significantly later. However, the small-leaved lime is characterized by very good adaptive ability.

In a study conducted by Kollas et al. (2011), with lime, they come to the conclusion that there is a contradiction with the hypothesis that plants of this species develop little and poor seed when grown in

regions with lower temperatures. They point out that the limit of the lowest temperatures for their development is determined by the ability to form a normal amount of viable seeds. Under unfavorable conditions, such as the effects of low temperatures the flowering, the spread of the pollen, growth of the pollen tube, fertilization, embryogenesis and seed maturation are severely hampered.

The main goal of the present study was to establish the differences between separate species of linden on the duration of several phenological stages of seedling, depends on the time of seed harvesting.

MATERIAL AND METHODS

The experiments were carried out in 2015-16 years with seeds of three species of linden large-leaved lime (*Tilia platyphyllos* Scop.), small-leaved lime (*Tilia cordata* Mill.) and silver linden (*Tilia tomentosa* Moench.). On the mother trees the beginning, mass and end of flowering as well as beginning and mass seeds (fruits) formation and seed maturing were observed. The presented data are referred to those trees from which seed was harvested to conduct the experiments.

The seeds harvested at 30, 45, 60, 75 and 90 days after the flowering of 35-40-year-old trees in the region of Plovdiv, Bulgaria. They were sown immediately after harvesting an open air bed at a distance of 20 cm between the rows and 7cm inside the row. The experiments were carried out by block method in 4 replicates per 100 seeds for each.

The main phenophases of seedling development such as beginning and mass of sprouting, beginning and mass of cotyledons formation, beginning and mass of development of first and third true leaves were established. The observations were carried out on each sowing seeds and developed seedlings. For the beginning of the above-mentioned phenophases was accepted their presence in 10% of the seeds or seedlings, while for mass it was in 75% (Dimova and Marinkov, 1999).

RESULTS AND DISCUSSION

The *Tilia* genus is characterized by an extremely wide variety of species (Radoglou et al., 2009). The main species that are distributed in Bulgaria and are widely used for landscaping and for the construction of urban and suburban parks are *Tilia platyphyllos* Scop., *Tilia cordata* Mill. and *Tilia tomentosa* Moench. Except the morphological features, they differ significantly during the occurrence of the duration of the main phenophases, especially the flowering and the formation of the seeds (Varaksin and Kladko (2010).

On the Table 1 are presents the results of observations for the beginning of flowering. The earliest flowering for both years of study was occurred in large-leaved lime (*Tilia platyphyllos* Scop.) -1-8 June or an average of 5 June. At the latest, the beginning of this phase was found in the silver linden between 9 June 2016 and 15 June 2015, on average on 12 June. It is noteworthy that early flowering of all three species of linden began in 2016, which may be due to the more favorable climatic conditions during the flowering and seed formation. These differences between the individual years are within the range of 4 days (in the small-leaved lime) to 7 days (in the large-leaved lime). It can be mention that the average date for the beginning of flowering is about 9.06.

The mass flowering (Table 2) on average for two years was occurred between 3 days for silver linden and 5 days for small-leaved lime after the beginning of flowering. The earliest dates are also occurring for large-leaved lime – 12 June 2015 and 5 June 2016. In small-leaved lime, this was observed with 4 to 7 days later and 6 days in the silver linden. Despite the earlier beginning of the flowering in the small-leaved lime, its mass appearance was later than the silver linden. This phenophase also occurs earlier in 2016. On average for the three species, the mass flowering occurs approximately on 13 June.

Table 1. Beginning of flowering in different linden species

Species	2015	2016	Average
<i>Tilia platyphyllos</i> Scop.	08.06.	01.06.	05.06.
<i>Tilia cordata</i> Mill.	11.06.	07.06.	09.06
<i>Tilia tomentosa</i> Moench.	15.06.	09.06.	12.06

Table 2. Appearance of mass flowering in different linden species

Species	2015	2016	Average
<i>Tilia platyphyllos</i> Scop.	12.06.	05.06.	09.06
<i>Tilia cordata</i> Mill.	16.06.	12.06.	14.06
<i>Tilia tomentosa</i> Moench.	18.06.	11.06.	15.06

Table 3. Appearance of end of flowering in different linden species

Species	2015	2016	Average
<i>Tilia platyphyllos</i> Scop.	14.06.	07.06.	11.06
<i>Tilia cordata</i> Mill.	18.06.	15.06.	17.06
<i>Tilia tomentosa</i> Moench.	21.06.	17.06.	19.06

The flowering of the linden studied species ends approximately 15 June (Table 3). The flowering period is the longest for the small-leaved lime - 8 days from the beginning of the flowering. This phenophase is shorter in the case of the large-leaved lime - for 6 days. On average, for the three species, the end of flowering was established around 16 June as well as in the case of the anterior phaeophasis, its end has been observed for the silver linden - between 17 and 21 June. This was established for large-leaved lime by 7 to 10 days earlier but the small-leaved lime occupied an intermediate position. The period from mass flowering until the end was about 4 days, mainly in silver linden.

The differences between separate species are also observed with regard to seed formation and maturation. The data are presented in Tables 4 and 5. The earliest, visible seed formation was found in the large-leaved lime – 13 June, followed by a small-leaved lime - six days later, and after 9 days this was also reported for the silver linden. The average for three species can be mentioned that the beginning of seed formation occurs on 18 June or about 4 days after the end of the flowering. Mass seed formation started between 30 June (for large-leaved lime) and 11 July (for silver linden) or approximately at 9 July, which is average 21 days after the seed has been set.

Table 4. Phenological observation on the seeds (fruits) formation

Species	Beginning			Mass		
	2015	2016	Average	2015	2016	Average
<i>Tilia platyphyllos</i> Scop.	16.06.	9.06.	13.06	4.07	27.06	30.06
<i>Tilia cordata</i> Mill.	21.06.	18.06.	19.06	11.07	6.07	8.07
<i>Tilia tomentosa</i> Moench.	24.06.	19.06.	22.06	14.07	8.07	11.07

Table 5. Maturation of the seeds (fruits) in the different linden species

Species	Beginning			Mass		
	2015	2016	Average	2015	2016	Average
<i>Tilia platyphyllos</i> Scop.	19.08.	10.08.	15.08	8.09.	30.08	5.09
<i>Tilia cordata</i> Mill.	24.08.	19.08.	22.08	12.09	8.09	10.09
<i>Tilia tomentosa</i> Moench.	28.08.	25.08.	27.08	20.09	16.09	18.09

The beginning of seed maturation average for two years of the study the earliest was occurred in the large-leaved lime - at 15 August, followed by the small-leaved lime and also by the silver-bearing with 7 and 12 days later, respectively. The most important phase in seed propagation is their mass maturation. It occurs in the period between 5-18 September, as the earliest it was observed also for the large-leaved lime. For full maturation after mass formation of the seeds are necessary approximately 65 days.

The linden seeds sprout comparatively difficult, as this process takes place over a long period (Chawla, 2004). According to Werker (2013), they are characterized by a long period of dormancy, due to their specific anatomical structure, which makes it difficult to absorb water and oxygen. Panayotov (2015) points out that the necessary conditions for normal germination and sprouting are, above all, the presence of optimal temperature, humidity, and access to oxygen.

In Table 6 show the results for the sprouting time of the studied linden species. It is necessary to point out that the seeds, harvested at 30, 45 and 60 days after flowering, have not emerged and therefore further interpreted data are only for seeds from 75 and 90 days. The beginning of sprouting in small-leaved lime for 75 and 90 days is not different and for both it was term occurred on 12 March. A similar trend is also considered for mass germination. Significant differences are observed in the other two species. Later on, the beginning of sprouting was observed in the seeds of 75 days where it was delayed by two days compared to the previous species. The differences in mass sprouting were weaker, with significant ahead, by 4 days, observed only for 90 days in *Tilia tomentosa* Moench.

Table 6. Effect of term of seeds collecting in species of genres *Tilia* on the their sprouting

Term (days)	<i>Tilia cordata</i> Mill.		<i>Tilia platyphyllos</i> Scop.		<i>Tilia tomentosa</i> Moench.	
	Beginning	Mass	Beginning	Mass	Beginning	Mass
75	12.03	23.03	14.03	24.03	14.03	23.03
90	12.03	23.03	13.03	23.03	10.03	19.03

The species differences are found in the next phase, the formation of cotyledons (Table 7). This phase first occurs in the species *Tilia tomentosa* Moench. at 3 April for the both terms, or after 20 days and 23 days from sprouting for days 75 and 90 respectively. On the next place were the seeds of 90-day of large-leaved lime and of small-leaved lime. In *Tilia platyphyllos* Scop. for the seeds harvested 75 days after flowering, the cotyledons were observed at the latest. The mass formation of the cotyledons was in the period 6 April (*Tilia tomentosa* Moench.) until 10 April (*Tilia platyphyllos* Scop.) or 14 and 17 days after a mass sprouting. The differences were also observed between the separate terms and it can be summarized that the cotyledons formed earlier for seeds, collected at 90 days after flowering.

The formation of the first true leaf, similarly to the previous phenophases, begun at the earliest for silver linden - 3 and 4 days after cotyledons for 90 and 75 days, respectively (Table 8). In these variants, the mass occurrence of these phases is accelerated. At the latest, despite the earlier term of

sprouting, the first true leaf was formed at *Tilia cordata* Mill. The longest period from the cotyledon phase to the first true leaf was established for the small-leaved lime, between 5 and 7 days.

Table 7. Effect of the term of harvest of the seeds of genres *Tilia* on the cotyledons development

Term (days)	<i>Tilia cordata</i> Mill.		<i>Tilia platyphyllos</i> Scop.		<i>Tilia tomentosa</i> Moench.	
	Beginning	Mass	Beginning	Mass	Beginning	Mass
75	06.04	09.04	07.04	10.04	03.04	06.04
90	06.04	08.04	05.04	07.04	03.04	07.04

One of the important phases of the development of the linden is the presence of a third true leaf (Table 9). The beginning of this stage indicates the possibility of subsequent normal development and growth of linden seedling. The data presented in this table referring to the results of the seeds, collected on the 75 day after flowering related only for 2016, because the seedlings of this variant in 2015 died immediately after the development of the first true leaf. Again the silver linden (*Tilia tomentosa* Moench,) has the fastest development. The beginning and mass development of a third true leaf begin at both terms of seed collection of 13 May and 18 May, respectively, or approximately 6 and 8 days after these first true leaf stages occur. A significant delay was observed for *Tilia cordata* Mill., especially for the beginning of the appearance of a third leaf as this requires four days more.

Table 8. Effect of the term of harvest of the seeds of genres *Tilia* on the first true leaf development

Term (days)	<i>Tilia cordata</i> Mill.		<i>Tilia platyphyllos</i> Scop.		<i>Tilia tomentosa</i> Moench.	
	Beginning	Mass	Beginning	Mass	Beginning	Mass
75	12.04	16.04	10.04	13.04	07.04	10.04
90	11.04	14.04	08.04	12.04	06.04	10.04

Table 9. Effect of the term of harvest of the seeds of genres *Tilia* on the third true leaf development

Term (days)	<i>Tilia cordata</i> Mill.		<i>Tilia platyphyllos</i> Scop.		<i>Tilia tomentosa</i> Moench.	
	Beginning	Mass	Beginning	Mass	Beginning	Mass
75*	17.05	20.05	14.05	18.05	13.05	18.05
90	14.05	18.05	14.05	20.05	13.05	18.05

CONCLUSIONS

At the earliest, the stages of flowering (beginning, mass, and end) are observed in the species *Tilia platyphyllos* Scop. (large-leaved lime). A similar trend is also noted for the formation and maturation of the seeds.

Early sprouting manifested the seeds, harvested at 90 days after flowering. The fastest it is for *Tilia cordata* Mill. However, the beginning and mass formation of the cotyledons first started in the seeds of *Tilia tomentosa* Moench.

The appearance of the first and third true leaves was also observed early for the species silver linden lime, and at the latest in *Tilia cordata* Mill., despite significant early sprouting.

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