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Rooting of sycamore (*Acer pseodoplatanus* L.) cuttings by etiolated shoots

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Abstract

The aim of the study is to investigate the possibility of shoots' etiolation and long-term auxin effect on rooting of stem cuttings from sycamore. 3-year-old seedlings and 40-year-old mature donor were used, phase-rejuvenated through cutting of the stem at the height of the root collar. Traditional and alternative rooting methods were studied as well. The alternative variant was an attempt for rooting of etiolated and de-etiolated shoots. In the beginning of May, etiolated shoots were obtained. The leaves from the first node were cut to base. The node was subject of auxin treatment with lanoline-based 8000 mg·L^{-1} IBA (Indole-3-butyric acid) paste. It was used through a single application after cutting up and long-term auxin treatment. The traditional methods for rooting did not give a positive result. Precondition for propagation by cuttings was i) donor plants darkening two months before the beginning of growing period to obtain etiolated shoots with one node; ii) treatment of the basal node in the beginning of May with 8000 mg·L^{-1} IBA paste and its protection with aluminium foil; iii) parallel uncovering of the donors for de-etiolation 4 weeks before planting. Rooting reached up to 72 %. Successful rooting was not achieved with cuttings from a mature donor. However, in 77.33 % of the etiolated cuttings with long-term treatment before separation from the donor plant callus was formed in the basis. Root caps were developed from the callus, lenticels and the basis of the cuttings. Their average number was 26.05 and reached up to 113. After planting of these cuttings root caps continued to grow and reached length up to 4 mm. Parallelly the above-ground part necrotised.

Key words: auxin, juvenile, mature donors.

Introduction

Sycamore (*Acer pseodoplatanus* L.) is one of the most fast-growing deciduous species. Its fast growth rate and high wood price turns it into economically attractive species to be grown (Hein et al. 2008, Vachide 2009). It is characterised by wide range of tolerance towards geographical, altitudinal and edaphic conditions (Joyce et al. 1998). Sycamore can be used for erosion protection afforestation and urban landscaping (Pasta et al. 2016), and for establishment of forest protection belts. It is wind and soil salinity resistant (Joyce et al. 1998). Due to these arguments Spiecker et al. (2008) recommend Sycamore to be widely used in the forestry in Europe.

Rooting sycamore cuttings is a difficult task (Dirr and Heuser 1987, Vachide 2009). According to Dirr and Heuser (1987), donor phasic status is of crucial importance, and cloning of mature donors using this method is practically impossible. The forming of adventives roots depends mostly on the genotype peculiarities (Rohr and Hanus 1987, Vachide 2009).

As optimal phenological status of the donor for preparation of cuttings it is recommended the shoots to be green (Jinks 1995). They have to be obtained straight after the growing begins in spring (Bertels 2012); or if they are half-lignified to be obtained in June and July (Spethmann 2007, Hartmann et al. 2010, Bertels 2012, Ewald and Naujoks 2015). The length of cuttings could be up to 20–35 cm (Hartmann et al. 2010, Ewald and Naujoks 2015).

Exogenic delivery of auxin is recommended as powder, water or alcoholic solution with concentration 2000–20,000 $mg \cdot L^{-1}$ (Hoogendoorn 1984, Spethmann 2007, Vachide 2009, Bertels 2012, Hartmann et al. 2010, Ewald and Naujoks 2015). The concentration of the auxins applied depends on the age of the donor specimen and its physiological status (Ermakov 1981, Dirr and Heuser 1987, Hartmann et al. 2010).

Besides the genotype, age and auxins, conditions during rooting are of great importance for the rhizogenesis. Peat (Hartmann et al. 2010), mixture of 2/3 peat and 1/3 perlite (Thompson et al. 2001) or sand (Ewald and Naujoks 2015) are usually used for substrate. It is recommended to keep high air humidity provided through a mist system (Ermakov 1981, Hoogendoorn 1984, Dirr and Heuser 1987, Jinks 1995, Thompson et al. 2001, Spethmann 2007, Hartmann et al. 2010, Ewald and Naujoks 2015). Thompson et al. (2001) determined switch-on regime system for 18 s every 30 min from 07:00 to 20:00 h every day. For good results, heating of the soil is also necessary (Hoogendoorn 1984, Bertels 2012).

Rooting is favoured to a large extent if donor plants are pruned and shaded before obtaining of the cuttings (Maynard et al. 1996). Etiolation of shoots or absence of light can induce or improve the percentage of rooting for species which under normal conditions are extremely difficult for vegetative propagation (Miske and Bassuk 1985; Maynard and Bassuk 1985, 1987, 1988, 1990; Podaras and Bassuk 1996; Sun and Bassuk 1999; Amissah and Bassuk 2007).

Adventive roots of green cuttings are set in the cambial area. Peripheral cells of the root germs form meristem cells later differentiated in vessel elements of the primary xylem. The formation of well-marked callus is not always observed and is not related to the formation of adventive roots. It can be definitely said that the callus formation results from the auxins effect (Vachide 2009).

In contrast to the generally accepted point of view, in rooting of cuttings, the callus formation in the potential root generation zone inhibits root formation. Large callus formations usually led to poor rooting (Vachide 2009). It reached up to 49–78 % (Jinks 1995, Thompson et al. 2001, Ewald and Naujoks 2015). Ewald and Naujoks (2015), however, pointed out that not all rooted cuttings are suitable for transplanting and that not all transplanted cuttings survive.

In an experiment of rooting of cuttings obtained from inoculated trees, they have been rejuvenated according to the stage. The percentage of rooting was 24 %. This low percentage was probably due to inoculated trees' fast ontogenetic ageing

(Thompson et al. 2001).

The low rooting rate of stem cuttings of some species has led to use of non-standard methods for improving the results. Such methods are shading and etiolation of donor plants. There are studies on lilac (*Bassuk* et al. *1985,* Maynard and Bassuk 1985, Miske and Bassuk 1985), *Acer griseum Franch, Acer saccharinum L., Betula papyrifera* Marsh., *Carpinus betulus L., Castanea mollissima* Blume*, Corylus Americana* Marshall.*, Pinus strobus L., Quercus coccinea* M.*, Quercus palustris* M.*, Quercus robur L., Quercus rubra L.* (Maynard and Bassuk 1985), on common beach and hornbeam (*Bassuk* et al. *1985), on Quercus bicolor* Willd. and *Quercus robur* (Amissah and Bassuk 2007), *Cornus alba L. (Pacholczak and Szydło 2010), Acer platanoides L. (Tomov 2015), etc.*

The aim of the present study is to investigate the possible effect of etiolation of shoots and long-term auxin treatment on rooting of stem cuttings from sycamore.

Material and Method

For determination of stage influence of the donor on rooting capability of cuttings, the following donors were used:

- phase young specimen – 3-year-old seedlings produced through sowing-seeds in a container with capacity 3 L;
- mature donor at the age of 40, phase-rejuvenated through cutting of the stem at the height of root collar, from which the stump shoots have been used as cuttings.

The effect of donor phenological status and exogenic auxin on rooting was studied using both traditional and alternative impact approach. The traditional one included cutting cuttings with length 7–12 cm during two terms:

- green, half-lignificated cuttings – phenophase of mature (summer) wood formation (beginning of July);
- mature cuttings – phenophase of induced dormancy (end of March).

The auxin impact on the rooting process was studied through 4 experimental variants: control – non-treated cuttings and treated with talcum-based indole-3-butyric acid (IBA) powder in concentrations 1000, 3000 and 8000 mg·L^{-1}.

The alternative variant was an attempt for rooting of etiolated shoots from two donor types. The containers and mature specimen were put in a darkened space (figs 1 and 2) before the start of growing

Fig. 1. Construction of crown darkening of phase mature donor.

Fig. 2. Construction of crown darkening of juvenile donors.

in the beginning of March for two months (until beginning of May). The space was established with a wooden construction around the donor covered with black mulching stuff Mypex.

In the beginning of May (1st May) etiolated shoots with at least two nodes were obtained. The mulching cloth was partially removed so the donor’s shoots to be lightened but with indirect sunlight.

The leaves from the first node were cut to the base.

To test the auxin impact on the rooting of experimental variants 8000 mg·L^{-1} IBA lanoline-based paste was used. The first node with leaves removed was treated with it.

Part of the etiolated cuttings were cut and immediately planted without auxin treatment (var. 1, control 1).

Second part of etiolated cuttings were cut, treated with auxin paste and immediately planted (var. 2, single auxin impact).

Third part of the etiolated cuttings were treated with auxin paste without being separated from the shoots. The treated base was banded by 3 cm wide aluminum foil. The treatment was 4 weeks (until 28 May) (var. 3 etiolated cuttings treated with IBA paste in the base for 4 weeks) before cutting.

On 4th May the chlorophyll content in the leaves and shoots of the lightened donors was recovered. The cuttings obtained from them were considered as de-etiolated.

Part of de-etiolated cuttings were cut and immediately planted without auxin treatment (var. 4, control 2).

Second part of de-etiolated cuttings were cut, treated with auxin paste and planted immediately (var. 5, de-etiolated cuttings, single auxin impact.

Third part of de-etiolated cuttings were treated with auxin paste without being separated from the shoots. The treated base was banded by 3 cm wide aluminum foil. The treatment was 4 weeks (until 31st May) when they were cut and planted (var. 6, de-etiolated cuttings treated in the base with IBA for 4 weeks).

The etiolated cuttings with long-term auxin impact were cut and planted on 28th May, and the de-etiolated on 31st May.

The following experimental variants were developed for the juvenile seedlings and the mature donor as well (Table 1).

Table 1. Experimental variants developed for the juvenile seedlings and mature donor.

Variant	Cuttings’ status
1	Control 1 – etiolated cuttings without auxin treatment
2	Etiolated cuttings, single treatment before planting
3	Etiolated cuttings, long-term treatment before separating of the donor
4	Control 2 – de-etiolated cuttings without auxin treatment
5	De-etiolated cuttings, single treatment before planting
6	De-etiolated cuttings, long-term treatment before separating of the donor

All cuttings were placed in a substrate of peat and perlite 1:1 (v/v). The peat parameters were: 0.5 mm particles; 20 $kg \cdot m^{-3}$ volume density; pH in water was 5.5–6.5; electrical conductivity 35–45 $mS \cdot m^{-1}$ ±25 %; enriched with fertilizer NPK (14:10:18) 1.5 $kg \cdot m^{-2}$. The experiment was conducted in a glasshouse, at a temperature of 22–24 ºC and relative humidity of 85–95 %, maintained by an automatic mist irrigation system.

Each experimental variant was carried out in 3 repetitions, 25 cuttings each.

The result from the rooting was registered a month after the cuttings' planting.

To prove the reliability of the differences obtained, software products SPSS, ANOVA, Post Hoc test were used at confidential interval 95%. To determine the degree of influence of various factors, dispersion analysis components were used: Fisher criterion (F value) and significance level (p-value).

Results and Discussion

The traditional approach in auto-vegetative propagation did not lead to positive results both for the mature donor and juvenile specimens. Rooting was not achieved (0 %) during the two experimental phenological terms and for all variants of exogenic auxin delivery.

Alternative methods for rooting of cuttings through etiolation, however, provoked rhyzogenesis induction from juvenile donors (Table 2).

The highest percentage of rooting was obtained in setting of de-etiolated cuttings from juvenile donor specimens – 71.7 ±3.3 %, treated with IBA 8000 $mg \cdot L^{-1}$ for 4 weeks (Table 2, var. 6, Fig. 3). This result is in accordance with the obtained by a number of authors (Maynard and Bassuk 1985; Bassuk et al. 1986; Maynard and Bassuk 1988, 1990, 1991) – the etiolation in combination with auxin treatment and banding improved the rooting of cuttings. In their investigation Maynard and Bassuk (1996) underlined that the formation of lignificated sclerids inhibits the rooting but the etiolation delayed the sclerids development. In addition, the auxins deactivated under the light influence and its switching off by banding increased the auxins' level and improved the rooting (Sun and Bassuk 1999). But Pacholczak et al. (2005) pointed out that in rooting of *Cotinus coggygria* cuttings the shading was more effective than etiolation. The improved rooting of shaded cuttings was accompanied by decreasing of soluble sugars', proteins and abscisic acid con-

Table 2. Rooting of cuttings from a juvenile donor.

Variant	Rooting, %	Average number of roots	Average length of the root, cm
1 control 1, etiolated without treatment	60.0 ±0.0 c	5.6 ±0.5 abc	2.2 ±0.5 a
2 etiolated, single auxin treatment	35.0 ±0.0 a	7.0 ±0.8 c	3.2 ±0.3 ab
3 etiolated, long-term auxin treatment	46.7 ±4.4 b	4.5 ±0.4 a	2.2 ±0.2 a
4 control 2, de-etiolated without treatment	46.7 ±3.5 b	4.4 ±0.2 a	5.1 ±0.3 c
5 de-etiolated, single auxin treatment	44.0 ±6.1 ab	5.4 ±0.4 abc	4.6 ±3.9 c
6 de-etiolated, long-term auxin treatment	71.7 ±3.3 d	5.8 ±0.5 bc	3.2 ±0.2 b

Note: Means ± Standard error within a column followed by the same letter are not significantly different according Least Significant Difference (LSD) multiple range test at $p \leq 0.05$.

Fig. 3. Rooted cuttings from juvenile donor.

tent, and with increasing of chlorophyll, free amino acids, polyphenolic acids and free indole-3-acetic acid. In rooting of *Berberis thunbergii* ‘Red Rocket’ and ‘Red Chief’ cuttings Pacholczak et al. (2006a, b) explained the shading positive effect with less sclerenchyma development which can decrease the barriers for root primordia.

Etiolated cuttings without auxin influence rooted to 60.0 % (var. 1).

In the third group, with identical results from statistical point of view, were the variants 3, 4, 5: control; etiolated cuttings single treatment after cutting up; etiolated cuttings long-term treatment before cutting up. Rooting there reached up to 44.0–46.7 %.

The least rooting was found in etiolated cuttings single auxin treatment – 35.0 %.

Obtained results showed two basic facts.

First, it is seen that juvenile sycamore specimens have the opportunity for secondary differentiation of tissues and are capable to develop adventive root rudiments, to form adventive root system.

Second, etiolation as a tool for provoking physiological stress leads to increasing of tissues’ morphogenesis activity. In support to this statement is the comparison between both controls in which rooting of de-etiolated cuttings (var. 4) reached 46.67 ±3.53 %, to differentiate from etiolated cuttings rooted 60.0 % (var. 1).

In the literature are given some general recommendations for donor plants shading before obtaining of the cuttings (Maynard et al. 1996). Etiolation of shoots or absence of light can induce or improve the percentage of rooting for species, which under normal conditions are extremely difficult for vegetative propagation (Miske and Bassuk 1985; Maynard and Bassuk 1985, 1987, 1988, 1990, 1996; Podaras and Bassuk 1996; Sun and Bassuk 1999; Hawver and Bassuk 2000; Amissah and Bassuk 2007; Tomov 2015, 2017). Pacholczak and Szydło (2010) investigating the rooting of *Cornus alba* varieties cuttings found out that the intensity of shading maters and recommended 50 % shading. But in our experiment the complete limitation of light led to positive results. Shading of 4-year-old dogwood (*Cornus alba*) cultivars stock plants before harvest led to statistically significant increase of rooted cuttings by 6–8 % (Pacholczak et al. 2017).

However Hawver and Bassuk (2000) pointed out that the etiolation should be applied in combination with additional technics for rooting improvement. Such could be auxin treatment, root restriction or root pruning, red light application on donors, etc. According to the authors end-of-day far red light led to decreasing of cytokinins which inhibit the rooting and as a result it improved.

Maynard and Bassuk (1985) reported about positive effect of etiolation and treatment with 8000 ppm IBA powder through banding in the basis of the shoots on rooting of young (1–4-year-old) donors from *Acer griseum* (34 %), *Acer saccharum* (36 %), *Betula papyrifera* and *Castanea mollissima* (100 %), *Carpinus betulus* (94 %), *Pinus strobus* (83 %), *Quercus coccinea* (46 %). For *Quercus rubra*, however, well-expressed effect of etiolation has not been observed, and there were no experiments with *Pinus mugo*. Tomov (2015, 2017) rooted cuttings from 4-year-old *Acer platanoides* seedlings through etiolation, banding 0.8 % IBA powder (37.7 %), as well.

For *Acer platanoides*, the best reported result was 75 % rooting of etiolated cuttings (Bassuk et al. 1986). The results of this experiment were identical with the findings of Bassuk et al. (1986) and confirm the positive effect of complete shading of the donor plants or different parts of the shoots (taping) on the rooting ability of cuttings. Maynard and Bassuk (1990) pointed out successful rooting of cuttings from 5-year-old *Acer griseum* seedlings (54 %) as a result of etiolation. They also reported (1996) about 73 % rooting of cuttings from 5-year-old seedlings of *Carpinus betulus* '*fastigiata*' after etiolation and treatment for 5 s into 9.8 mM IBA in 50% aqueous ethanol.

Etiolation and banding could improve the rooting percentage of softwood cuttings from *Quercus robur*, *Q. macrocarpa* Mich and *Q. bicolor* (Amissah and Bassuk 2007). The authors reported that the influence on the rooting had the environmental conditions and more significant was the effect in field-grown cuttings in comparison with greenhouse-grown cuttings. Negative impact on rooting had cuttings' aging.

Amissah and Bassuk (2004) showed the importance of solvents when IBA was used for treatment of etiolated shoots without banding and outlined the advantages of ethanol and acetone, and rejected the positive influence of catechol (1,2-dihydroxybenzene). Our investigations showed that the lanoline as a base for the auxin paste did not lead to toxic effect. It could be pointed out as an advantage that the paste is more resistant to meteorological influences and does not dry out.

In their research Maynard and Bassuk (1992) pointed out that the rooting potential decreases if the time of their cutting is delayed related to greening after etiolation. For *Carpinus betulus* during a period of 8 weeks positive effect was not observed. Our studies showed that for sycamore the rooting potential of cuttings was kept high if cutting was realised 4 weeks after greening. The same period was also suitable in rooting of etiolated cuttings of *Acer griseum* (Maynard and Bassuk 1990).

The analysis of root system development of juvenile cuttings showed that highest average number of roots in present in etiolated cuttings with single treatment with IBA after cutting up – 7.0 ±0.8 (var. 2). From statistical point of view, however, this variant did not differ from the de-etiolated cuttings, single treated with IBA after cutting up (var. 5), cuttings after etiolation, treated long-term with IBA before separation (var. 6), as well as non-treated etiolated cuttings (var. 1), where the average number of roots was from 5.4 ±0.4 to 5.8 ±0.5 (Table 2). Amissah and Bassuk (2004) reported significant increasing of the root average number in etiolated cutting of *Quercus bicolor* depending on the IBA solvent and found the positive effect of ethanol and acetone.

According to the length of roots (Table 2) it was determined that with biggest average length were de-etiolated cuttings – control 2, var. 4 (5.1 ±0.3 cm) and those single treated with IBA after cutting up - 4.6 ±3.9 cm (var. 5).

Differences of various indices obtained and discussed in various variants were with statistically proved significance (Table 3), significance level less than 0.05 %.

Successful rooting was not achieved with cuttings from a mature donor. However, interesting occurrence of morphogenesis was observed by the etiolated cuttings, treated long-term before separation (Table 4). In 73.3 % (from 68 to 84 %) of the cuttings, callus was formed in the basis as early as before their separation from the donor plant. Root caps were developed from the callus, lenticels and the basis of the cuttings. Their average number was 26.1 and reached up to 113. After planting of these cuttings root caps continued to grow and reached length up to 4 mm but parallelly above-ground part necrotised (Fig. 4). In some studies the negative impact of the callus on rhizogenesis was observed and it is recommended usage of band to fix up which inhibit callus formation eg velcro (Maynard and Bassuk 1987). Maynard and Bassuk (1985) successfully rooted by etiolation and treatment with 8000 ppm IBA powder through banding in the shoots' basis of mature donors from *Carpinus betulus* (72 %),

Table 3. Statistical significance of the criteria for assessment of juvenile donors rooting.

Criteria	Statistical indices	
	Fisher criterion (*F* value)	**Significance level (*p*-value)**
Rooting, %	12.7	0.003
Average number of roots	3.4	0.005
Average length of roots, cm	15.0	0.000

Table 4. Reaction of cuttings from mature donor.

Callused cuttings, %			Number of roots			Necrotised cuttings, %		
min	**max**	**mean**	**min**	**max**	**mean**	**min**	**max**	**mean**
68	84	73.3±4.8	10	113	26.1±1.9	16	32	22.7±4.8


Fig. 4. Formed callus (left) and root rudiments (right) of cuttings from mature donor.

Corylus americana (87 %) and *Quercus robur* (36 %). Amissah and Bassuk (2005) showed the positive effect on the rooting of *Quercus bicolor* and *Quercus macrocarpa* cuttings if shoots developed after preliminary pruning of stump root stocks to soil level. This approach was applied in our study as well aiming at stage rejuvenation of donor specimen.

Conclusions

The traditional methods for auto-vegetative propagation did not lead to a positive result in rooting of stem cuttings from sycamore.

Precondition for auto-vegetative propagation was: darkening for two months before the beginning of growing period in order to obtain etiolated shoots with one node; treatment of the basal node in the beginning of May with IBA paste with concentration 8000 mg·L^{-1} and protection with aluminium foil; parallel uncovering of the donors for de-etiolation for 4 weeks before planting. In these conditions rooting reached up to 72 %.

For rooting mature donors' cuttings optimisation in growing conditions should be applied, which would allow further development of the root system.

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