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Field collection date and concentrations of indole-3-butyric acid (IBA) effect on the vegetative propagation of London planetree

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Abstract: The London planetree (*Platanus acerifolia* (Ailton) Wild.) is an exotic tree species native to Asia and the Northern Hemisphere, with relevance in urban landscaping and timber potential. This study evaluated the effects of different collection seasons and the use of indole-3-butyric acid (IBA) at various concentrations on the rooting of semi-woody cuttings of *P. acerifolia*. Cuttings (10 cm) were collected in autumn, winter, spring, and summer, treated with IBA (0, 3000, and 6000 mg L^{-1}), and maintained in vermiculite for 90 days under shaded nursery conditions. The hypothesis was that collection season would affect rooting and that the auxin could stimulate adventitious root formation. Results showed that collection season significantly influenced all evaluated parameters, while the use of IBA did not show a significant effect on rhizogenesis. Summer 2015–2016 was the most favorable season, with 60.8% of cuttings rooted and the highest number of shoots, due to accelerated vegetative growth and lower tissue lignification. Winter 2015 showed higher mortality (79.2%) and lower rooting. Therefore, the use of cuttings collected in summer is recommended to maximize vegetative propagation of *P. acerifolia*, ensuring higher rates of root and shoot formation, which are essential for successful field establishment.
Keywords: Cuttings, *Platanus acerifolia*, rooting, seedlings, tree.

1. Introduction

London planetree (*Platanus acerifolia* (Ailton) Wild.) is a tree species of large size and fast growth. Asia and the Northern hemisphere are its natural environment, typical of subtropical and temperate climates. It can reach heights up to 40 m and has an ornamental aspect, deciduous foliage, and a rounded and leafy crown. This species is commonly used in parks, reforestation, windbreak, and carpentry, for furniture and floors due to its high physical and mechanical resistance (Ono et al., 1994, Dias et al., 1999; Rosa et al., 2018).

London planetrees are cultivated in Brazil, mainly in colder regions, such as Paraná, Santa Catarina, and the Rio Grande do Sul. It is primarily used for urban and ornamental purposes, for its shade and beauty, due to its resistance to urban pollution and good development on compacted soils (Santos and Santos 2019).

According to Rosa et al. (2018), *P. acerifolia*, in rural areas, is used as a hedge or a source of shade in different areas such as chicken broiler, swine, cattle, and goat farms, providing thermal comfort for animals during summer. Besides, the decidual trait of the species permits solar radiation to enter rural installations during winter, providing ventilation, heating, and disinfection to these environments.

Thus, the use of new techniques to multiply *P. acerifolia* cuttings is fundamental. Nevertheless, this tree presents low efficiency in seedlings production through its seeds (García et al., 2013). Hence, the vegetative propagation with cuttings is an alternative to produce seedlings (Nicoloso et al., 1999; Dias et al., 1999; García et al., 2013).

Cuttings are economically more viable due to the multiplication process of desirable genotypes, low cost, and reduced production period (Dantas et al., 2016). Cuttings are also more practical. They have a higher yield, produce seedlings in all seasons (identical to the parent-plant), have a lower juvenile phase, among other benefits (Xavier et al., 2013; Hartmann et al., 2018).

The formation of adventitious roots is fundamental for the multiplication process through cuttings (Stuepp et al., 2016). However, endogenous and exogenous factors are decisive for rooting. Other factors to be considered are water, phytohormones, carbohydrate level, minerals, light, temperature, humidity, and substrate (Xavier et al., 2013; Hartmann et al., 2018).

In this context, plant growth regulators may be used, which interact and stimulate the root development of plants (Rickli et al., 2015). Nowadays, a synthetic auxin, known as indole-3-butyric acid (IBA), is one of the plant regulators most used to produce several species seedlings because it has low toxicity, better solubility, and higher stability than other synthetic plant regulators (Emer et al., 2016; Véras et al., 2018).

It is essential to highlight that carbohydrate content and consistency of cuttings vary according to the season (Latoh et al., 2018). The branches are more herbaceous during spring and summer and have few carbohydrate reserves because growth is faster. On the other hand, during fall and winter, cuttings are more lignified and have more carbohydrate reserves (Stuepp et al., 2017; Almeida et al., 2017; Taiz et al., 2017; Hartmann et al., 2018).

The vegetative propagation of London planetrees is an alternative to stimulate knowledge about this species, especially for the timber industry. Therefore, this study aims to evaluate the effect of seasons on the rooting of *P. acerifolia*, using different IBA concentrations.

2. Materials and Methods

2.1. Study location

The study was developed in the forest tree nursery of the Universidade Tecnológica Federal do Paraná (UTFPR-DV – Federal Technological University of Paraná), in the southwest of the state of Paraná, at the city of Dois Vizinhos, (25°44'03'' S, and 53°03'10" W, 500 m altitude). According to the Köppen classification, the climate of the region is subtropical, Cfa-type, with temperatures ranging from -3 °C to 18 °C in the coldest month, constantly humid with rains in all months. The temperatures of the hottest month are higher than 22 °C, and at least four months have temperatures higher than 10 °C (Alvares et al., 2014).

2.2. Material collection and preparation of cuttings

We collected semi-woody branches of the same parent plant of *P. acerifolia* in four seasons at the UTFPR-DV (25°42'12.30" S, and 53°05'53.81" W, 550 m altitude). After each material was collected from the parent plant, the material was taken to the forest tree nursery UTFPR-DV and kept under adequate conditions. The branches were covered with newspapers and moistened to prevent dehydration. In the beginning, we opted to collect material during the coldest periods of the day, which were the dawn, morning, or twilight. On the nursery, cuttings were prepared from the semi-woody branches with 10 cm in length and around 6 mm in diameter, with an angled cut at the base and a straight cut at the apex, without leaves, containing between two and three sprouts.

Collection dates of branches were the first fortnight of April (fall) 2015, July (winter) 2015, November (spring) 2015, and January (summer) 2015/2016. After the preparation, the bases of cuttings were immersed in alcoholic solutions (50% v/v) of indole-3-butyric acid (IBA) with concentrations of 0, 3000, and 6000 mg L^{-1} for 10 seconds.

2.3. Experimental Design

A completely randomized design was used with a 4x3 factorial scheme [four collection seasons (fall, winter, spring, and summer) with three IBA concentrations (0; 3000; and 6000 mg L^{-1})]. Each treatment contained four repetitions with twelve cuttings per repetition, resulting in 144 cuttings per collection season and a total of 570 cuttings for all seasons.

2.4. Storage of cuttings

Cuttings were conditioned in polypropylene tubes of 120 cm³, containing a vermiculite substrate of fine granulometry. Afterward, the tubes were stored in trays, kept in a shade house of the forest tree nursery (UTFPR-DV), under room temperature and air humidity. Climatic data of the meteorological station of UTFPR-DV were used as a parameter. The station pertains to the Instituto Nacional de Meteorologia (INMET - National Institute of Meteorology). Based on such data, an average was determined of the temperature (mean, low, and high) and air humidity during the research period between April/15 and March/2016 (Fig. 1).

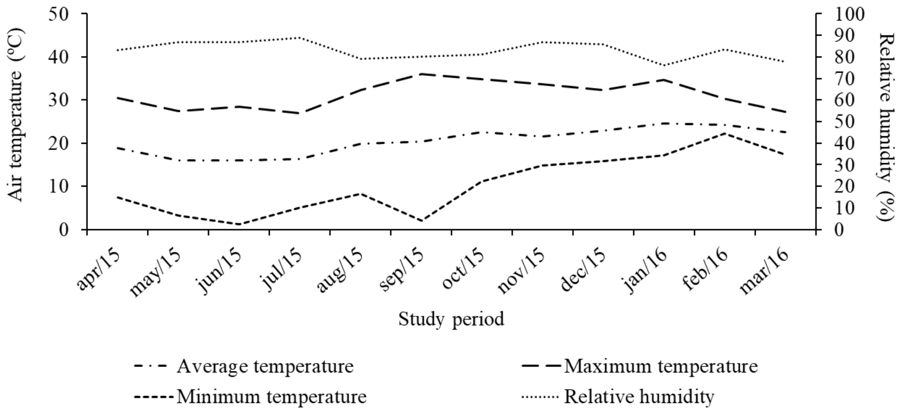

Figure 1. Air temperature (°C) (average, minimum and maximum) and relative air humidity (%) during the period (months) of the study (April / 15 to March / 16) referring to the four seasons of vegetative propagation *P. acerifolia*. Dois Vizinhos, PR, UTFPR, INMET, 2015-2016.

The environment luminosity provided a shading of 50% solar transparency in black coloration. Irrigation occurred five times each day: during mornings (8:00, 10:00, and 12:00) and afternoons (15:00 and 17:00) for 15 minutes in each period. The irrigation system was semi-automatic with a motorized pump of 110 HP and sprinklers with a flowrate of 0.45 $m^3.h^{-1}$. The material was kept at the shade house for 90 days.

2.5. Physical analysis of the substrate

The substrate (vermiculite) was conditioned in kraft paper packages and taken to the Silviculture Laboratory of the UTFPR-DV. It was stored in a drying greenhouse for 48 hours at 60º C. After drying, and the substrate physical analysis was made by the

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Microscopy Laboratory I (UTFPR-DV) following the methodology of Fretz et al. (1979), obtaining; as a result, the physical attributes (Table 1).

Table 1 – Physical analysis of the vermiculite substrate used in cutting *P. acerifolia*. UTFPR, Dois Vizinhos - PR, 2017.

Substrato	DS(g.cm³)	DU (g.L)	EPT	ARCC	EACC
			..................%		
VER	0.2363	236.3	74.5	65.02	7.94

DS – dry density; DU - wet density; EPT - total porous space; ARCC - water retention in field capacity (microporosity); EACC - air space in field capacity (macroporosity); (VER) vermiculite.

2.6. Evaluations

After 90 days, the following parameters were evaluated: percentage of cuttings rooted (RC) (for roots higher than 0.1 cm); percentage of cuttings with calluses (CC) (deposition of meristematic cells in the basis of non-rooted cuttings); percentage of dead cuttings (DC) (completely dry cuttings); alive cuttings (AC) (cuttings without roots and green color); percentage of sprouts cuttings (SC) (presence of sprouts on cuttings); the number of roots (NR) (count of the number of roots above 0.1 cm per cutting); length of the three larger roots (LTLR) (mean of the three larger roots of each cutting); the number of sprouts (NS) (count of sprouts present in each cutting).

2.7. Data analysis

The data were submitted to the Shapiro-Wilk normality test (p-value > 0.05) and Bartlett variance homoscedasticity test to verify the necessity of transformation by arcsine $\sqrt{x/100}$ for the percentage of alive cuttings and by the square root $\sqrt{x+1}$ for the variable number of sprouts per cutting. Afterward, the means with and without transformation were submitted to the analysis of variance ANOVA ($p<0.05$) and the comparison of means by the Tukey test at 5% probability for the qualitative factor, using the software SISVAR 5.6 (Ferreira, 2014).

3. Results

The results indicated no significant interaction between the collection season and the IBA concentrations for the parameters studied (Table 2). However, the factors were analyzed separately, and only the factor collection season was significant in all parameters: percentage of rooted cuttings (RC), alive cuttings (AC), dead cuttings (DC), number of roots (NR), length of the three larger roots (LTLR), percentage of cuttings sprouts (SC), and number of sprouts (NS) (Table 2). Nevertheless, the rate of cuttings with calluses (CC) did not provide significant data in this study.

Table 2 – Summary of variation analysis containing mean square values for variables: percentage of rooted cuttings (RC); number of roots (NR); length of the three largest roots (LTLR), percentage of dead cuttings (DC), percentage of live cuttings (AC), percentage of cuttings sprouts (SC), and number of sprouts (NS), collection seasons (CS) and IBA concentrations (IBA) in *P. acerifolia* cuttings. Dois Vizinhos, PR, UTFPR, 2016-2017.

SV	DF	RC	NR	LTLR	DC	AC(a)	SC	NS(b)
		%		Cm	%	%	%	
CS	3	2328.46*	57.12*	86.71*	5173.39*	992.30*	850.00*	4.70*
IBA	2	84.14^{ns}	18.44^{ns}	35.13^{ns}	83.72^{ns}	64.52^{ns}	81.25^{ns}	0.45^{ns}
CS x IBA	6	74.75^{ns}	30.30^{ns}	40.55^{ns}	178.86^{ns}	75.49^{ns}	189.58^{ns}	0.07^{ns}
Error	36	118.0	24.37	40.33	329.47	130.80	73.61	0.28
Total	47							
CV (%)		33.66	29.16	32.21	28.13	44.00	32.32	34.00

^{ns} not significant at 5% probability, *Significant at 5% probability. SV = source of variation; DF = Degrees of freedom. (a)Data transformed by arc sine ($\sqrt{x / 100}$). (b) Data transformed by the square root ($\sqrt{x+1}$).

The percentage of rooted cuttings (RC) was significantly higher for the collection season S (summer 2015-2016), obtaining 60.83% of cuttings rooted, differing from the other season that presented inferior results (Fig. 2A).

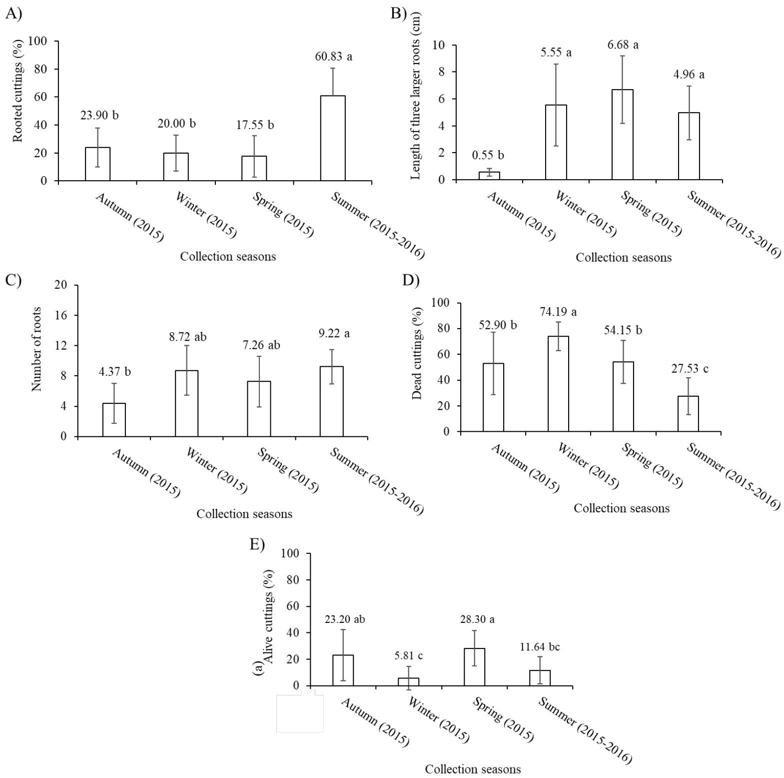

Figure 2 – Average data: **A.** Percentage of rooted cuttings; **B.** Length of the three larger roots; **C.** number of roots; **D.** Percentage of dead cuttings; **E.** percentage of alive cuttings. [(a)]Data transformed by arc sine ($\sqrt{x / 100}$). Means followed by the same letter do not differ significantly by Tukey test at 5% probability.

Regarding the length of the three larger roots (LTLR), F (fall/2015) obtained lower results than the other seasons and stood out negatively with only 0.55 cm for LTLR (Fig. 2B). S was also superior regarding the number of roots (9.22), differing statistically from F, with only 4.37 roots (Fig. 2C).

Regarding the percentage of alive cuttings (AC), Sp (spring/2015) (28.33%) was statistically higher than S (11.67%) and W (5.83%). This result is due to the low rooting and the high mortality observed in that season (Fig. 2D). The percentage of dead cuttings (DC) in S (27.50%) did not differ statistically from the other seasons, presenting the lower mortality rate. However, W (winter/2015) (74.17%) showed a higher percentage of dead cuttings (Fig. 2E).

Regarding the parameters, percentage of sprouts cuttings (S) (Fig. 3A) and the number of sprouts (NS) (Fig. 3B), S stood out again. Regarding cuttings with sprouts, S (23.30%) differed significantly from Sp (5.80 %) and W (5.80 %), while it presented higher values for the number of sprouts (1.87) than the other treatments.

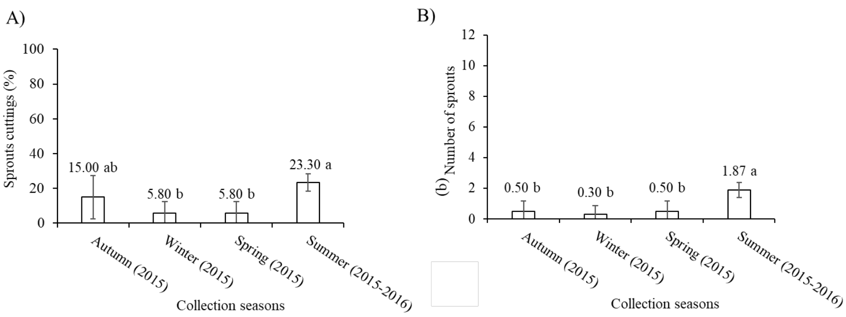

Figure 3 – Average data: **A.** Percentage of sprouts cuttings; and **B.** Number of sprouts. [(b)] Data transformed by the square root *($\sqrt{x+1}$)*. Means followed by the same letter do not differ significantly by Tukey test at 5% probability.

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4. Discussion

Regarding CR, the results obtained differ from Nicoloso et al. (1999a), who obtained better outcomes for the rooting of *P. acerifolia* in cuttings collected during winter. According to Rosa et al. (2018), *P. acerifolia* is a forest species (woody) with temperate characteristics, and it was expected that the best collection date would be during winter (carbohydrate reserves). Moreover, Hartmann et al. (2018) highlight that the cuttings have higher carbohydrate reserves that would permit better rooting during winter.

Nevertheless, the opposite occurred in our study since cuttings collected during summer presented better rooting of *P. acerifolia* cuttings. This result may be explained by the summer being a season where the branches are more herbaceous and faster plant growth (Stuepp et al., 2017; Hartmann et al., 2018).

In this aspect, besides it may present lower carbohydrate reserves, the tissues during summer have less lignification, promoting the formation of adventitious roots in the cuttings, which may have enhanced better rooting of London planetrees (Taiz et al., 2017; Hartmann et al., 2018; Kerbauy 2019). Other studies also highlight that summer is the best season to prepare the cuttings, such as Pivetta et al. (2012) obtained 80.73% rooting success for oleander cuttings (*Nerium oleander L.*). On the other hand, Hilgert et al. (2020) found values close to 35% for pecan tree cuttings (*Carya illinoinensis*) collected during summer.

Another relevant aspect is the temperature during the root formation process. Summer has higher temperatures, which accelerates tissue growth and, consequently, enhances the rooting of cuttings (Hartmann et al., 2018; Hilgert et al., 2020). Thus, according to Almeida et al. (2017), the metabolism decelerates with low temperatures, which reduces the capacity to develop root tissues, requiring a more extensive period or impeding cuttings' rooting.

Regarding LTLR, the results differ from those found by Silva et al. (2012). During fall, the authors observed that semi-woody cuttings of olive cultivars (*Olea europaea*) presented 2.1 cm in root length. It also differed from Stuepp et al. (2017), where *Ilex paraguariensis* cuttings collected during the four seasons obtained values close to 27 cm during fall.

Fall is favorable to the rooting of most forest species (woody) because there is an immediate supply of reserves (carbohydrate) since the plant is in dormancy, favoring the translocation of photosynthates (Oliveira et al., 2012; Stuepp et al., 2017). However, our study found different results from the studies as fall was unfavorable for *P. acerifolia* cuttings. This fact may be related to the conditions of the parent plant during the collection of branches to prepare the cuttings (Hartmann et al., 2018). Bhatla and Lal (2018) states that the physiological and nutritional condition of the parent plant can interfere in physiological activity and carbohydrates synthesis. Besides, it may influence the involvement and determination of morphogenic responses, affecting the length and number of roots.

The different temperatures over the year may have influenced the number of roots (Hartmann et al., 2018). In this aspect, an interaction may occur among the factors: temperature, photoperiod, and auxin concentrations; besides other hormones in the rhizogenesis process of cuttings (Bhatla and Lal 2018; Hartmann et al., 2018; Hilgert et al., 2020).

According to Taiz et al. (2017), the temperature directly affects the metabolism of plants, and the higher the temperature, the faster the chemical reactions, which may favor root development. According to Zem et al. (2015), the best result in summer this period is of intense vegetative growth and high physiological activity, which favors the development of roots in most species.

Regarding the mortality of cuttings, Zem et al. (2015) state the percentage of dead cuttings is influenced by the collection season due to alterations in the physiological balance on longer or shorter days.

Stuepp et al. (2017) found different results while studying yerba mate cuttings during the four seasons, obtaining higher mortality in summer (95%). Sá et al. (2018) state that some factors are linked to mortality, such as the dislocation of photoassimilates and other essential substances for the metabolism related to the collection season.

Collection season also influences the parameter AC. Mabizela et al. (2017) report that alive cuttings are affected in some seasons, depending on the species and the best period to collect the propagative material, as it may modify the rooting potential.

The result of spring as the best season for AC of London planetrees may be explained, according to Fragoso et al. (2015), by the occurrence during this season of active points of growth in parent plants, which are related to auxin and co-factors synthesis. This fact results in the differentiation of tissues by being a period of higher vegetative development and consorted to the high metabolic activity. Hartmann et al. (2018) state that the most active growth phases in plants begin during summer-spring. Besides, the reserve material is available, which subsequently provides root development.

The collection period influences the emergence of sprouts (Oliveira et al., 2012; Hilgert et al., 2020). Summer presented the best results, which may be explained by Almeida et al. (2017), who reported that growth in this season is faster. Moreover, Hartmann et al. (2018) report that the physiological balance of parent plants, temperature conditions, and energy (carbohydrate) reserves distribution influence the emergence of sprouts.

On the other hand, no IBA concentration demonstrated significant interaction since the means were close in all parameters evaluated. Our results diverge from those found by Dias et al. (1999) and Nicoloso et al. (1999) that studied cuttings with the application of IBA. In such studies, IBA significantly affected rooting. Rosa et al. (2018) report that IBA expressively increased the rooting of *P. acerifolia* cuttings.

However, our study found that IBA concentrations did not influence the vegetative propagation of the London planetree. Therefore, further studies are required concerning this species. We also highlight that the literature about the vegetative propagation of the species is scarce and outdated. Nevertheless, the season with higher temperatures (S) provided a better stimulus for rooting of London planetree cuttings, constituting an alternative for vegetative propagation by cuttings.

5. Conclusions

The summer collection period proved to be the most suitable for the propagation of London planetree (*P. acerifolia*), resulting in higher rooting and sprouting rates.

Indole-3-butyric acid (IBA) did not influence the increase in rhizogenesis of the species.

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