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Collection period and indolebutyric acid on the rooting of adult pecan plant cuttings

Abstract – The objective of this work was to evaluate the effect of the collection period and of the exogenous application of indolebutyric acid (IBA) on the rooting of cuttings from adult pecan (*Carya illinoinensis*) plants. The experiment was carried out in a greenhouse with intermittent mist irrigation, from December 2017 to October 2018. The experimental design was completely randomized, in a 4x5 factorial arrangement, with four collection periods (spring, summer, autumn, and winter) and five concentrations of IBA (0, 2,000, 4,000, 6,000, and 8,000 mg L^{-1}). For the cuttings collected during summer and autumn, there was a linear relationship between rooting and the increase of the IBA concentration. The best results for rooting, callus formation, mean number of roots, mean root length, and root dry and fresh mass are obtained for the cuttings collected during summer, with the application of 8,000 mg L^{-1} IBA on the base of each cutting, under a mist irrigation system. The rooting of cuttings from adult pecan plants with the application of IBA is possible, being an alternative propagation method for the species.

Index terms: *Carya illinoinensis*, callus, clonal propagation.

Período de coleta e ácido indolbutírico no enraizamento de estacas de plantas adultas de nogueira-pecã

Resumo – O objetivo deste trabalho foi avaliar o efeito do período de coleta e da aplicação exógena de ácido indolbutírico (AIB) no enraizamento de estacas de plantas adultas de nogueira-pecã (*Carya illinoinensis*). O experimento foi realizado em casa de vegetação com irrigação por nebulização intermitente, de dezembro de 2017 a outubro de 2018. O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 4x5, com quatro períodos de coleta (primavera, verão, outono e inverno) e cinco concentrações de AIB (0, 2.000, 4.000, 6.000 e 8.000 mg L^{-1}). Para as estacas coletadas durante o verão e o outono, observou-se relação linear entre o enraizamento e o aumento da concentração de AIB. Os melhores resultados para enraizamento, formação de calo, número médio de raízes, comprimento médio de raízes, e massa seca e fresca de raízes são obtidos para as estacas coletadas durante o verão, com a aplicação de 8.000 mg L^{-1} de AIB na base de cada estaca, em sistema de irrigação por nebulização. O enraizamento de estacas de plantas adultas de nogueira-pecã com a aplicação de AIB é possível, sendo um método de propagação alternativo para a espécie.

Termos para indexação: *Carya illinoinensis*, calo, propagação clonal.

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Introduction

Carya illinoinensis (Wangenh.) K.Koch, popularly known as pecan, is a species native to North America that is grown in several countries (Zhang et al., 2015). In Brazil, the largest cultivated area, with more than 5,000 hectares, is located in the state of Rio Grande do Sul (Hamann et al., 2018).

Although pecan seedlings can be produced from seeds, the obtained plants will differ in the orchard due to genetic variability (Vahdati et al., 2020). In addition, when propagated by this method, the species presents slow growth and a long juvenility period. For these reasons, the propagation system currently used commercially consists of grafting with seed rootstocks (Fronza et al., 2018). However, rootstocks derived from pecan seeds of open-pollinated plants also show a great variability, causing differences in grafting, growth, and performance between plants (Cao et al., 2019).

In this scenario, maintaining homogeneity among plants through vegetative propagation is a vital point for seedling production of forest species (Stuepp et al., 2014). Among the main techniques used for vegetative propagation, is the rooting of cuttings technique, which is an exclusively clonal propagation method that maintains all the desirable characteristics of the mother plant (Hartmann et al., 2011). However, the results of this procedure differ according to the collection period of the cuttings, such as seasons of the year, mainly due to variations in the meristematic activity rate and tissue lignification of the plant (Fachinello et al., 2005).

Besides being influenced by the collection period of the propagating material, rooting also differs with plant development. Hartmann et al. (2011) found that the rooting capacity of cuttings of several species, such as olive (*Olea europaea* L.), eucalyptus (*Eucalyptus* spp.), and apple [*Malus domestica* (Suckow) Borkh.], decreased after the transition from the juvenile to the adult phase because of the accumulation of sclereid cells. Rickli et al. (2015) also reported that cuttings obtained from regrown stumps had a greater potential to be used in vegetative propagation due to the induction of vigorous buds by an alteration in hormonal balance. Therefore, it is important to stimulate the formation of propagating material with juvenile characteristics, which can be done by rescuing juvenile material from adult plants through pruning, allowing a reduction in ontogenetic age (Wendling et al., 2014).

The rhizogenesis process of cuttings is also affected by other factors, such as the concentration of auxins, especially in species that are difficult to root. Auxins have the ability to stimulate the cell division process, causing the emission and elongation of lateral roots (Enders & Strader, 2015). Among those plant hormones, indolebutyric acid is the most commonly used due its greater root-inducing capacity (Pacurar et al., 2014).

The objective of this work was to evaluate the effect of the collection period and of the exogenous application of indolebutyric acid (IBA) on the rooting of cuttings from adult pecan plants.

Materials and Methods

The cuttings for the experiment were collected from 14 pecan mother plants, with 38 years of age, planted at a 10x12-m spacing at the orchard of the agronomic experimental station of Universidade Federal do Rio Grande do Sul (UFRGS), in the municipality of Eldorado do Sul, in the state of Rio Grande do Sul, Brazil (30°07'08"S, 51°39'59"W, at an average altitude of 46 m). Before the collection, a drastic pruning just above the grafting point of the plants was carried out in July 2017, leaving approximately 30 cm of each lateral shoot for the emission of new shoots. The experiment was conducted under intermittent mist irrigation in a greenhouse, which belongs to the Department of Horticulture and Forestry of Escola de Agronomia of UFRGS, from December 2017 to October 2018.

The shoots that originated from the cuttings were collected during the four seasons of the year, in the morning, from 8 to 10 a.m., due to the high moisture of the propagation material, which had been previously rolled in paper and moistened with sterile distilled water. After collected, the cuttings were packed in polystyrene boxes to be transported to the experimental site.

Considering plant development, the collected shoots were: herbaceous in spring, semi woody in summer and autumn, and woody in winter, with different ages from budding to the collection period (Table 1). Therefore, for standardization, all the propagation material was homogenized in a single lot after each collection in each season, using cuttings from the central part of the shoots of each mother plant. Two buds were left at the top and bottom part of the cuttings, which were later cut into a bevel above the apical bud; the buds had 8 to 10 mm in diameter and were distanced from each other 10 to 12 cm. To minimize the evapotranspiration of the cuttings, the leaves were reduced to one, which was cut in half, with six leaflets; however, since the species is deciduous, the winter cuttings had no leaves.

The experimental design was completely randomized, in a 4x5 factorial arrangement, consisting of four collection periods (spring, summer, autumn, and winter) x five concentrations of IBA (0, 2,000, 4,000, 6,000, and 8,000 mg L^{-1}), with four replicates of ten cuttings per plot, totalizing 40 cuttings per treatment. The treatments consisted of combinations of the IBA concentrations with the different collection periods.

IBA was chosen for the study because it is a synthetic auxin effective in promoting rooting, is not degraded by light, and has an easy dissolution. For an improved dissolution, the IBA solutions were made with hydroalcoholic solutions with indole-3-butyric acid with 99% purity (Vetec Química Fina, Duque de Caxias, RJ, Brazil); 50% ethyl alcohol was used as a solvent and distilled water, to complete the remainder of the solution, as adapted from Fachinello et al. (2005). For a better penetration, the base of each collected cutting was kept in contact with the IBA solution for 10 s (Zem et al., 2016).

After the application of the treatments with different IBA concentrations, the cuttings were placed in plastic trays – with 50 cells with 100-mL volume each, filled with carbonized rice hull substrate – and were kept inside a greenhouse with intermittent mist irrigation. The substrate was chosen based on its high aeration space and low water retention, indicated for an ambient for cuttings under mist irrigation. The irrigation system was composed of Fogger sprinklers, with four nozzles and a flow rate of 8.7 m^3 h^{-1}, spaced at 50 cm.

The trigger times for intermittent mist irrigation differed according to the season of the year and time of day. From November to May, irrigation was performed from 7 a.m. to 7 p.m., every 5 min for 15 s, and from 7 p.m. to 7 a.m., every 15 min for 15 s. From June to October, it was carried out from 7 a.m. to 7 p.m., every 8 min for 15 s, and from 7 p.m. to 7 a.m., every 25 min for 15 s. The average temperature and relative humidity inside the greenhouse were recorded on a daily basis (Figure 1), using the AK174 digital thermo-hygrometer (Akso Produtos Eletrônicos Ltda., São Leopoldo, RS, Brazil).

All plants from all treatments were assessed 60 days after being cut. The parameters evaluated and calculated were: cuttings with a callus formation (%), rooting (%), survival of cuttings (%), leaf retention (%), sprouting (%), mean number of roots per cutting, mean root length per cutting (cm), and mean root fresh and dry weights (mg). A graduated ruler was used to measure mean root length. To determine root mass, roots were previously washed in running water to

Table 1. Approximate age of cuttings (AC) after sprouting (days after drastic pruning) and dates of the installation of the experiment and of the evaluation of the cuttings collected from 38-year-old pecan (*Carya illinoinensis*) adult plants at different seasons of the year and treated with indolebutyric acid.

Variable	Collection period / Type of cuttings			
	Spring / Herbaceous	Summer / Semi woody	Autumn / Semi woody	Winter / Woody
AC (days)	40	90	140	270
Installation date	12/13/2017	02/01/2018	03/23/2018	08/01/2018
Evaluation date	02/11/2018	04/02/2018	05/22/2018	10/02/2018

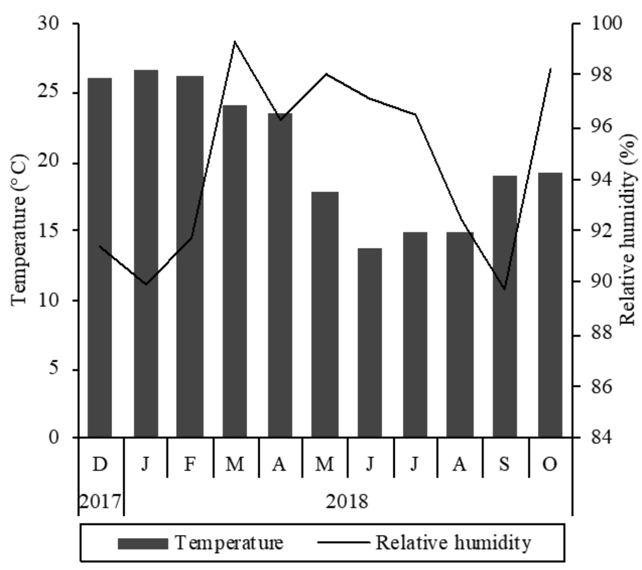

Figure 1. Average temperature and relative humidity inside the greenhouse with intermittent mist spraying from December 2017 to October 2018, in the municipality of Porto Alegre, in state of Rio Grande do Sul, Brazil.

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remove any substrate and then fresh mass was weighed and placed in a drying oven, at 65°C, until constant weight, in order to weigh root dry mass; both fresh and dried roots were weighed on a precision analytical scale (0.0001 g).

The data were subjected to the analysis of variance (Anova), using the model that presented a significant difference for the F-test, at 5% probability. When the interaction factors were significant, the data were subjected to the polynomial regression analysis, and, when only the collection period was significant, means were compared by Tukey's test, at 5% probability. The variables callus formation, rooting, leaf retention, and sprouting were transformed into $\sqrt{x + 1}$, and survival cuttings, into arcsine $\sqrt{(x)/100}$, with results presented in the original values, in order to normalize the data and conduct a statistical analysis. Person's correlation was also performed for callus formation and rooting. The statistical analysis was carried out using the statistical software SigmaPlot, version 10.0 (Systat Software Inc., Chicago, IL, USA), and Sisvar, version 5.6 (Ferreira, 2015).

Results and Discussion

There was no interaction between the factors tested for survival of cuttings, sprouting, and leaf retention, but there was a significant effect of seasons due to the different temperatures and plant tissue characteristics. The lowest values for survival of cuttings (18.5%) and leaf retention (16.5%) were obtained for the cuttings collected in spring (Table 2), which was mainly attributed to their herbaceous constitution, making them extremely tender and, consequently, sensitive to the greenhouse temperatures, leading to a greater dehydration and deterioration. Fachinello et al. (2005) concluded that cuttings dehydrate and wilt specially because of the high transpiration caused by high temperatures. For peach [*Prunus persica* (L.) Batsch] cuttings, with low lignification and leaf presence, Oliveira et al. (2003) also found a higher mortality at elevated temperatures due to increased transpiration, observing the dehydration and rapid degradation of the energy reserves of the cuttings even when an irrigation system with intermittent mist was used.

The extremely tender herbaceous cuttings, collected in spring, are characteristic of pecan, which, compared with other temperate climate fruit species, is considered of late budding, beginning in September and October (Fronza et al., 2015).

Due to their woody composition, the cuttings collected in winter showed a superior survival of 95% (Table 2), which can also be attributed to the vegetative rest period of the species for the storage of reserve compounds.

The highest sprouting percentage of 85% was also obtained for the cuttings collected during winter, mainly due to the increase in temperature during the months of the installation of the experiment (Figure 1) and to the mobilization of reserve substances for buds during the dormancy period, stimulating sprouting. Similar results of 70% sprouting average were reported by Ohland et al. (2009) for cuttings of the Roxo de Valinhos common fig (*Ficus carica* L.) cultivar, collected during winter, in August.

For the variables callus formation and rooting, there was an interaction between the evaluated factors. The polynomial regression analysis showed an increasing linear behavior with the increase of the IBA concentrations in the summer and autumn collection periods (Figure 2). However, there was no regression adjustment for callus formation and rooting with the exogenous application of auxin during spring and winter. It should be noted that, as results, only the averages for each variable were presented.

The greatest callus formation of 35% was obtained for summer cuttings, with the application of 8,000 mg L^{-1} IBA (Figure 2 A). The highest rooting of 30% was also found for summer cuttings with the application of the same concentration of IBA (Figure 2 B).

Table 2. Survival, leaf retention, and sprouting of pecan (*Carya illinoinensis*) cuttings from adult plants, 60 days after being cut, collected at different seasons of the year in the municipality of Eldorado do Sul, in the state of Rio Grande do Sul, Brazil(1).

Collection period	Survival (%)	Leaf retention (%)	Sprouting (%)
Spring	18.5c	16.5b	16.0c
Summer	73.5b	61.5a	47.0b
Autumn	66.5b	57.5a	40.5b
Winter	95.0a	-	85.0a
CV (%)	11.25	7.29	11.48

(1)Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. CV, coefficient of variation.

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Likewise, Gustafson Jr. (1973) observed the positive effect of 8,000 mg L^{-1} IBA on cuttings of a 30-year-old Coy pecan cultivar, which resulted in the highest average of 37.5% of rooted cuttings. This positive effect on rooting shows that the application of IBA is essential for the successful propagation of this species through cutting, especially during summer, when better results are obtained.

However, as rooting increased linearly up to the 8,000 mg L^{-1} concentration, it was not possible to estimate the saturation value, from which there would be a decline in rooting. Moreover, it was also not possible to infer if rooting would increase with even higher concentrations. According to Hartmann et al. (2011), the increase in rooting as a response to the application of auxins reaches a maximum point, after which any subsequent addition may be inhibitory. This way, it may be possible to obtain superior results with concentrations higher than 8,000 mg L^{-1} IBA, as well as a saturation point for propagation through the rooting of pecan cuttings.

All rooted cuttings showed the presence of calluses, with a strong correlation of 0.85 between the variables callus formation and rooting. Although the callus is a parenchyma cell mass that can occur by itself, in species that have difficulty in rooting, roots can be formed in the callus region (Hartmann et al., 2011). Due to this root formation, the presence of callus was important for the rooting of cuttings from adult pecan plants. However, according to Fachinello et al. (2005), in some species, callus formation is not a sign of root formation, but of healing of a cut or a barrier to their development.

For the mean number of roots and mean root length in summer and autumn, there was an increasing linear behavior with the increase of the IBA concentrations after the polynomial regression analysis (Figure 3). However, there was no regression adjustment for mean number of roots and root length with the exogenous application of auxin to the cuttings collected in spring and winter.

The concentration of 8,000 mg L^{-1} IBA applied to the cuttings collected during summer promoted a superior number of roots, with a mean of 4.6 roots per stem. (Figure 3 A). This is an important characteristic for the production of seedlings because it favors their survival and development. Kilkenny et al. (2012) also observed a linear growth between the number of roots and the increase of IBA concentrations for cuttings of *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson.

The highest mean root length was also obtained with 8,000 mg L^{-1} IBA applied to the cuttings collected during summer, with an average of 4.4 cm (Figure 3 B). According to Tonietto et al. (2001), the application of IBA not only induces but also anticipates the formation

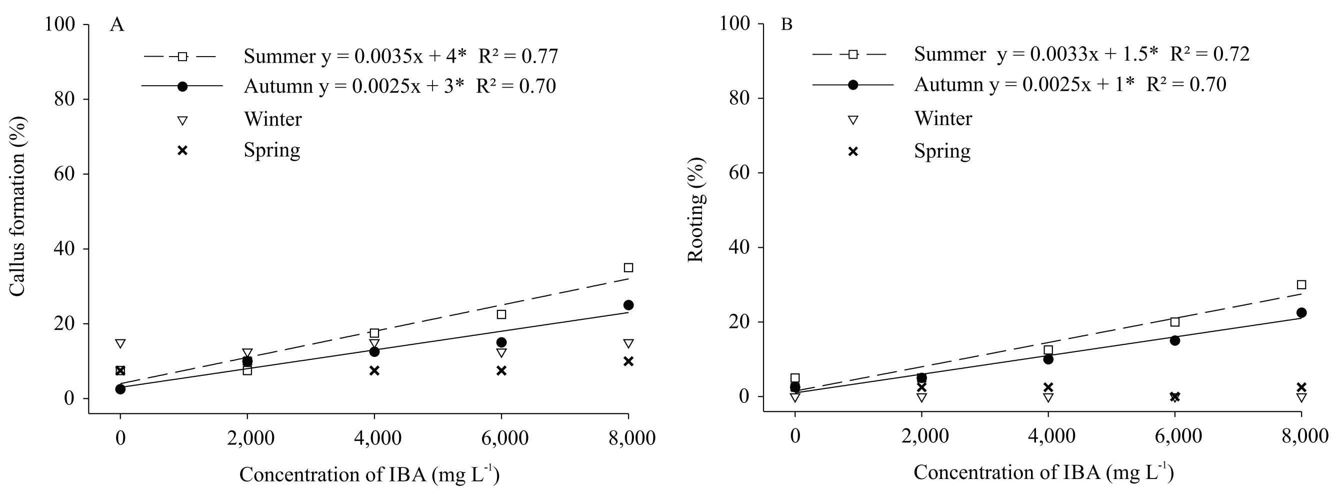

Figure 2. Callus formation (A) and rooting (B) of pecan (*Carya illinoinensis*) cuttings from adult plants subjected to different indolebutyric acid (IBA) concentrations and collected at different seasons of the year, when kept in a greenhouse with intermittent mist irrigation, in the municipality of Porto Alegre, in the state of Rio Grande Sul, Brazil. *Significant at 1% probability.

of roots, allowing more time for their development. A positive effect of IBA application on root length was also reported by Babaie et al. (2014), who found that cuttings of *Ficus binnendijkii* (Miq.) Miq. responded to the increase of the exogenous application of IBA.

The concentration of 8,000 mg L^{-1} IBA also resulted in a higher number of roots and in a longer root length for the pecan cuttings, directly influencing the propagation of the species (Figure 4 D). When rooting occurred with the lowest IBA concentrations, there was only callus formation or the emission of a few roots with a shorter length than those obtained with the highest concentration (Figure 4 A).

The analyzed variables were also influenced by the internal temperature of the greenhouse, which varied mainly with the months of the year (Figure 1). According to Hartmann et al. (2011), there is an interaction between temperature, photoperiod, and concentration of auxins and other hormones in the rhizogenesis process of cuttings. Fachinello et al. (2005) pointed out how temperature is an important factor for rooting – high temperatures, for example, favor cell division and the rooting of cuttings; however, they also increase transpiration and cause wilting, especially in herbaceous cuttings.

The best results for the variables linked to rooting, such as callus formation, rooting, mean number of roots and mean root length, were obtained for the cuttings collected during summer. These positive results are probably due to the higher average temperature of the season and associated with the higher lignification of the cuttings, as well as to their greater amount of leaves, which is related to seasons and drastic pruning. During autumn, when the greenhouse temperature started to drop, the cuttings collected showed a decrease in callus formation, rooting, mean number of roots, and root length. During winter, there was also a lack of root induction due to even lower temperatures. The obtained results reveal the importance of temperature in the process of root formation and of the application of IBA during the different collection periods. The highest rooting percentage found by Denaxa et al. (2012) was of 76% for the cuttings of the Arbequina olive cultivar collected during summer, followed by 40% for those collected in autumn. According to Almeida et al. (2017), low temperatures slow down the metabolism of the cuttings, consequently reducing their capacity to form root tissues.

The difference in the results obtained for the cuttings collected during autumn and summer may be related to the deciduous nature of the species. It may also be associated with the beginning of the dormancy period in autumn, triggered by a temperature drop, initiating the process of senescence. According to Hartmann

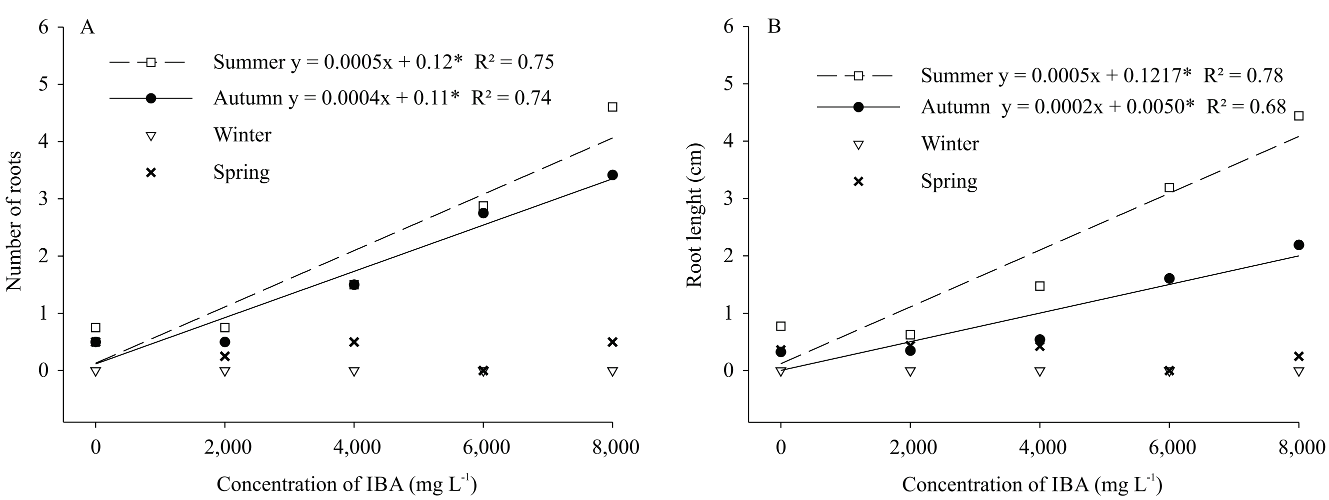

Figure 3. Average number of roots (A) and average root length (B) of pecan (*Carya illinoinensis*) cuttings from adult plants subjected to different indolebutyric acid (IBA) concentrations and collected at different seasons of the year, when kept in a greenhouse with intermittent mist irrigation, in the municipality of Porto Alegre, in the state of Rio Grande do Sul, Brazil. *Significant at 1% probability.

et al. (2011), in deciduous plants, at the moment of dormancy onset, there is an accumulation of phenols and inhibitors that cause a reduction in the activity of young tissues and vascular cambium, leading to a decrease in rooting.

The cuttings collected during winter showed little callus formation and no root formation. During winter, deciduous plants, such as pecan, are in a state of dormancy, with more lignified shoots and a lower physiological activity (Marangon & Biasi, 2013). Therefore, the fact that the mother plants were already in this state explains the null results for root formation. Similarly, Izhaki et al. (2018) found that *Diospyros virginiana* L. cuttings collected during seasons that promote tissue activity had a higher rooting rate, which decreased with the advance of the growing season. In the present study, the pecan cuttings collected during winter showed a higher lignification of the propagation material, which is characteristic of deciduous species in this season of the year.

The variables fresh mass and dry mass of roots were influenced directly by the IBA concentrations and collection periods, with an interaction between factors. There was also a linear increase with the increased IBA concentrations for the cuttings collected during summer and autumn (Figure 5). The highest average results of 1,146.75 mg for fresh root mass (Figure 5 A) and 578.79 mg for root dry mass (Figure 5 B) were obtained for the cuttings collected during summer and with the application of 8,000 mg L^{-1} IBA.

Regarding the variables fresh and root dry mass, superior results were obtained for the cuttings collected during summer with the application of 8,000 mg L^{-1}, mainly because the other variables related to rooting – number and length of roots – showed similar increases in the cuttings collected at the same time. Pio et al. (2003) reported a similar effect on cuttings of common fig, with a linear increase of dry root weight up to the highest IBA concentration.

According to Dutra et al. (2002), the application of IBA can promote better results in the formation of the root system, both quantitatively, through the percentage of rooted cuttings, and qualitatively, through the dry weight and number of roots. The effect on root system quality is important for the acclimation and subsequent development stages of seedlings. In relation to pecan cuttings, especially for those collected during summer, there was a positive effect using 8,000 mg L^{-1} IBA, which was quantitative, for rooting, and qualitative, for the variables related to the quality of the root system, such as mean number of roots and mean root length. The quality of the root system is an important factor because it allows evaluating if a seedling derived from a cutting can be used for planting (Silva et al., 2014).

The data found in the present study show the possibility of pecan propagation by the rescue of propagating material from adult plants and the application of IBA. Although pecan is a species that presents poor rooting of cuttings (Casales et al., 2018), a greater uniformity is obtained when plants

Figure 4. Rooting of cuttings of pecan (*Carya illinoinensis*) adult plants during summer after the application of 2,000 mg L^{-1} (A), 4,000 mg L^{-1} (B), 6,000 mg L^{-1} (C), and 8,000 mg L^{-1} (D) indolebutyric acid (IBA)

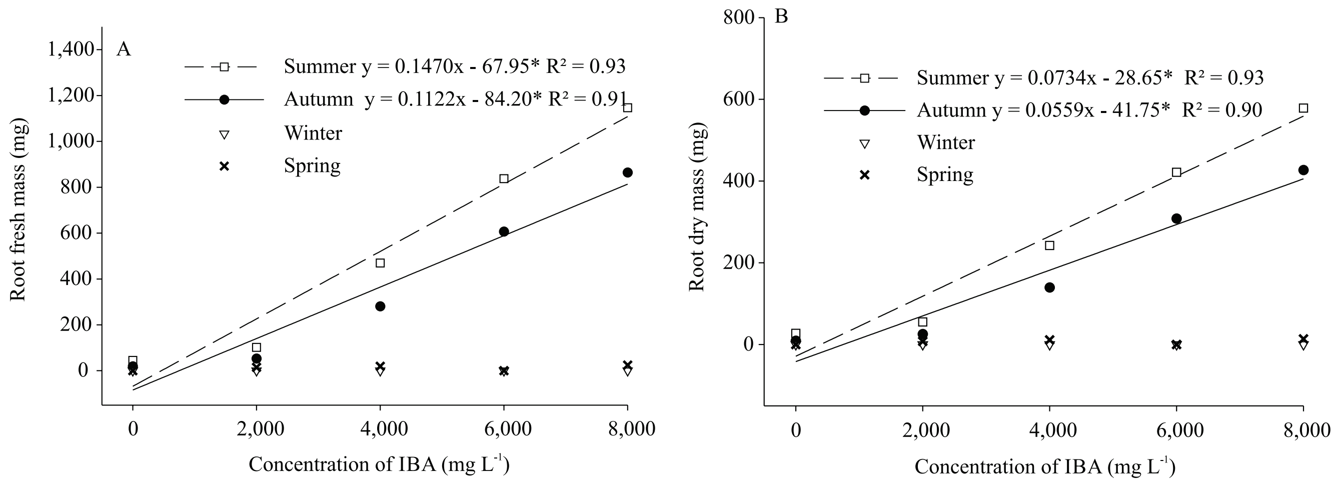

Figure 5. Average fresh (A) and dry (B) mass of roots of pecan (*Carya illinoinensis*) cuttings from adult plants subjected to different indolebutyric acid (IBA) concentrations and collected at different seasons of the year, when kept in a greenhouse with intermittent spray irrigation, in the municipality of Porto Alegre, in the state of Rio Grande do Sul, Brazil. *Significant at 1% probability.

are propagated by this method. However, the period (season) when the cuttings are collected and the IBA concentration used can provide different results.

Due to the linear behavior observed for the studied variables, especially for rooting, there is a need for complementary studies with the application of concentrations higher than 8,000 mg L^{-1} IBA. This way, it would be possible to estimate the maximum concentration that would allow increasing the percentage of pecan rooted cuttings and that could be used as a future alternative for the propagation of the species.

Conclusions

1. Rooting of pecan (*Carya illinoinensis*) cuttings from adult plants with the application of indolebutyric acid (IBA) is possible, being an alternative propagation method for the species.
2. The best results for rooting, callus formation, number of roots, root length, and root dry and fresh mass are obtained for the cuttings collected during summer, with the application of 8,000 mg L^{-1} IBA on the base of each cutting under a mist irrigation system.

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