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Rooting of Eugenia uniflora cuttings: substrate, seasonality, auxine and reinvigoration methods

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Abstract

Eugenia uniflora is a species that presents great genetic variability, due to its main form of propagation, the seminal, which can make homogeneous crops unfeasible for certain desirable characteristics. Therefore, the objective of this work was to develop a protocol for vegetative propagation of *Eugenia uniflora* by stem cuttings. Substrates (commercial substrate, vermiculite and sand), indolbutyric acid concentrations, reinvigoration methods (drastic pruning and serial mini-cutting) and harvesting times (winter, summer, autumn and winter) of the stems were compared. The highest rooting percentage and quality of the root system in cuttings were obtained when commercial substrate and with some reinvigoration method were used (20%). The mini-cuttings technique was more efficient, reaching almost 50% of rooting when collected in summer. Stem cuttings from plants that did not undergo any kind of reinvigoration, regardless of the time of collection, did not result in root formation. No anatomical changes were observed between cuttings from reinvigorated and non-reinvigorated plant material, indicating that the rhizogenic process did not occur in the cuttings originated from plants without reinvigoration because of biochemical and/or physiological reasons. The treatment of the stem cuttings with IBA did not result in increase of rooting, therefore the use of this plant regulator is not recommended.

Keywords: anatomy, brazilian cherry, rooting cofactors, vegetative propagation

Introduction

Eugenia uniflora (Brazilian cherry) is a species with great economical potential (Chaves et al., 2013). Apart from its fruitful potential, in recent years, it has aroused great interest, mostly because of its fruits and leaves, secondary metabolites with antioxidant, antibacterial, antifungal, insecticide, and anthelmintic actions (Moura et al., 2018), which boost its use, mainly by the pharmaceutical and cosmetic industry.

The Brazilian cherry presents great variability in the composition of bioactives, due to its main form of propagation, the seminal. The sexual propagation, or by seeds, promotes segregation of the plant characteristics (Moura et al., 2007), which makes it difficult to establish homogeneous commercial orchards. The asexual propagation techniques constitute an alternative to promote the uniformity of the physical-chemical characteristics of the species.

The conventional cutting and its derived techniques, such as the mini-cuttings technique, are the most used, due to low cost, apart from promoting large quantities of good-quality plants in lesser time. In these methodologies segments from the source plant are detached and then stimulated to form adventitious roots (Hartmann et al., 2011). However, the formation of roots from cuttings is influenced by several external and internal factors, as well as their interactions (Lattuada et al., 2011): substrate, plant growth regulators, variations in the environmental conditions and phenological stage/ stadion.

Another determining factor in the cutting method is the age of the source plant. Mature source plants generally present greater difficulties in rooting of cuttings. According to Dias et al. (2012), the physiological, biochemical and anatomical characteristics of these source plants present themselves in a differentiated

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manner from young source plants.

One way to minimize the effect of these characteristics present in mature plants is to refer to physiological age, a process called reinvigoration. In reinvigorating, some techniques can be used, including drastic hard pruning and mini-cuttings technique (Peña et al., 2015a; 2015b).

E. uniflora, according to Franzon et al. (2010), as in the vast majority of the Myrtaceae family species, the percentages of rooting through the cuttings method are not satisfactory. Therefore, the objective of this study is to answer some questions: Is there influence from the substrate type in the rooting of the Brazilian cherry tree cuttings? What is the best concentration of IBA in the rhizogenic process of the species? Does the rooting of the *E. uniflora* cuttings respond to the time of the material collection? Do reinvigoration methods promote greater percentages of rooting? Should the influence from the reinvigoration methods in the formation of roots occur, anatomically, do the reinvigorated and non-reinvigorated materials present differences?

Material and Methods

The material used in experiments 1, 2 and 3 was collected in the area of medicinal plants at the Experimental Station of Canguiri from the Federal University of Parana, in the municipality of Pinhais – PR, from Brazilian cherry trees (*Eugenia uniflora*) aged between 10 and 12 years according to the year of collection (2016, 2017 and 2018). In experiment 4 the material was collected in July 2018 and January 2019 from a clonal garden composed of plants produced via conventional cutting, originating from experiment 1.

The cuttings/mini-cuttings were made designed with the length of 8 cm and 5 cm, respectively, keeping two leaves in half at the apex. A bevel cut was performed at the base of the cutting and a perpendicular one at the apical portion.

The cuttings/mini-cuttings were immersed in hydroalcoholic solutions (50%) of indolebutyric acid (IBA) for 10 seconds.

In experiments 2, 3 and 4, the cuttings/mini-cuttings were planted in propylene tubes of 53 cm^3 containing commercial substrate.

In all experiments the cuttings were kept in greenhouse for 120 dayswith intermittent irrigation (1 minute every 15 minutes), average temperature 25 ± 2°C and relative humidity equal to 85%.

The percentage of rooted cuttings/mini-cuttings was evaluated, as well as the number of roots per cutting/ mini-cutting, length of roots per cutting/mini-cutting (length of the three longest roots per cutting/mini-cutting), percentage of cuttings/mini-cuttings with calli (alive, rootless, with a build-up of indifferentiated cells mass at the basal region), percentage of living cuttings/ mini-cuttings (without root and/or callus formation), percentage of dead cuttings/mini-cuttings, percentage of cuttings/mini-cuttings with leaf retention (kept the initial leaves) and the percentage of cuttings/mini-cuttings with sprouts.

The results of all experiments were subjected to the analysis of variance, the averages being compared by the Tukey test (P>0.05) with the aid of the statistical programme ASSISTAT version 7.7 (Silva & Azevedo, 2016).

Experiment 1: Evaluation of substrate type in rooting of E. uniflora semi-hardwood cuttings.

The semi-hardwood cuttings were prepared from sproutings originating from drastic pruning at 1.20 m of source plants, carried out in September 2016, with the collection of cuttings in December 2016.

The experiment was implanted in completely casualized outline, in factorial arrangement (3 x 3), with four repetitions containing 10 cuttings per experimental unit. The treatments consisted of three concentrations of IBA (0, 1000 and 2000 mg L^{-1}) and three substrate types (commercial substrate, medium vermiculite and sand), totalling 9 treatments.

Experiment 2: Evaluation of the seasonality effect on the rooting of E. uniflora semi-hardwood cuttings.

The cuttings were collected from annual sprouting of source plants selected between the months of March and November 2018.

The experiment was implanted in a completely casualized outline, in factorial arrangement (5 x 4), with four repetitions containing 15 cuttings per experimental unit. The treatments consisted of five concentrations of IBA (0, 500, 1000, 2000, and 3000 mg L^{-1}) in four collection times (summer, autumn, winter and spring 2018), totalling 20 experiments.

Experiment 3: Evaluation of the drastic pruning effect on the rooting of E. uniflora herbaceous cuttings.

In this experiment Brazilian cherry trees were pruned at approximately 30 cm from the ground in September 2018 and the collection of sproutings, for the design of the cuttings, carried out in January 2019.

The experiment was implanted in a completely casualized outline, with four repetitions containing 15 cuttings per experimental unit. The treatments consisted of five concentrations of IBA (0, 500, 1000, 2000 and 3000

mg L^{-1}).

Experiment 4: Evaluation of the rooting of E. uniflora mini-cuttings in different collection times

The seedlings, originating from experiment 1, were planted in vases of 3.5 L in August 2017 and kept under direct sun with irrigation at an interval of four times a day, for 6 minutes, and fortnightly fertirrigation (50 mL of nutritious solution composed of 4 g L^{-1} of ammonium sulphate, triple superphosphate and potassium chloride and 1 g L^{-1} of FTE BR-12).

The experiment was implanted in a completely casualized outline, in factorial arrangement (2 x 5), with four repetitions containing 15 mini-cuttings per experimental unit. The treatments consisted of two collection times of formed mini-cuttings (winter 2018 and summer 2019) and five concentrations of IBA (0, 500, 1000, 2000 and 3000 mg L^{-1}).

Post-experiment anatomical analyses of cuttings/mini-cuttings

After the evaluation of experiments 2, 3 and 4, three samples of approximately 2 cm of the rooted propagules basal region, with calli and alive only were collected. The samples were fixed in FAA 50 for 24 hours at room temperature (Johansen, 1940). The samples were then gradually dehydrated in graded ethanol series, infiltrated and included in hydroxyethylmethacrylate (Technovit®) according to the manufacturer's introductions. Transverse sections (12 µm) were obtained on a rotary microtome (Olympus® CUT 4055). Photographs were generated using a digital camera (Sony Cybershot P72) coupled with a light microscope (Zeiss).

Results and Discussion

In experiment 1 there was no interaction between the substrate type and the IBA concentrations (Table 1). The variables cuttings with sprouts and cuttings with calli were not analysed statistically because their data was not homogeneous.

The only variable in which the effect of the IBA concentrations was observed was the alive cuttings. With the rise in the IBA concentrations, a smaller survival tendency of the cuttings was observed. For the factor substrate type, it is noted that there is a better quality of radicial systems of cuttings subjected to the commercial substrate, when comparing the values of root length. Sand was the substrate type with the lowest averages.

The influence of substrate type in the cuttings rhizogenic process is directly associated to its porousness, which influences in the aeration and the water retention (Hartmann et al., 2011), being vermiculite and commercial substrate recommended to many species. Sand presents difficulty to cuttings rooting mostly due to its disuniformity in water retention and distribution (Lima & Ohashi, 2016). Pio et al. (2005) analysing physical characteristics of different substrate types, observed that commercial substrate and vermiculite present similar porousness (35.17 and 32.70%), indicating that this aspect was not responsible for the difference observed between the two substrates.

Table 1. Rooted semi-hardwood cuttings, number and length of roots per cutting and alive cuttings of *E. uniflora* due to different substrate types and IBA concentrations (April/ 2017).

Substrate	IBA (mg L^{-1})			Average
	0	1000	2000	
Rooted Cuttings (%)^{NS}				
commercial	20.00	12.00	16.00	16.00a
Vermiculite	10.00	4.00	6.00	6.67a
Sand	6.00	6.00	6.00	6.00a
Average	12.00a	7.33a	9.33a	
Number of Roots per Cutting^{NS}				
commercial	1.42	0.27	0.85	0.85a
Vermiculite	0.60	0.20	0.60	0.47a
Sand	0.90	0.60	0.27	0.59a
Average	0.97a	0.36a	0.57a	
Lenght of Roots per Cutting (cm)				
commercial	6.68	1.53	5.15	4.45a
Vermiculite	1.20	0.65	0.65	0.83b
Sand	0.43	0.95	0.33	0.57b
Average	2.77a	1.04a	2.04a	
Alive Cuttings (%)				
commercial	18.00	12.00	6.00	12.00a
Vermiculite	38.00	18.00	12.00	22.67a
Sand	14.00	26.00	16.00	18.67a
Average	23.33a	18.67ab	11.33b	

Averages followed the same letter do not differ statistically at 5% of probability by the Tukey test. NS = non-significant; CV = coefficient of variation.

The presence and content of essential nutrients are important when choosing the substrate. Pereira & Peres (2016) and Almeida et al. (2017) highlight the influence of nutrition on the survival and rooting of cuttings. According to the authors, macro and micronutrients are essential to the biosynthesis of macromolecules and to other important physiological processes in the rhizogenic process and the quality of the radicial system. The commercial substrate presents macro and micronutrients of slow release and peat, differently from vermiculite which is considered an inert substrate. The period between the implantation and the evaluation of the experiments, 120 days, may have exhausted the reserves present in the cuttings influencing in a lower rooting percentage. Therefore, the presence of nutrients and organic matter in the commercial substrate may have promoted better results in the quality of the radicial system formed.

In experiment 2, seasonality effect and the IBA concentrations in the rootings of Brazilian cherry tree cuttings (Table 2), there was not also interaction between the factors in the evaluated characteristics. At no collection time, rooting in the cuttings was observed. However, the formation of calli in greater quantity in summer and in winter was observed. Generally, in species with difficult rooting, the formation of calli precedes the appearance of roots (Bernardes Jr et al., 2017), indicating that a longer permanence in the greenhouse could bring about the cuttings rooting. The difference in percentage of calli observed within the seasons of winter, summer and autumn may be associated with a greater formation of sproutings in this last season. The inclination of a cuttings reserve towards the formation of sprouts may negatively influence the formation of calli and/or roots that depend on these reserves for the cellular divisions to occur and then develop (Lima et al., 2018).

Table 2. Semi-harwood *E. uniflora* cuttings with calli, with sproutings, with leaves, alive or dead, due to different collection times and IBA concentrations.

Season	IBA (mg L^{-1})					Average
	0	500	1000	2000	3000	
	Cuttings with Calli (%)					
Summer/ 2018	21.67	13.34	6.67	6.67	8.34	11.33ab
Autumn/ 2018	6.67	6.67	5.00	3.33	3.34	5.00b
Winter/ 2018	16.82	19.32	4.55	17.50	12.50	14.14a
Spring/ 2018*	0.00	0.00	0.00	0.00	0.00	0.00
Average	11.29a	9.83a	4.06a	6.88a	6.05a	
	Cuttings with Sproutings (%)					
Summer/ 2018	8.33	8.33	5.00	6.67	1.67	6.00b
Autumn/ 2018	31.67	21.67	23.34	16.67	23.33	23.34a
Winter/ 2018	4.77	7.50	9.09	10.00	10.00	8.27b
Spring/ 2018*	1.67	3.34	1.67	1.67	1.67	2.00b
Average	11.61a	10.21a	9.78a	8.75a	9.17a	
	Cuttings with Leaves (%)					
Summer/ 2018	26.67	23.34	21.67	18.33	13.33	20.67b
Autumn/ 2018	26.67	41.67	35.00	36.67	25.00	33.00a
Winter/ 2018	9.55	5.00	4.54	20.00	7.50	9.32c
Spring/ 2018*	0.00	0.00	0.00	0.00	0.00	0.00
Average	15.72a	17.50a	15.30a	18.75a	11.46a	
	Alive Cuttings (%)					
Summer/ 2018	55.00	71.67	65.00	60.00	38.33	58.00a
Autumn/ 2018	65.00	60.00	68.34	80.00	61.67	67.00a
Winter/ 2018	28.86	38.86	53.18	35.00	27.50	36.68b
Spring/ 2018*	3.33	3.34	1.67	3.33	3.34	3.00c
Average	38.05a	43.47a	47.05a	44.58a	32.71a	
	Dead Cuttings (%)					
Summer/ 2018	23.34	15.00	28.34	33.33	53.33	30.67c
Autumn/ 2018	28.33	33.33	26.67	16.67	35.00	28.00c
Winter/ 2018	54.32	41.82	42.27	55.00	60.00	50.6.8b
Spring/ 2018*	96.67	96.67	98.33	96.67	96.67	97.00a
Average	50.67a	46.71a	48.90a	50.42a	61.25a	

Averages followed by the same letters do not differ statistically by the Tukey test at 5% of probability. CV = coefficient of variation; * = data non-used by ANOVA

Other variables influenced by seasonality were the permanence of leaves, percentage of living and dead cuttings after 120 days in the greenhouse. The highest averages were observed in cuttings collected in autumn and the lowest collected in spring. The variables cuttings with calli, with sproutings, and with leaves from

the material collected in spring, were not included in the statistical analyses for they present variance equal to nought.

As for the influence of different IBA concentrations, significant differences were not observed in any of the evaluated variables.

In cuttings collected in spring a high rate of mortality was observed (97.00%) (Table 2). At the time of the material collection, November 2018, high temperatures and low precipitation were registered. According to Ventura et al. (2019), plants subjected to thermal and hydrological stresses suffer biochemical, physiological, and metabolic alterations. Plants subjected to these sorts of stresses may present reduction in the photosynthetic rate and increase in the respiration (Taiz & Zeiger, 2017). The unbalance between these two physiological processes may trigger the consumption of reserves from the source plants and consequently influence in the lesser survival of the cuttings.

The lack of cuttings rooting can be related to the ontogenetic age of the source plant. The cuttings used in this experiment were originated from annual sproutings of source plants at approximately 11 years of age. The accumulation of inhibitors and the diminution of rooting cofactors generally are observed with the increase of plant age (Lattuda et al., 2011), allowing to influence negatively in the rooting of cuttings originating from older plants.

In the cuttings originating from sproutings after drastic pruning at 30 cm from the ground (Experiment 3) significant differences were not found as to evaluated variables when subjected to different IBA concentrations used in the experiment (Table 3).

Although non-significant, the variables results of rooted cuttings (RC), number of roots (NR) and root length (RL) indicate that drastic pruning stimulates the production of adventitious roots and a good formation of the radical system when compared to the results presented in Experiment 2 (annual sproutings). These results corroborate with the ones presented by Peña-Peña (2014), when comparing the rhizogenic potential of Brazilian cherry tree cuttings originating from annual sproutings and prunings. According to the author, pruning induces the production of sprouts that present greater chances of rooting.

In Experiment 4, the interaction between the two factors (collection time and IBA concentration) was observed as to the variables of rooted mini-cuttings and alive mini-cuttings. The variables of number of roots and mini-cuttings with calli were not analysed statistically for their variance was equal to nought. In this experiment the highest rooting averages were observed in the summer collection without the application of auxin (Table 4).

When factors are compared individually, no significant difference in variables was found when the mini-cuttings were subjected to different IBA concentrations.

In the summer collection the best values were observed, with the exception of the variable of alive mini-cuttings. This result may have been influenced by higher average of rooted mini-cuttings and with calli, since mini-cuttings presenting roots and/calli are not accounted for in this variable.

Table 3. Rooted herbaceous cuttings (RC), number of roots per cutting (NR) and average root length per cutting (RL) of *E. uniflora* originated from drastic pruning at 30 cm from the ground due to different IBA concentrations (January/ 2019).

Variables	IBA (mg L^{-1})					CV%
	0	500	1000	2000	3000	
RC (%) ^{NS}	11.67	21.67	25.00	30.00	21.67	50.71
NR ^{NS}	1.25	1.13	1.29	1.33	1.15	29.13
RL (cm) ^{NS}	5.05	4.47	4.16	4.33	4.68	53.04

CV= coefficient of variation; NS= non-significant.

Table 4. Rooting, alive propagules, with leaves and dead due to the collection time and the IBA applications in *E. uniflora* mini-cuttings.

Season	IBA (mg L^{-1})					Average
	0	500	1000	2000	3000	
	Rooted Mini-cuttings (%)					
Winter	10.00bA	7.50bA	12.50aA	12.50aA	15.00aA	11.50
Summer	48.33aA	29.17aAB	23.34aAB	10.84aB	21.67aAB	26.67
Average	29.17	18.33	17.92	11.67	18.33	
	CV%= 69.48					
	Alive Mini-cuttings (%)					
Winter	70.00aA	65.00aA	62.50aA	65.00aA	65.00aA	65.50
Summer	41.67bC	45.00bBC	56.67aAB	73.33aA	68.33aAB	59.99
Average	55.83	55.00	59.58	69.17	66.67	
	CV%= 19.99					
	Mini-cuttings with Leaves (%)					
Winter	55.00	52.50	57.50	55.00	52.50	54.50 b
Summer	83.34	75.83	74.99	76.67	79.17	78.00 a
Average	69.17 a	64.17 a	66.25 a	65.83 a	65.83 a	
	CV%= 17.16					
	Dead Mini-cuttings (%)					
Winter	17.50	25.00	25.00	22.50	20.00	22.00 a
Summer	10.00	22.50	13.34	13.33	10.00	13.83 b
Average	13.75 a	23.75 a	19.17 a	17.92 a	15.00 a	
	CV%= 62.47					

Averages followed by the same upper-case letters for line and lower-case for column do not differ at 5% of probability by the Tukey test.

CV = coefficient of variation; NS = non-significant.

In experiments 1, 3 and 4 recommended techniques were adopted to obtain greater vigor of the plants used in these experiments, drastic pruning and mini-cuttings technique. The results obtained in these experiments were numerically superior to those observed in cuttings from Brazilian cherry trees that were not subjected to these treatments (Experiment 2).

Similar results were obtained from other species subjected to reinvigoration methods aiming at the cuttings vegetative propagation. *Vochysia bifalcata (*Vochysiaceae) when subjected to conventional cutting, with the collection of propagules directly from the source plant, did not present rooting (Danner et al., 2010), however, when the species was subjected to reinvigoration methods, such as drastic pruning, 81.0% of rooting was obtained (Rickli et al., 2015). Bitencourt et al. (2009) comparing two types of cuttings (cuttings from annual sproutings and cuttings from re-sproutings) of the Yerba Mate, showed that the material reinvigorated by pruning presented higher percentages of rooting (65.5%) in relation to the one from annual sprouings (8.5%). Wendling et al. (2016) worked with propagules originating from clonal minigardens of *Araucaria angustifolia* (Araucariaceae) and obtained 53.7% of rooting in mini-cuttings, higher values of rooting of cuttings originating from branch sproutings which do not surpass 28.0% of rooting observed by Wendling & Brondani (2015).

Thefore, it's evident that techniques which allow reinvigoration are efficient at the rhizogenic process for species of difficult rooting such as *E. uniflora*. According to Wendling et al. (2014), these techniques allow alterations in these tissues and they favour root formation of cuttings originated from adult material.

The reinvigoration methods induce a hormonal balance alteration, thus stimulating new sproutings. The new sprouts present higher levels of auxins, rooting cofactors and lower growth inhibitors levels, thus enabling a higher percentage of radicial induction (Rickli et al., 2015; Latoh et al., 2018). According to Dias et al. (2015) and Rickli et al. (2015), apart from physiological changes that favour the rooting of cuttings originated from reinvigorated material, anatomical alterations can also be observed, such as reduction in lignifiation and increase in cambial activity.

In all the treatments, the vascular cambium is active: drastic pruning (Figures 1A, 1B); mini-cuttings technique (non-documented) and annual sproutings (Figures 3A, 3B). The vascular cambium activity, according to Medrado et al. (1995), favours the proliferation of parenchymatic cells, which present high disdefferentiation level, enabling calli and roots formation. However, despite the cambium activity, the rooting was nought in the cuttings originated from annual sproutings collected throughout the four seasons and relatively low in the other experiments.

The anatomical analysis also allowed to observe the secondary growth in cuttings, characterized by the presence of secondary xylem and phloem (Figures 1A,

1B, 3A, 3B). The primary phloem lignification was also observed (Figures 1, 2A, 2B). The lignification of the primary phloem, according to Spicer & Groover (2010), leads to its inactivation and is also an evident characteristic in stems which have secondary growth established. The primary phloem lignification forms a continuous ring of pericyclic fibers around the stem.

In the present work, both the propagules originated from plants that were not subjected to reinvigoration methods and those originated from plants that underwent such stimulus, presented this continuous fiber ring around the stem. In literature, it is common to make a direct association between the presence of a pericyclic fiber ring with the absence of adventitious roots formation (Lima et al., 2011). Nevertheless, it's noted that such structure was not capable of stopping the production of roots in reinvigorated plants cuttings (Figures 1C, 2A) indicating that the rhizogenic process did not occur in the cuttings originated from plants without reinvigoration because of biochemical and/or physiological reasons.

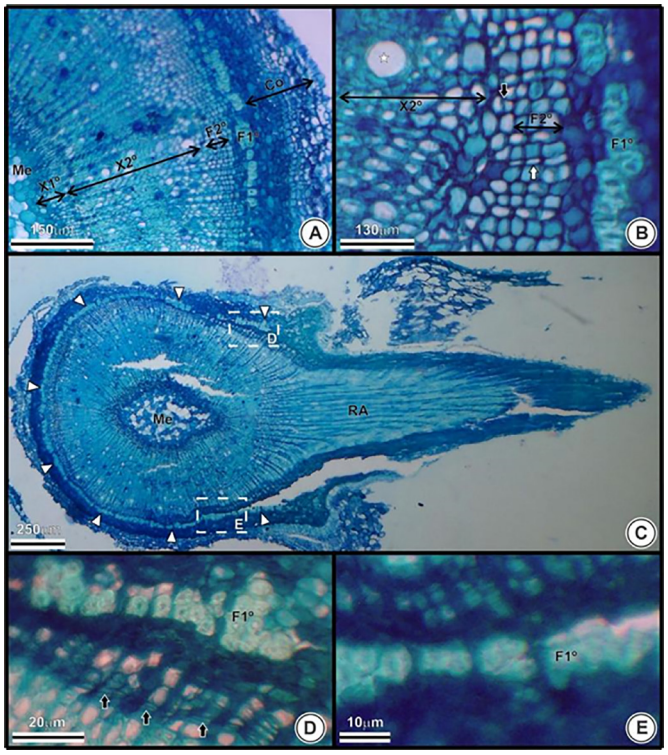

Figure 1. Semi-harwood stem cuttings originated from drastic pruning sproutings (30 cm) of *E. uniflora* (transverse sections). A: secondary growth; B: vascular cambium; C: rooted cutting; D and E: lignified primary phloem. Co = cortex; F1° = primary phloem; F2° = secondary phloem; X2° = secondary xylem; X1° = primary xylem; Me = pith; black arrow = vascular cambium; star = vessel element; white arrow = parenchymatic ray; RA = root; white arrowhead = pericyclic fibres ring.

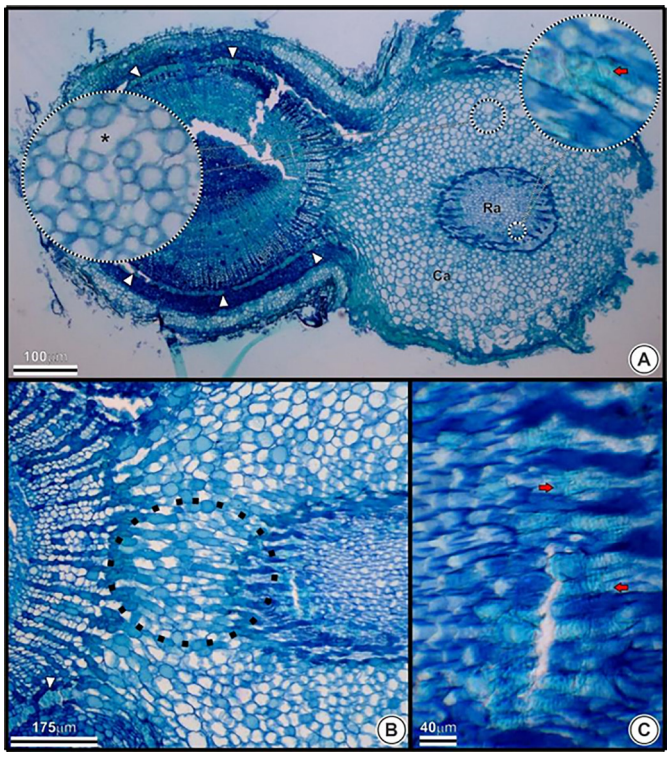

Figure 2. Stem mini-cuttings of *E. uniflora* (transverse section). A: cutting with callus and root, details of callus and root enhanced; B: connection between root and vascular cylinder; C: thickening of cell wall of vessel element cell. (*) = intercellular space; white arrowhead = pericyclic fiber ring; Ca = callus; Ra = root; red arrow = vessel element cell.

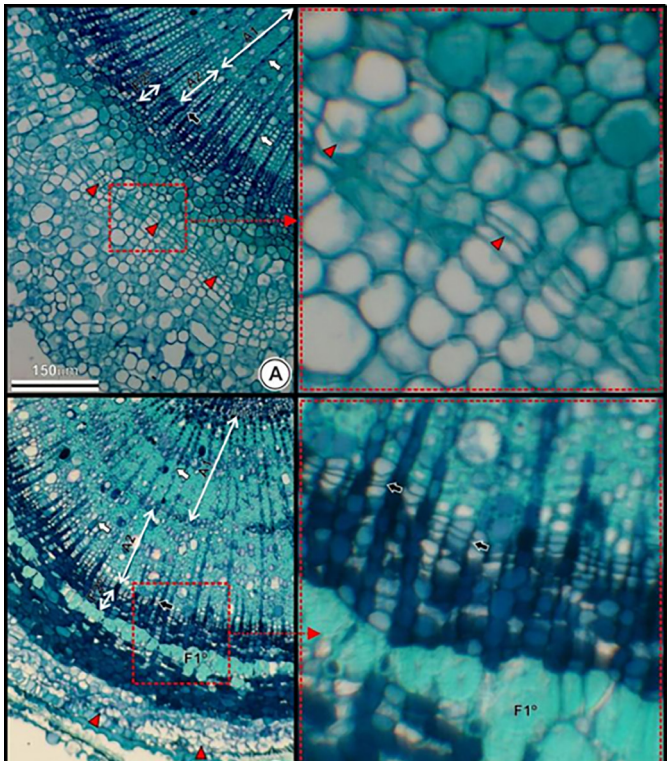

Figure 3. Semi-hardwood stem cuttings originated from annual sproutings of *E. uniflora* (transverse sections). A: phellogen differentiation; B: lignified primary phloem indicating a more advanced stage of the secondary growth. A1 and A2 = secondary xylem, growth rings; white arrow = parenchymatic ray; black arrow = vascular cambium; red arrowhead = phellogen; F1° = primary phloem; F2° = secondary phloem

The rhizogenic process has great influence on the concentration of phyto-hormones, sugars, proteins and phenolic compounds (Ferriani et al., 2010). According to authors, alterations in the quantities of these compounds in different stages of source plants development brings about higher or lower rooting rates of propagules originated in these materials. As reinvigoration methods aim to decrease physiological age, biochemical and physiological alterations are expected, thus augmenting the rooting rate.

Conclusions

Under the conditions this work was carried out, it was possible to conclude that the commercial substrate promotes a better quality of radical system in cuttings of *E. uniflora*. The IBA application is not necessary for the obtainment of the species cuttings/mini-cuttings rooting. The most responsive propagules to the rooting process are obtained from source plants subjected to reinvigoration methods. The anatomical analysis did not highlight anatomical difference between the reinvigorated and non-reinvigorated material. The presence of pericyclic fiber does not hinder the formation of adventitious roots.

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