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Article

'Cerejeira da mata' and 'guabijuzeiro' propagation by air layering

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Abstract

'Guabijuzeiro' and 'cerejeira da mata' are plant species from the Myrtaceae family, with many difficulties in asexual multiplication. Thus, the aim of this study was to evaluate 'cerejeira da mata' (Eugenia involucrata DC.) tree and 'guabijuzeiro' [Myrcianthes pungens (Berg) Legrand] tree propagation by air layering, using different IBA concentrations (0, 1000 , 2000 and 3000 mg L-1) and materials to wrap the substrate (transparent plastic, black plastic and transparent plastic + aluminum foil). The experimental design for both experiments was a randomized blocks, in a 3 x 4 factorial (wrapping material x IBA concentration), with three repetitions of five air layering each. After 180 days, the percentage of rooting, length and number of roots were evaluated. Sixty days after rooting the percentage of survival plants were evaluated. The air layering technique was not efficient in the 'guabijuzeiro' propagation. This technique could be used in 'cerejeira da mata' plants without the IBA application and using transparent plastic, but with low performance.

Keywords: *Myrcianthes pungens* (Berg) Legrand; *Eugenia involucrata* DC.; Asexual propagation.

Introduction

The South of Brazil is characterized by high diversity of native fruit tree species, such as 'guabijuzeiro' (*Myrcianthes pungens*) and 'cerejeira da mata' (*Eugenia involucrata*). This species could be of economic interest due to its sensory and nutraceutical properties, but still little exploited, being an alternative to increase the property income (Hellwig & Ueno, 2009).

The 'guabijuzeiro' [*Myrcianthes pungens* (Berg) Legrand] is a perennial plant, varying from 15 to 25 meters in height, presenting as characteristic the spinescent apex of the leaf, whose flowering occurs between the months of September to January, and fruiting from December to April. The plant occurrence ranges from the States of São Paulo to Rio Grande do Sul in Brazil, being found in all forest formations, except in the Atlantic Forest and Coastal Restinga (Fior et al., 2010).

The 'cerejeira do mato' or 'cerejeira do Rio Grande' (*Eugenia involucrata* DC) presents predominantly the occurrence in the semi-deciduous forest and araucaria forests (Sausen et al., 2009). Fruiting occurs from October to December (Cripa et al., 2014). The fruits of this species are used for both 'in natura' consumption and for processing, obtaining sweets and jellies. In popular medicine it presents compounds to combat diarrhea, rheumatism and digestion,

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through the ingestion of the tea made from its leaves (Golle et al., 2012).

Currently, like most native fruit trees, the 'cerejeira do mato' tree and the 'guabijuzeiro' are basically propagated by seeds, due to the difficulties to form the adventitious roots in vegetative propagation, through the cutting method (Golle et al., 2012; Lattuada et al., 2010). However, these species present some characteristics that negatively influence the implantation of commercial orchards when using seedlings propagated by seeds, such as genetic segregation, plants with a long juvenile period (Souza et al., 2011). It should also be considered the fact of recalcitrance in the seeds (Alegretti et al., 2015).

In this way, vegetative multiplication techniques must be adopted, establishing adequate and efficient protocols, with satisfactory results.

One of the methods that already presented such results, but with another species of the same family and with the same problems of obtaining clones was with the air layering technique in 'jabuticabeira' (Danner et al., 2006; Sasso et al., 2010; Cassol, 2013). This technique is considered to allow a greater chance of root formation due to the rooting process, which occurs with the branch attached to the mother plant, offering all favorable conditions for differentiation and later rhizogenesis.

The formation of roots during the watering process is positively influenced by the greater availability of hormones and the realization of the branch ring, which prevents the carbohydrates, hormones and other substances produced by it from being transported to other parts of the plant, concentrating on the region for differentiation and subsequent rooting. In addition, as xylem is not affected during annealing, being water and mineral elements normally available (Siqueira, 1998), increasing the chances of adventitious root formation.

However, some studies (Danner et al., 2006; Castro & Silveira, 2003) indicate the use of growth hormones as auxins to accelerate the rhizogenesis process, since they present greater efficiency in seedlings obtaining.

Fachinello et al. (2005) recommended, for greater rhizogenesis, higher temperature in roots formation location than the environment, since it can be a stimulus for division and differentiation of the exchange meristems, due to the greater metabolic activity in the cells. In the case of air layering, the substrate heating is more difficult than in cuttings, where they normally use heated water to increase the substrate temperature, being important the test of materials to increase and maintain the substrate temperature that will cover the layering or reduces the exchange with the medium. Thus, the objective of this study was to evaluate the propagation of 'cerejeira da mata' (*Eugenia involucrata* DC.) and 'guabijuzeiro' [*Myrcianthes pungens* (Berg) Legrand] trees by air layering and with different IBA concentrations and materials in the air layering wrap.

Materials and methods

Two experiments were carried out at the Research and Teaching Unity at the Federal Technological University of Parana – Campus Dois Vizinhos, PR, Brazil. The first experiment was carried out with three adult trees of 'cerejeira da mata' (*Eugenia involucrata*), with 10 years and propagated by seeds, located in the arboretum of the institution, being the experiment performed in June 2012, when they were in the vegetative period. The second experiment was performed with three adult plants of 'guabijuzeiro' (*Myrcianthes Pungens*), with 10 years, propagated by seeds and located in a rural property, being the experiment performed in February 2012, at the end of the fruiting stage of the species.

The experimental design of both experiments was a randomized blocks design, in a 3 x 4 factorial scheme (wrapping material x IBA concentration), with three replicates of five air layering points per experimental unit.

To perform the air layering, branches with 6-8mm in diameter were initially selected. During the process, a specific knife was used, making a complete ring with 1.5 cm wide that was removed from each branch to the exchange region. At each end of the site where the ring was withdrawn, 4 drops (0.2 mL) of dilute indolebutyric acid (IBA) solution were placed according to the

concentration of the treatment.

The IBA solutions applied in the exchange region were 0 (control), 1,000, 2,000 and 3,000 mg.L^{-1}. The IBA applied was diluted in alcohol, and after the dilution a distilled water in the same proportion was added, forming a solution with a volume of 1:1 v/v (alcohol + distilled water). Thus, four solutions were prepared, differentiating only in the diluted IBA concentration.

Subsequently to the IBA application, the exposed area was wrapped with commercial substrate and Moistened with Plantmax®, to obtain an oval shape with 5 to 7 cm in diameter of the air layering. For this, the substrate was coated with a transparent plastic, black plastic or transparent plastic + aluminum foil on the outside, constituting the wrappings materials to be tested. After wrapping, the air layerings were tied at the ends with galvanized wire.

In both experiments, the moisture content of each substrate was weekly controlled, with the application of water with the aid of a needle syringe at the end.

After 180 days of the experiments implantation, the rooting percentage (%), length (cm) and average number of roots were evaluated. The length of the adventitious roots was obtained using a millimeter ruler, measuring the three largest roots, and the average number of roots was obtained by counting all the adventitious roots emitted from the air layering. The rooted air layers were detached from the mother plant and planted in 5 L pots containing the same commercial substrate. Vessels with sprinklers were kept in greenhouses with six daily shifts of sprinkler irrigation. After 60 days of transplanting, the survival percentage was evaluated.

The data of the evaluated variables were previously submitted to the Lilliefors Normality test, with transformation for all analyzed variables. The transformations were realized using arcsen $\sqrt{x/100}$ and $\sqrt{x+1}$ for variables with percentages and observed values, respectively. With transformation, data was submitted to variance analysis and to the Duncan's test (a = 0.05) for the qualitative factor and regression for the quantitative, using the Sanest® software.

Results and Discussion

For 'guabijuzeiro', it was not possible to obtain rooting using the air layering method, except for the treatment, using 3,000 mg L^{-1} of IBA (Figure 1), with mean of 3.5%.

Despite the fact that just one treatment presented rooted, this is an indicative of adventitious root formation with the air layering, which was not possible by cutting. However, is possible that the tested IBA concentrations for 'guabijuzeiro' were low, requiring other studies with higher concentrations. This fact was proven by Danner et al. (2006) in 'jabuticabeira', obtaining the best root percentages using the concentration of 4000 mg L^{-1} of IBA, which is a species with difficulties to form adventitious roots and from the same family as the 'guabijuzeiro'.

In addition, the period of the experiment implantation coincided with the 'guabijuzeiro' flowering, which can explain this experiments results, since the air layering rooting is related to

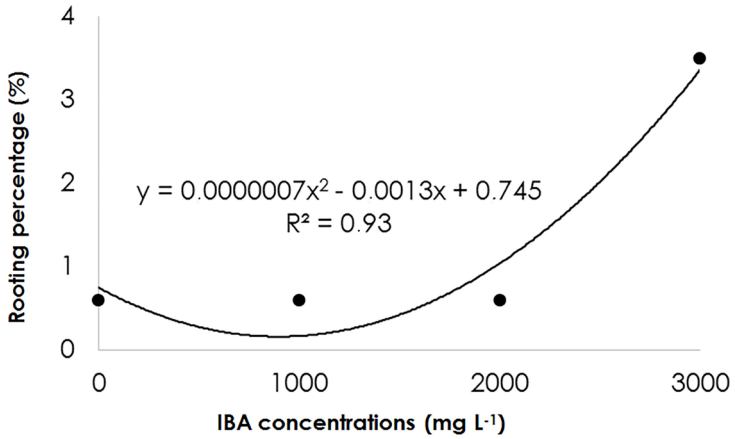

Figure 1. Rooting percentage of 'guabijuzeiro' using air layering according to different IBA concentrations.

the physiological condition of the matrix plant, in which the hormonal balance and the content of reserve substances, especially with regard to the favorable C / N balance.

As the flowering of plants is a factor that affects the hormonal balance and the content of reserve photoassimilates, it can be inferred that the plants at this period presented a low C / N ratio.

The C / N ratio is one of the main factors that stimulated rooting, and it increase directly and proportionally affect the rooting increase (Pizzato et al., 2011; Loss et al., 2009). The C / N ratio can be strongly influenced by the reduction of the nitrogen amount during the storage of reserve substances, which makes this material more ready to the best occurrence of adventitious root formation (Leandro & Yuyama, 2008).

According to Duarte (1991), the rooting variation throughout the year probably occurs due to variations in the content of the cofactors and to the formation and accumulation of rooting inhibitors. Therefore, it is also believed that the fact of non-root formation may be associated with the presence of high levels of rooting inhibitors, such as those derived from ellagic acid, which present a higher endogenous concentration in flowering and are antagonistic to rhizogenesis.

For 'cerejeira da mata' trees it can be verified a significant interaction between IB concentration x wrapping material only for the variable mean number of roots per air layering (Table 1). For the other variables, no significant results were obtained with interaction in each isolated factor.

Table 1. Mean number of roots using air layering in 'cerejeira da mata' trees according to the IBA concentration and to the wrapping material tested.

IBA concentrations (mg.L^{-1})	Wraping materials		
	Transparent	Black	Transparent + Aluminium foil
0	0.3 a AB*	0.0 a B	0.3 a AB
1000	0.0 b B	0.39 ab B	1.22 a A
2000	1.17 a A	0.3 a B	0.43 a AB
3000	0.3 b AB	1.64 a A	0.0 b B
CV (%)		20.54	

*In each line, means followed by the same lowercase letters and in each column, by the same uppercase letter are not statistically different, according to Duncan´s test ($p \leq 0.05$).

In Figure 2, it can be observed that transparent plastic and transparent plastic + aluminum foil with 1833 and 1333 mg L^{-1} of IBA resulted in maximum points, with mean number of roots of 0.89 and 0.94 cm, respectively. The use of black plastic with 500 mg L^{-1} of IBA resulted in a minimum point of 0.04 cm.

According to the results of tests with wrapping materials, the highest number of roots was with the use of transparent plastic with 2000 mg L^{-1} of IBA, not statistically different from the concentrations of 0 and 3000 mg L^{-1}. For the black plastic, the highest average was using 3000 mg L^{-1}, different from the use of transparent plastic + aluminum foil, which presented the highest averages with 1000 mg L^{-1} followed by 0 and 2000 mg L^{-1}. For the IBA concentrations, it was verified that, with the use of 0 and 2000 mg L^{-1} the wrapping materials did not influenced the results. Using 1000 mg L^{-1}, the highest results were with black plastic and transparent plastic + aluminum foil. With 3000 mg L^{-1} the highest results were using black plastic (Table 1).

These differences among IBA concentration and the wrapping material, observed in the present study can be directly related to different responses to exogenous auxin with the substrate temperature, since it can vary according to the material used. Thus, it is proposed that the materials black plastic and transparent plastic + aluminum foil achieved the highest means due to the highest accumulation or conservation of temperature, which favors the faster cellular division and consequent differentiation.

The rooting percentage, mean root length and survival percentage presented means of 10.56%; 0.96 and 1.86%, respectively.

The lower rooting percentage was also observed by Oliveira et al. (2008) in avocado trees

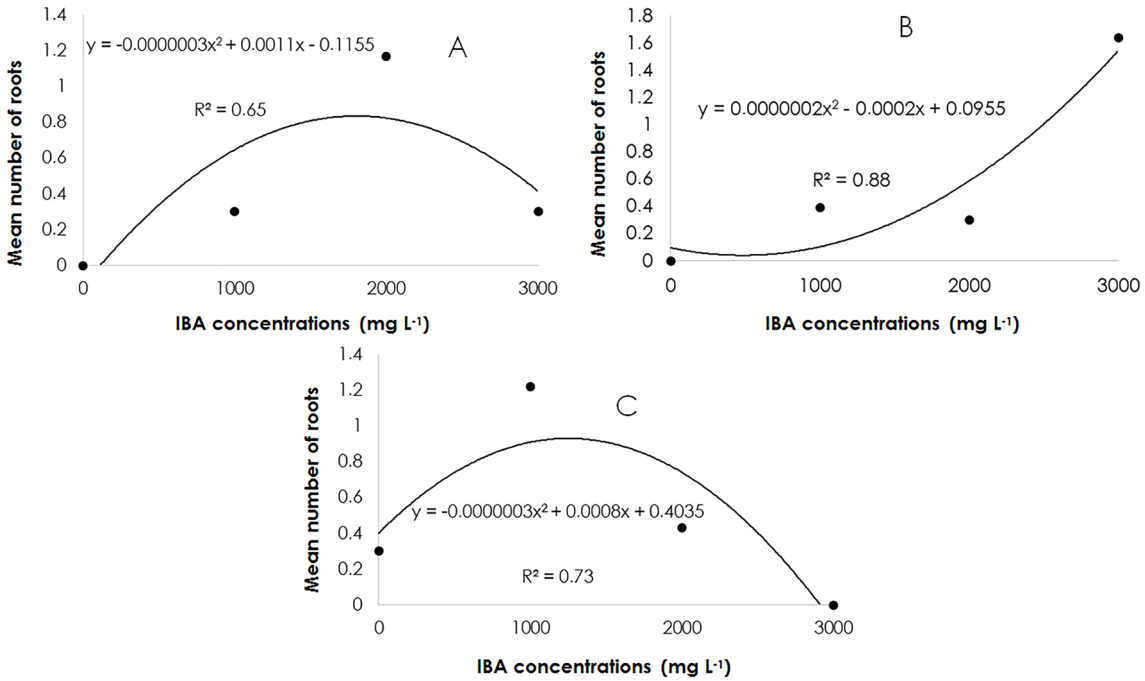

Figure 2. Mean number of roots of 'cerejeita da mata' submitted to air layering according to IBA concentration and the wrapping materials: transparent plastic (A), black plastic (B) and transparent plastic + aluminum foil (C).

(*Persea americana* Mill.) using IBA concentrations of (0, 1000, 3000 and 5000 mg L^{-1}) in young and adult plants.

It was observed that only 1.86% of the roots from 'cerejeira da mata' survived to the transplanting, which may be related to the small number of roots formed (<2.0). Therefore, it is advisable to evaluate the watering process in future studies with a longer time than 180 days, since it is believed that it is not enough to allow the formation of adequate number of roots and in other periods.

In general, the 'cerejeira da mata' tree, despite the low rooting, was the one that presented greater capacity of adventitious rhizogenesis when compared to 'guabijuzeiro'. However, for both fruit trees it is necessary to test other means that can stimulate the formation of roots with air layering.

For 'guabijuzeiro', the air layering is not an efficient technique, but for the 'cerejeira da mata' tree, it is possible to use the technique without the IBA application and with transparent plastic, but with low performance. The use of higher IBA concentrations for the 'cerejeira da mata' tree is recommended for future studies aiming to increase the rhizogenesis.

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