

NOTAS

***Ficus enormis* cuttings rooting:  
concentrations of indole butyric acid and seasonal variations**

Enraizamiento de esquejes de *Ficus enormis*:  
concentraciones de ácido indol butírico y variaciones estacionales

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SUMMARY

Plants producing fruits for many months of the year, or at times when few species bear fruits, are highly valuable for restoration of degraded ecosystems due to their ability to attract and support dispersing fauna. An example of this are native fig trees such as *Ficus enormis*. Thus, in view of the difficulties for *F. enormis* seedlings production, we aimed at developing a method of cuttings propagation by evaluating the rooting and root vigor of cuttings from a field clonal garden. The experiment was established between June/2015 and May/2016 at Laboratory of Forest Species Propagation of Embrapa Florestas, in Colombo-PR. We evaluated collection of cuttings during three seasons (winter, spring and summer) and application of concentrations of indole-3-butyric acid - IBA (0, 1000, 2000, 3000, 4000, 5000 mg L<sup>-1</sup>). After 45 days of cuttings establishment in an acclimatized greenhouse, the best results for rooting, number of roots, average length of the three largest roots per cutting and maintenance of leaves and shoots were found in spring and summer. Using plant regulator did not influence the rooting or root vigor of propagules; the time of the year was the predominant factor to induce rooting. The results show that the cuttings technique with propagules from a clonal garden is efficient for producing *F. enormis* plants and can serve as a basis for the species propagation.

*Key words:* epicormic shoots, native species of Brazil, plant propagation, root vigor, vegetative propagation.

RESUMEN

Las plantas que producen frutos durante muchos meses del año, o en momentos en que pocas especies dan frutos, son de gran valor para la restauración de ecosistemas degradados debido a su capacidad para atraer y apoyar la fauna dispersora. Un ejemplo de esto son las higueras nativas como *Ficus enormis*. Por las dificultades para la producción de plántulas de *F. enormis*, fue evaluada la propagación de esquejes mediante enraizamiento y vigor de sus raíces de un jardín clonal de campo. El experimento se estableció entre junio/2015 y mayo/2016 en el Laboratorio de Propagación de Especies Forestales de Embrapa Florestas, en Colombo-PR. Se evaluó la recolección de esquejes durante tres estaciones (invierno, primavera y verano) y la aplicación de concentraciones de ácido indol-3-butírico - IBA (0, 1000, 2000, 3000, 4000, 5000 mg L<sup>-1</sup>). Después de 45 días de establecimiento de esquejes en un invernadero climatizado, los mejores resultados para el enraizamiento, el número de raíces, la longitud promedio de las tres raíces más grandes por corte, el mantenimiento de las hojas y los brotes se encontraron en primavera y verano. El uso del regulador de la planta no influyó en el enraizamiento y el vigor de las raíces de los propágulos, y la época del año fue el factor predominante para inducir el enraizamiento. Los resultados muestran que la técnica de esquejes con propágulos de un jardín clonal es eficiente para producir plantas de *F. enormis* y puede servir como base para la propagación de esta especie.

*Palabras clave:* brotes epicórmicos, especies nativas de Brasil, propagación de plantas, vigor de raíz, propagación vegetativa.

INTRODUCTION

In Brazil, degraded ecosystems have often been restored by planting mixed plots of tree species and by phy-

sically protecting the area (Rodrigues *et al.* 2009). When well designed and executed, mixed plots can help restore such areas. In this case, species are chosen to favor natural succession (Carpanezzi and Nicodemo 2009). In ge-

neral, plants that produce fruits for many months of the year, or in times when few species bear fruits, are highly valuable. Thus, an important step to increase the chances of successful restoration of degraded areas is choosing the species to be used (Volis 2016). However, many important species are not available in nurseries, which is frequently an obstacle. Such absence is due to the lack of information about the collection, processing, storage and germination of seeds, caused by the high heterogeneity and complexity of ecophysiological strategies that native species present (Pilon and Durigan 2013).

One example of this are the native fig trees of the *Ficus* genus, such as *Ficus enormis* Mart. ex Miq., which are considered important components in ecosystem dynamics as they attract dispersing fauna (Carpanezzi and Nicodemo 2009). *Ficus enormis* (family Moraceae) is an early secondary, evergreen species (Marmontel *et al.* 2013) that naturally occurs in the Northeast, Midwest, Southeast and Southern regions of Brazil, and is found in Caatinga, Cerrado and Atlantic Forest Biomes (REFLORA 2019). Adult trees are large, commonly reaching 25 m in height and up to 1.80 m in diameter at breast height (Pelissari and Romaniuc Neto 2013). Despite its importance in restoration, this species is frequently not found in nurseries due to its small sized seeds and the difficulty in processing and handling them.

Due to the difficulties in *F. enormis* seedlings production, rooted cuttings appear as a viable and relatively fast alternative of asexual reproduction, becoming more efficient as propagation protocols are established. Including vegetatively propagated seedlings is justified by the importance of including functional groups in restoration works, and the genetic basis of the species can be supplied by collecting cuttings from many matrices (Carpanezzi and Carpanezzi 2006). The factors responsible for successful rooting cuttings, as substrates, rooting environment, type of propagule and use of plant regulators, vary for each species (Santos *et al.* 2011), and it is essential to obtain viable propagules with good rooting capacity.

The maturation of propagules (ontogenetic ageing) is a determining factor in the adventitious rhizogenesis process. Propagules with a higher degree of juvenility tend to have superior rooting and root vigor (Stuepp *et al.* 2018). One technique used to maintain the juvenility of propagules is continuous pruning of the stock plants aiming at the induction of epicormic shoots (Heide 2018). However, few studies have evaluated the effect of this in native tree species of Brazil. Environmental influences, promoted by the different seasons, can also act on the endogenous levels of auxin of mother plants, influencing the cuttings rooting (Tombesi *et al.* 2015). For some species that have low endogenous concentrations of plant hormones linked to rhizogenesis, the application of synthetic auxins may contribute to increase the amount of free auxins in the propagules, favoring the process of root induction, and thus, the success of vegetative propagation (Pacurar *et al.* 2014). The addition of auxin has been verified in many

species and the necessary concentrations vary from species to species (Santos *et al.* 2011).

Thus, aiming at developing an efficient protocol for *F. enormis* rooted cuttings production, the objective of this study is to evaluate the effect of different concentrations of the synthetic auxin indole-3-butyric acid (IBA) on rooting and root vigor of stem cuttings from epicormic shoots derived from a clonal field garden, during three different seasons.

## METHODS

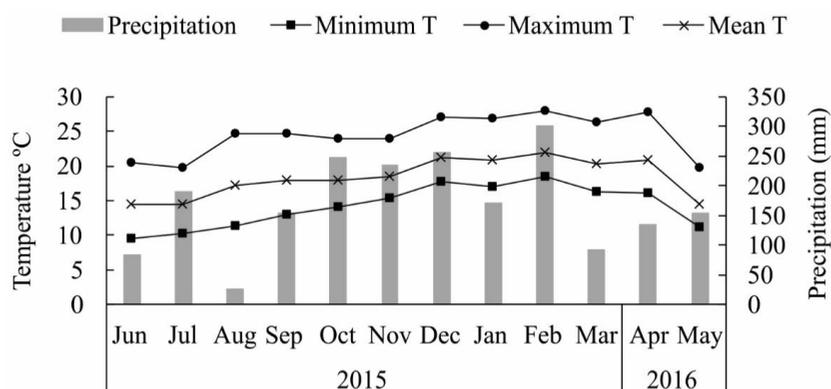
The experiment was established between June/2015 and May/2016 at Laboratory of Forest Species Propagation of Embrapa Florestas, in Colombo-PR (25°20' S and 49°14' W, 950 m). The local climate is Cfb type (Köppen classification), with average annual temperature of 16.6°C and average annual rainfall of 1596 mm, without water deficit and with frosts occurring in autumn and winter (figure 1).

The cuttings were obtained from stock plants established in 2001 in a field clonal garden, with 2 m x 1 m spacing, in the city of Colombo-PR, Brazil. To reinvigorate the stock plants, they were drastically pruned in March/2015, lowering them to an average height of 0.3 m from the soil to induce the emission of epicormic shoots. From this, shoots were collected for cuttings preparation and rooting in winter/2015, spring/2015 and summer/2016. Stem cuttings with 8±1 cm in length and diameter ranging from 0.4 ± 0.2 mm were made, maintaining two leaves in the terminal portion, reduced to 1/3 of their original surface area.

Cuttings received phytosanitary treatment with sodium 0.5 % hypochlorite solution for 10 minutes (bactericidal action) and were washed in running water for 10 minutes. Subsequently, the cuttings base was treated with indole-3-butyric acid (IBA) at concentrations of 0, 1000, 2000, 3000, 4000 and 5000 mg L<sup>-1</sup>, diluted in hydroalcoholic (1:1 v/v) solution for 10 seconds. Rooting was carried out in 110 cm<sup>3</sup> tubes, filled with medium-sized vermiculite and partially carbonized rice husk (1:1, v/v). Afterwards, the cuttings were established in a greenhouse, with intermittent misting and controlled temperature (20 °C to 30 °C) and relative humidity over 90 %.

After forty-five days, we evaluated: percentage of rooted cuttings, number of roots/cutting, length of the three largest roots/cutting (cm), percentage of cuttings with calluses, percentage of cuttings with roots and calluses, percentage of living cuttings that did not present root induction or callus formation, percentage of dead cuttings with necrotic tissues, percentage of cuttings with original leaves (living cuttings that maintained original leaf) and percentage of cuttings with shoots.

The experiment was set in a completely randomized design, with a factorial arrangement of 3 x 6 (3 seasons x 6 IBA concentrations), with four replications. Each replication consisted of 20 cuttings. The homogeneity of variances was verified using the Bartlett test and, when the ANOVA test showed statistical significance ( $P < 0.05$ ), the Tukey



**Figure 1.** Maximum, minimum and average temperatures and accumulated rainfall in Colombo-PR, Brazil region between March/2015 and May/2016 (SIMEPAR/PR).

Temperaturas máximas, mínimas y medias y precipitaciones acumuladas en la región de Colombo-PR, Brasil, entre marzo/2015 y mayo/2016 (SIMEPAR/PR).

test was used to determine significant differences ( $P < 0.05$ ) between individual means. To verify the relationship between percentage of rooted cuttings and the variables leaf maintenance and shoot emission, as well as with average temperatures of the region (figure 1), a Pearson's correlation analysis ( $P < 0.01$  and  $P < 0.05$ ) was performed.

## RESULTS

The ANOVA revealed a significant interaction between season and plant regulator factors for number of roots per cutting and percentage of cuttings with shoots. However, regarding factors individual analyses, seasons significantly affected all variables analyzed. For IBA application, there was a significant difference for number of roots per cutting and percentages of cuttings with calluses and with shoots.

In general, the variables that responded to the interaction between season and plant regulator presented higher values in spring and summer, under the concentrations of  $4000 \text{ mg L}^{-1}$  and  $5000 \text{ mg L}^{-1}$  (table 1).

These results are confirmed by the individual analysis of the factors. These revealed higher rooting percentages, number of roots and average length of the three largest roots per cutting and percentages of leaf maintenance and shoot emission in the warmer seasons (figure 2). On the other hand, we observed the highest percentages of cuttings with calluses, living cuttings without roots or calluses and dead cuttings in winter, however with values below 20 %.

The correlation analysis between average temperatures of each season and rooting percentages showed a positive correlation of 0.95. Cuttings rooting presented positive correlations of 0.70 and 0.60 in relation to leaf maintenance and shoot emission, respectively.

Another important result refers to high callus formation in rooted cuttings, especially in spring and summer

(figure 3). In these seasons, more than 80 % of the rooted cuttings presented callus formation.

## DISCUSSION

Studies indicate that during the hottest periods of the year, many plants experience intense vegetative growth, thus, produce higher amounts of auxins, carbohydrates and rooting cofactors (Salmi and Hesami 2016). High temperatures, along with higher precipitation and radiation, directly interfere in growth and development of apical meristems, which are the main sites for auxin synthesis in plants (Zhao 2018). However, during the winter, when temperatures are lower, woody plants become dormant to protect their meristems by reducing exchange rate activities and increasing concentrations of phenolic compounds and inhibitors, which temporarily ceases their growth (Ding and Nilsson 2016). Thus, the highest concentrations of endogenous auxins, possibly present in the parent plants during spring and summer, and lowest concentration of inhibitors may have been responsible for the production of more vigorous shoots, favoring the highest rooting percentages. In fact, the correlation analysis between average temperatures of each season and rooting percentages showed a positive correlation close to 1.00, which has also been verified in other studies (Fragoso *et al.* 2015). Similarly, the higher number of roots values and average length of the three largest roots per cutting demonstrate the high root vigor of the cuttings collected in these seasons, which may reflect on the stability, survival and development of seedlings in the field (Stuepp *et al.* 2017).

Nevertheless, even the lowest rooting percentages obtained in winter were higher than 60 %, regardless of the IBA application, which is possibly related to the vigor of the used cuttings (Nascimento *et al.* 2018). As already mentioned, materials with a higher degree of juvenili-

**Table 1.** Averages of number of roots per cutting and percentage of cuttings with shoots in *F. enormis* cuttings, installed in three seasons under six concentrations of indole-3-butyric acid (IBA).

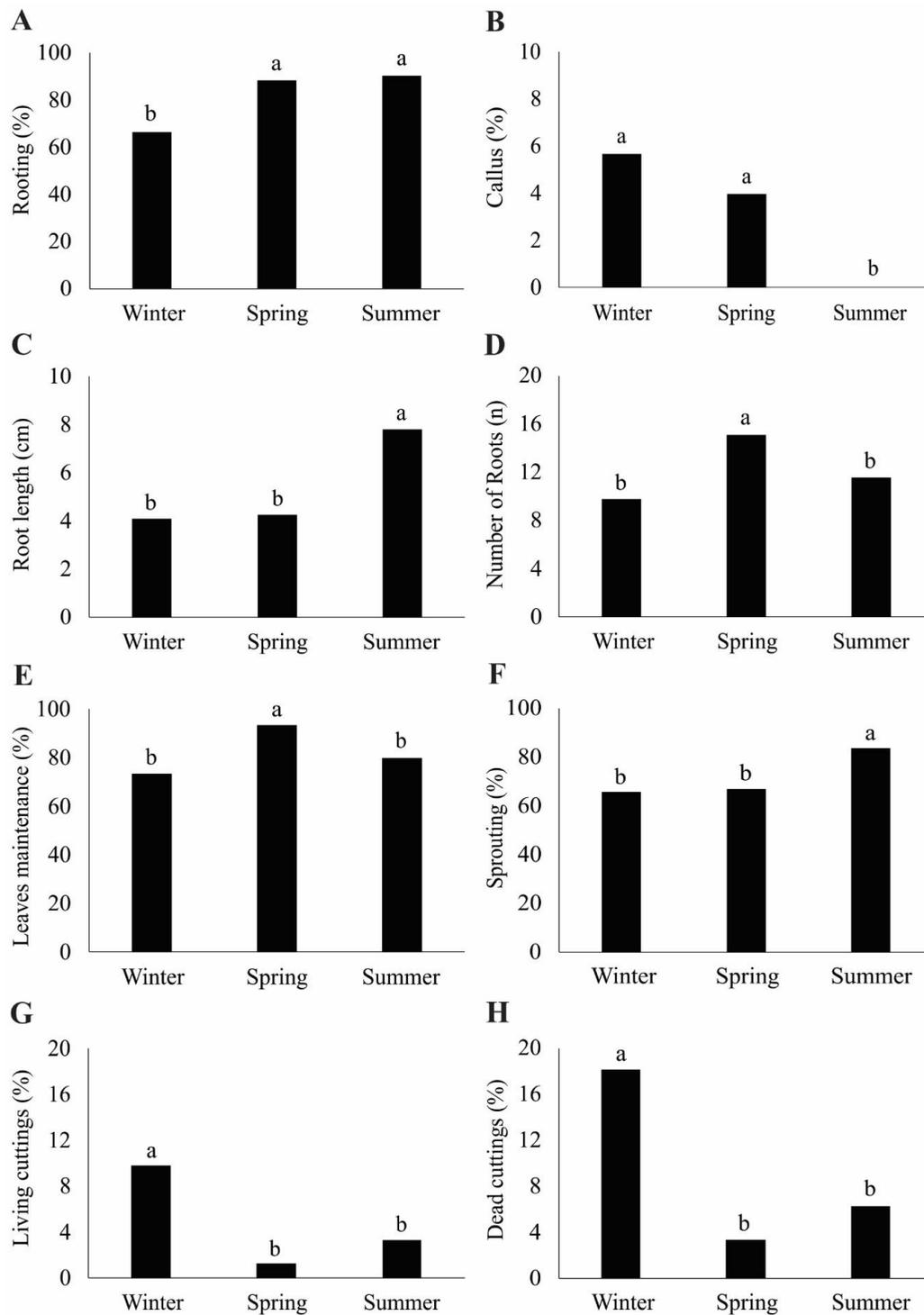
Promedios de número de raíces por esqueje y porcentaje de esquejes con brotes en esquejes de *F. enormis*, instalados en tres estaciones bajo seis concentraciones de ácido indol-3-butírico (IBA).

Number of roots per cutting						
IBA (mg L <sup>-1</sup> )	Winter		Spring		Summer	
0	6.28	a A	10.91	c A	10.43	a A
1000	7.89	a B	12.16	bc AB	13.54	a A
2000	9.04	a A	12.38	bc A	12.14	a A
3000	12.49	a A	13.50	bc A	11.80	a A
4000	11.70	a B	18.22	ab A	11.55	a B
5000	11.30	a B	23.53	a A	10.00	a B
Coefficient of variation = 24.83 %						
Percentage of cuttings with shoots (%)						
IBA (mg L <sup>-1</sup> )	Winter		Spring		Summer	
0	62.50	a AB	45.00	bc B	87.50	a A
1000	60.71	a AB	41.25	c B	77.50	a A
2000	69.64	a A	66.25	abc A	81.25	a A
3000	66.07	a B	92.50	a A	77.50	a A
4000	58.93	a B	83.75	a AB	91.25	a A
5000	76.79	a A	72.50	ab A	87.50	a A
Coefficient of variation = 20.51 %						

Averages followed by the same lower case letter in column and capital letter on the line do not differ among them at  $P < 0.05$  using Tukey test.

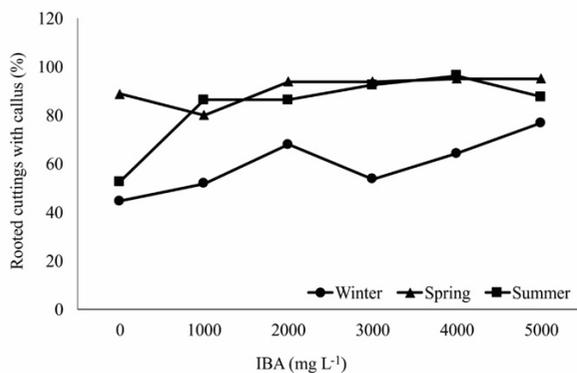
ty tend to have higher concentrations of free auxins and, consequently, superior rooting and root vigor (Stuepp *et al.* 2018). In *F. enormis*, the high rooting percentages, regardless of the IBA application, suggest that the presence of auxins was not a limiting factor. The high concentration of free auxins in propagules with a higher degree of juvenility may be related to the reduced activity of peroxidase enzymes in these materials, which would be responsible for the degradation of endogenous auxins (Wendling *et al.* 2014). Studies also suggest that different auxin transport mechanisms may be involved in the larger availability of this hormone in juvenile propagules, favoring the high rooting percentages of these materials compared to propagules with a higher degree of maturity (Nakhooda *et al.* 2011, Stuepp *et al.* 2017). In the present study, the high vigor of the material used also demonstrates the efficiency of continuous pruning of *F. enormis* stock plants established in a field clonal garden to provide propagules with a higher degree of juvenility and good aptitude for adventitious rooting. Nonetheless, in the Mixed Ombrophilous Forest, frosts can affect *F. enormis* shoots in a clonal garden. Therefore, it is often not possible to obtain cuttings in the coldest autumn-winter period, usually from May 15 to the end of August.

Similarly, the variables leaf maintenance and shoot emission were high (above 60 %) during all three seasons, although, significantly higher values were obtained in spring and summer when compared to winter (figure 2). As observed in other species (Fragoso *et al.* 2015, Tombesi *et al.* 2015), these results show a positive relationship between the variables leaf maintenance and cutting rooting, which presented a 70 % correlation. This relationship is, possibly, due to the supply of carbohydrates and hormones present in leaves, demonstrating the importance of maintaining them as essential metabolites for the rooting process (Tombesi *et al.* 2015). Similarly, regarding the emission of shoots, the positive correlation with rooting suggests that the process of shoot formation was not harmful to root system induction in *F. enormis* during any of the evaluated seasons. In some species, the emission of shoots prior to rooting can lead to the depletion of carbohydrates and auxins present in the propagules that would be required in the rhizogenesis process, making it difficult to form adventitious roots (Hartmann *et al.* 2011). In *F. enormis*, however, the rooting of the cuttings started very early, about 2-3 weeks after installation, favoring the formation of roots prior to the shoots emission.



**Figure 2.** Averages of rooting (A), callus formation (B), average length of the three largest roots per cutting (C), number of roots per cutting (D), leaf maintenance (E), shoot emission (F), living cuttings without roots or calluses (G) and dead cuttings (H) in *F. enormis* cuttings installed in three seasons.

Promedios de enraizamiento (A), formación de calos (B), longitud promedio de las tres raíces más grandes por esqueje (C), número de raíces por esqueje (D), mantenimiento de hojas (E), emisión de brotes (F), esquejes vivos sin raíces o callosidades (G) y esquejes muertos (H) en esquejes de *F. enormis* instalados en tres estaciones.



**Figure 3.** Percentage of cuttings with roots and calluses for *F. enormis*, installed in three seasons and under six concentrations of indole-3-butyric acid (IBA).

Porcentaje de esquejes con raíces y callosidades para *F. enormis*, instalados en tres estaciones y bajo seis concentraciones de ácido indol-3-butírico (IBA).

The analysis of callus formation revealed values below 6% in all seasons. However, the high presence of calluses in rooted cuttings, especially in spring and summer (> 80%), indicates possible indirect root formation (Rasmussen and Hunt 2010). The presence of calluses is undesirable for plant propagation, as their presence in the vascular connection region may limit the appropriate rhizogenesis process, compromising root functionality (Monder *et al.* 2017). However, this relationship varies depending on the species. The indirect formation of the root system is common during the regeneration of many plant species and has been considered a consequence of the maturation of vegetative propagules (Wendling *et al.* 2014). For *F. enormis*, however, more in-depth studies are needed to understand these changes and their consequences on the vegetative propagation of the species.

The lowest percentages of living cuttings without roots or calluses and dead cuttings in spring and summer are related to the highest percentages of cuttings rooted in these seasons. These results show the efficiency of the cuttings technique for vegetative propagation of *F. enormis* from epicormic shoots and the importance of using suitable propagules for rooting, particularly regarding the vigor and juvenility of the stock plants. It is important to highlight that there was no nutritional intervention in the stock plants in the field. The adequate supply of macro and micronutrients can favor the increase of the vegetative vigor of the stock plants and provide propagules with better morphophysiological and anatomical qualities (Zerche and Druge 2009). Non-nutritional replacement under long periods of management, however, can lead to the exhaustion of the stock plants, making their ability to supply propagules unfeasible.

The results found here consolidate the best options regarding the time of the year and support that IBA is not ne-

cessary for rooting of *F. enormis* cuttings maintained in a field clonal garden. Along with results about other aspects, such as substrates, phases and environments (Fragoso *et al.* 2016), a guide for the daily production of *F. enormis* cuttings could be established in commercial nurseries.

## CONCLUSIONS

Under the conditions in which the present work was carried out, it is possible to conclude that the vegetative propagation of *F. enormis* from epicormic shoots from clonal field garden is a viable method for this species seedlings production, without any plant regulator needed.

The use of plant regulator has no influence on rooting and root vigor of cuttings, being the time of the year the preponderant factor for root induction in cuttings of *F. enormis*.

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