ISSN 0104-7760
v.25 n.1 2019

CERNE

Bruno Nascimento[1a+], Alexandra Cristina Schatz Sá[1b], Gabriel Souza[1], Mariane de Oliveira Pereira[1], Marcio Carlos Navroski[1e]

NITROGENATED FERTILIZATION FAVORS VEGETATIVE RESCUE AND PROPAGATION OF *Ilex paraguariensis*

NASCIMENTO, B.; SÁ, A. C. S..; SOUZA, G.; PEREIRA, M. O; NAVROSKI, M. C. Nitrogenated fertilization favors vegetative rescue and propagation of *Ilex paraguariensis*. **CERNE**, v. 25, n. 1, p. 76-83, 2019.

Keywords:
Complete girdling
Cuttings
Fertilization
Mate tree
NPK

HIGHLIGHTS

250 g of N or 500 g of NPK rapidly increase epicormic shoots growth in I. paraguariensis.

250 g of NPK have better results for epicormic shoots growth in long therm.

The longer the epicormic shoots stays in the mother tree, the better for cutting.

I. paraguariensis cuttings are very sensitive to the loss of moisture

Historic:
Received 07/1/2018
Accepted 16/02/2019

ABSTRACT

The vegetative growth of plants depends on the nutritional conditions of the soil, mainly in relation to the N element. This element favours the formation of cytokines and auxins, essential hormones for sprouting and rooting, respectively. The objective of this study was to evaluate the influence of nitrogen fertilization of *Ilex paraguariensis* mother trees on epicormic sprouts emission and rooting of its cuttings. Five treatments of fertilization were applied on the mother trees: control; 250 g of urea (N); 250 g of NPK (5-20-10); 500 g of NPK, and; 250 g of NPK with 30 g of micronutrients. Two evaluations (at 240 and 480 days) were carried out for: trees with sprouts emission (%), average sprouts number and their average length (cm). Sprouts were collected at both vegetative evaluations after the application of the experiment (August 2016) and sectioned into cuttings. Cuttings variables were evaluated: survival (%); rooting (%), callus formation (%) and average root number. The best response to epicormic sprouts emission was obtained with 250 g of NPK at 480 days for trees with sprouts (>75%), sprouts number (4.4) and sprouts length (9.9), but considerable results were also obtained at 240 days with urea. For cuttings, the best results were also obtained from sprouts obtained at 480 days for most treatments (>80% survival and rooting). Nitrogen fertilization is essential for a successful vegetative rescue and propagation by cuttings of *I. paraguariensis*, and longer periods may be able to boost these results.

[+]Correspondence:
brunonascimento.ef@gmail.com

DOI:
10.1590/01047760201925012613

[1] University of Santa Catarina State, Santa Catarina, Brazil - ORCID: 0000-0001-6333-134X[a], 0000-0003-1532-8765[b], 0000-0002-2645-9763[e]

INTRODUCTION

Present in South America, and significantly in the southern states of Brazil, *Ilex paraguariensis* Saint Hilaire, family Aquifoliaceae, has great economic importance for this region, having ample potential of use, from the pharmaceutical industry to consumption in form of teas (Dartora et al., 2013). In 2017, the production of *I. paraguariensis* leaves drove economically more than R$ 400 million, increasing approximately 2% of production, which reflected more than 6% in economic gains compared to the previous year (IBGE, 2017).

To attend the markets demand of *I. paraguariensis*, it is necessary to ensure the continuous productivity of its raw material, which may require greater control over plant nutrition, although its needs are not yet well described (Santin et al., 2015). The N and P elements are the most important for the vegetative growth of this species, for sprouting, leaves, roots and seedling growth (Gaiad et al., 2006; Santin et al. 2008a; 2013). The lack of the N element can directly hinder the formation of growth regulating hormones, compromising the vegetative development (Kiba et al., 2011; Kojima et al., 2009).

By providing an increase in plant nutrition with N, a higher cytokine formation occurs in the plant (Kojima et al., 2009), responsible for stimulating the development of lateral buds, new sprouts and leaves (Fukaki, Tasaka, 2009; Santin et al., 2008b). The intentional injury caused to the stem of woody plants, such as girdling techniques, promote an accumulation of cytokine below the affected area, increasing its sprouting significantly (Hartmann et al., 2011). However, using only the N element may not guarantee the vegetative development of this species (Santin et al., 2008a), requiring the insertion of P, responsible for energy production and growth (Santin et al., 2013).

Alternatively, the high presence of N for plant nutrition decreases the total amount of auxin in the plant (Kojima et al., 2009). This class of hormone is responsible for root development, capable of promoting cell differentiation and promoting new roots (Fukaki; Tasaka, 2009). The cuttings process, in the permanence of leaves, promotes hormonal imbalance between auxin and cytokine levels, with an accumulation of the first one, stimulating the formation of roots in the basal part of the cuttings (Hartmann et al., 2011; Nogueira et al., 2017).

In addition to the nutritional characteristics of the mother tree, the ability to vegetative propagate woody species is associated with its degree of maturation, i.e., its ontogenetic age. The ontogenetic age is inversely proportional to the physiological age, which the juvenile characteristics of seedlings are maintained in the dormant buds near the plant base, having a greater vegetative vigour (Hartmann et al., 2011; Santin et al., 2008b; Stuepp et al., 2017). The girdling techniques are effective for the induction of juvenile sprouts of aged *I. paraguariensis* (Santin et al., 2008b), being an essential technique for the success of its cuttings propagation (Nascimento et al., 2018).

Considering the possibility of obtaining more vigorous sprouts for cuttings, the objective of this study was to evaluate the interaction between different fertilizations based on the N element and the epicormic sprout emission on *I. paraguariensis* by the complete girdling technique, also determining the rooting of its cuttings in two periods of vegetative material collection after fertilization.

MATERIAL AND METHODS

Characteristics of the study area

The field experiment was conducted in a native area in the municipality of Urupema, in Santa Catarina state. The study area is located in the Catarinense mountain range at approximately 1.400 meters above sea level, at the approximate coordinates of 28°17'38''S; 49°55'54''W.

This region has a humid temperate climate (Cfb), with an average annual precipitation around 1.800 mm, well distributed throughout the year. The average annual temperature is 13 °C, with a very distinct winter and summer. The average temperature for these two stations is 8 °C and 18 °C, respectively. Being characteristic of the region, there can be snow and minimum temperatures of up to -14 °C (EMBRAPA, 1998).

The region belongs to the Altomontana Mixed Ombrophilous Forest, being characterized by the presence of the species *Araucaria angustifolia* (Bertol) Kutze (from 1.000 meters above sea level), forming groupings in association with other species (Martins-Ramos et al., 2011).

The chemical characteristics of the soil (Table 1) were determined from chemical analysis at the Laboratory of Soil Analysis of the University of Santa Catarina State (UDESC) of Lages. Three samples of approximately 20 cm depth were collected at different points at the experiment site.

Experiment of mother trees fertilization

The native mother trees of *I. paraguariensis* were dispersed at the experiment site, aged between 15 and 20 years, with an average diameter of 7.4 cm, selected based on their phytosanitary quality.

TABLE 1 Chemical properties of the soil in the experimental area of *I. paraguariensis* vegetative rescue, in Urupema, Santa Catarina..

pH	OM	Clay	CEC pH 7,0	Al	H + Al	Ca + Mg	Ca	K	P	Fe*	Mn*	Cu*	Zn*
H2O (1:1)	- - - % - - -		- - - ----- - ------ - $cmol_c \cdot dm^{-3}$ - - - - - - -----------					- - - ----- ---------------- - $mg \cdot dm^{-3}$ - - - - - ------------					
4.3	11.6	8.0	49.6	6.3	48.6	2	0.2	138	12	154	0	0	1.4

*Results obtained by the analysis of soil micronutrients.

Previously to the application of the fertilization treatments, cleaning was carried out around the selected trees, performing crowning with 1 m radius, removing the spontaneous vegetation present and exposing the soil surface. The fertilization was conducted by spreading the fertilizer superficially over the exposed soil and later incorporated. For the control (non-fertilization) treatment, only the cleaning and girdling were conducted.

TABLE 2 Formulation and composition of the fertilization treatments applied to *I. paraguariensis* mother trees s.

Fertilization treatments	Formulation	Composition
Control	-	-
250 g of urea	45% of N	112.5 g of N
250 g of NPK	5-20-10	12.5 g of N; 50 g of P; 25 g of K
500 g of NPK	5-20-10	25 g of N; 100 g of P; 50 g of K
250 g of NPK + 30 g of micronutrients	5-20-10 + 30 g of micronutrients	12.5 g of N; 50 g of P; 25 g of K; + 30 g of micronutrients*

*The composition of each 30 g of the Fetrilon® brand fertilizer contained: 4.0% of Mn (1.2 g), 4.0% of Fe (1.2 g), 1.5% of Cu (0.45 g), 1,5% of Zn (0.45 g), 0.5% of B (0.15 g) and 0.1% of Mo (0.03 g). Moreover, its composition had two macronutrients, being: 1.9% of Mg (0.57 g) and 3.0% of S (0.9 g).

The complete girdling (100%) of the stem of the selected mother trees was accomplished with a machete at a height of 20 cm from the ground. A ring of 5 cm wide was removed, only the outer shell being removed, not affecting the internal wood. Both cleaning, fertilization and girdling occurred in the same period of August 2016.

The following variables were evaluated at 240 days (April 2017) and 480 days (December 2017): Trees with the presence or not of sprouts below the girdle (%); average number of sprouts formed, and; average length of sprouts (cm). The experiment was conducted in a completely randomized design, consisting of three replicates of three trees each.

Rooting of cuttings obtained from the fertilization treatments

The collection of plant material occurred simultaneously in the sprouting evaluations, at 240 days and 480 days after the mother trees fertilization. Therefore, two cuttings experiments were implemented in two different seasons, in April 2017 and the December 2017, analysed separately.

At both periods, the larger sprouts (>10 cm) were collected, arranged in a Styrofoam box with water (to reduce water loss), and transported to the Forest Nursery of the University of Santa Catarina State, in the municipality of Lages.

Sprouts were sectioned into cuttings, cut with pruning shears, approximately 7 cm long, with at least one leaf cut in 50% of its area. Due to the low availability of material, the entire portion of the sprout was used, from the basal portion (more lignified) to the apical portion (more herbaceous).

The cuttings total number and the average number of cuttings produced per sprout were obtained for both sprouts collection. We collected all sprouts from the mother trees for the first cuttings experiment (240 days) and only the longest ones for the second cuttings experiment (480 days). Smaller sprouts were kept to sustain their growth and to avoid affecting even more the *I. paraguariensis* mother trees physiologically and its vegetative growth, removing only what was necessary for the cuttings experiment. No statistical analysis was applied for these evaluations due the high difference of material quantity.

The cuttings were put to root in 180 cm^3 tubes with commercial substrate mix (Maxfertil® - mixture of composted *Pinus* bark and peat, pH between 5.3 and 6.5) and thin vermiculite (Terra Nobre®) in a ratio of 1:1, with addition of 6 g L^{-1} of slow-release fertilizer (Osmocote®, in NPK 15-9-12 composition). The trays, containing the tubes and cuttings, were allocated in a mini tunnel system, with monthly average temperatures between approximately 15 and 25 °C, constant humidity close to or greater than 90% (Figure 1), and automatic irrigation by micro sprinkler, composed of four daily irrigations of five minutes each.

We evaluated the first cuttings experiment (240 days) after 90 days of installation (July 2017), due to changes in its environmental storage conditions, which affected the cuttings survival. The second cuttings experiment (480 days) was evaluated after 120 days of installation (April 2018).

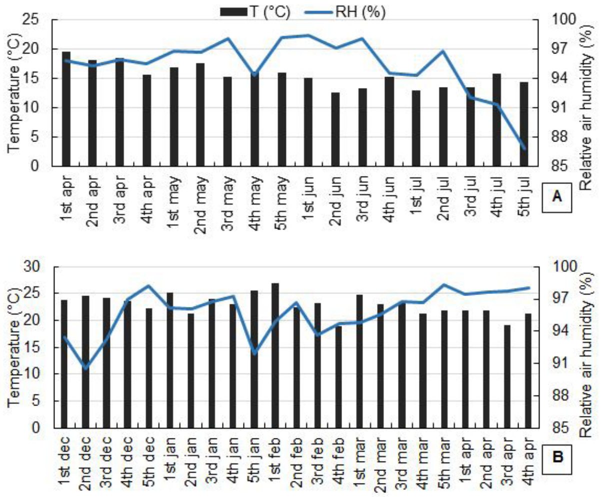

FIGURE 1 Weekly averages of temperature (°C) and relative air humidity (%) in the mini tunnel during the first cuttings experiment (A) (material collected at 240 days – 2017) and second cuttings experiment (B) (material collected at 480 days – 2017/2018).a.

In both periods we evaluated: Survival (%); rooting (%); callus formation (%), and; number of roots. Survivors were cuttings that had live wood, old leaves or young sprouts, rooted or not. For calluses, the cuttings were considered alive, with undifferentiated cell mass at the base, rooted or not. The percentage of rooting was considered on the total, not only on the surviving cuttings. Those with induction of root primordia of at least 2 mm in length were considered as rooted.

The experiment was carried out in a completely randomized design, with five replications of at least three cuttings. There was a difference in the number of cuttings per replicate due to the distinct amounts of sprouts produced by the fertilized mother trees. No comparison was made with the control treatment at the first cuttings (240 days) due to the insufficient number of sprouts.

Statistical analyses

Normality (Shapiro-Wilk) and homoscedasticity (Bartlett) of the residuals from both experiments were evaluated. Analysis of variance (One-Way ANOVA – $P<0.05$), Tukey (normal data – $P<0.05$) and Kruskal-Wallis (non-normal data – $P<0.05$) were applied by the statistical program R Developmental Core Team (2016) software.

RESULTS

Experiment of mother trees fertilization

A statistical difference was detected between the treatments at both sprouting evaluations (Table 3).

TABLE 3 Average of mate mother trees with sprouts from complete girdling (%), average sprout number and average sprout length (cm) according to different fertilizations in two evaluation periods.

Fertilization treatments	Trees with sprouts (%)	Average sprout number	Average sprout length (cm)
First evaluation (240 days)			
Control	14.8 ± 5.8** c*	0.2 ± 0.1 b	0.3 ± 0.1 c
250 g of urea	63.0 ± 6.7 a	1.7 ± 0.1 a	2.9 ± 0.8 a
250 g of NPK	40.7 ± 7.4 abc	1.5 ± 0.6 ab	0.9 ± 0.4 bc
500 g of NPK	59.3 ± 9.2 ab	2.8 ± 0.7 a	1.8 ± 0.4 ab
250 g of NPK***	25.9 ± 4.9 bc	0.7 ± 0.2 b	0.7 ± 0.2 bc
Second evaluation (480 days)			
Control	44.4 ± 17.6 bc	1.1 ± 0.5 bc	4.4 ± 0.1 bc
250 g of urea	66.7 ± 16.7 a	2.4 ± 0.8 b	7.5 ± 0.7 a
250 g of NPK	77.8 ± 14.7 a	4.4 ± 1.1 a	9.9 ± 1.5 a
500 g of NPK	66.7 ± 16.7 a	4.2 ± 1.2 a	7.8 ± 0.6 a
250 g of NPK***	55.5 ± 17.6 b	3.3 ± 0.8 ab	6.6 ± 0.7 b

*Averages followed by the same letter do not differ by Kruskal-Wallis non-parametric test ($P<0.05$); **Standard error; ***Treatment of 250 g of NPK with 30 g of micronutrients.

Considering the first evaluation (240 days), the treatment with 250 g of urea obtained the best results for trees with new sprouts (63.0%), average number of sprouts per tree (1.7) and with a longer average length (2.9 cm). Treatments with 250 g and 500 g of NPK had similar results with 250 g of urea, but slightly lower. However, the treatment of 250 g of NPK with micronutrients and control were the least representative (up to 25.9% for sprouts emission, 0.7 sprouts formed of 0.7 cm).

For the second evaluation (480 days), the difference between treatments was reduced. Both treatments with 250 g of urea, 250 g of NPK and 500 g of NPK presemted the highest sprouts emission (up to 77.8%), of longer lengths (up to 9.9 cm). However, the average number of sprouts produced was lower (2.4) for the treatment with 250 g of urea than those mentioned (up to 4.4) and 250 g of NPK with micronutrients (3.3). Again, for sprouts emission (up to 55.5%) and length of sprouts (up to 6.6 cm) the treatments with 250 g of NPK with micronutrients and control had the lowest averages.

Observing the evaluation periods, all the treatments increased in all variables, as a consequence of the later evaluation. In this aspect, the treatment with 250 g of NPK obtained the highest gains for sprouts emission (increase of 37.1%), number of sprouts (increase of 2.9) and sprouts length (increase of 9.0 cm).

Rooting of cuttings obtained from the fertilization treatments

A considerable difference between the fertilization treatments was obtained for cuttings production (table 4).

The treatment with 250 g of NPK obtained the highest total numbers of cuttings produced for both evaluations periods (63 and 30) and average number

TABLE 4 Total number of cuttings and average cuttings number produced according to each fertilization treatments applied to the *I. paraguariensis* mother trees.

Fertilization treatments	Cuttings total number		Average cuttings number per sprout	
	240 days	480 days	240 days	480 days
Control	0*	12	0*	0.5
250 g of urea	30	24	1.1	0.7
250 g of NPK	63	30	1.9	0.7
500 g of NPK	42	11	1.0	0.2
250 g of NPK**	15	27	1.4	0.7

*Not evaluated due to lack of sprouts for cuttings; **Treatment of 250 g of NPK with 30 g micronutrients.

of cuttings produced per sprout for the first evaluation (240 days – 1.9). The differences between evaluations variables is due that only the longest sprouts were collected for the second cuttings evaluation (480 days). No sprouts were obtained for the control treatment during the first material collection (240 days), due the lack of vegetative material long enough for cuttings.

A statistical difference was detected between the treatments for both cuttings evaluations (Table 5).

TABLE 5 Averages of survival (%), rooting (%), callus formation (%) and number of roots of *I. paraguariensis* cuttings comparing fertilized mother trees in two periods.

Fertilization treatments	Survival (%)	Rooting (%)	Callus formation (%)	Number of roots
First cuttings (240 days after fertilization; 90 days after cuttings)				
Control	-***	-	-	-
250 g of urea	80.0 ± 7.4** a*	63.3 ± 8.9 a	23.3 ± 7.0 a	3.4 ± 0.7 a
250 g of NPK	30.2 ± 5.8 b	22.2 ± 5.3 b	7.8 ± 3.4 b	1.6 ± 0.5 a
500 g of NPK	64.3 ± 7.4 ab	45.2 ± 7.7 ab	33.4 ± 7.4 a	2.7 ± 0.6 a
250 g of NPK****	40.0 ± 13.1 b	26.7 ± 11.8 b	33.3 ± 12.6 a	1.6 ± 0.8 a
Second cuttings (480 days after fertilization; 120 days after cuttings)				
Control	83.3 ± 11.2 a	75.0 ± 13.1 a	66.7 ± 14.2 a	7.5 ± 1.9 b
250 g of urea	75.0 ± 9.0 a	75 ± 9.0 a	62.5 ± 10.1 a	16.4 ± 3.9 a
250 g of NPK	90.0 ± 5.5 a	73.3 ± 8.2 a	83.3 ± 6.9 a	14.1 ± 4.0 a
500 g of NPK	81.8 ± 12.2 a	81.8 ± 12.2 a	63.6 ± 15.2 a	18.1 ± 5.2 a
250 g of NPK****	81.5 ± 7.6 a	44.4 ± 9.7 b	62.9 ± 9.5 a	4.9 ± 1.9 b

*Averages followed by the same letter do not differ among themselves by the Tukey test ($P<0.05$); **Standard error; ***Not evaluated due to lack of sprouts for cutting; ****Treatment of 250 g of NPK with 30 g micronutrients.

Considering the first cuttings experiment (collection of plant material at 240 days, evaluation at 90 days), similar to the fertilization experiment, the treatment with 250 g of urea obtained the best results, mainly survival (80.0%) and rooting (63.3%). The treatment with 500 g of NPK achieved similar results to the best treatment. However, the treatment with 250 g of NPK and 250 g of NPK with micronutrients obtained the lowest percentages of survival (up to 40.0%) and rooting (26.7%).

For the second cuttings experiment (material collected at 480 days, evaluation at 120 days) there were significant changes in all treatments. Survival remained above 75.0% in all treatments, while rooting reached more than 80.0% for the treatment with 500 g of NPK. Rooting was only lower (44.4%) for 250 g of NPK with micronutrients, even lower in relation to control (75.0%).

Relating the two cuttings experiments, all treatments increased positively in all variables, becoming very statistically similar.

DISCUSSION

Experiment of mother trees fertilization

The application of the complete girdling technique on *I. paraguariensis* trees to promote epicormic sprouts emission rarely leads to their death (Santin et al., 2008b). The species has a great capacity for healing this type of injury (Nascimento et al., 2018), what was also observed in this experiment.

The complete girdling technique alone can promote a large variation in the number of *I. paraguariensis* trees with sprouts, number of sprouts and their length, and may be related to factors such as pruning (Santin et al., 2008b) and mother trees age (Bitencourt et al., 2009). The association of pruning and girdling promotes a greater number of trees with sprouts emission (more than 80.0%), with a higher number of sprouts (>4) and length (>16 cm) for a period of 270 days (Santin et al., 2008b). On the other hand, advanced ages (>80 years) can negatively affect the number of trees with sprouts emission (<45.0%), but are still able to guarantee a higher number of sprouts (>4) and longer length (15 cm) for a period of 180 days (Bitencourt et al., 2009). In this perspective, the gains obtained by the fertilization of the *I. paraguariensis* mother trees would still be inferior for sprouts length, even considering the best treatments during the second evaluation (480 days). It is possible that the study population is adapted to the adverse environmental conditions, reflecting in a slow vegetative development (Nascimento et al., 2018).

We observed a high organic matter (OM) in the soil of the experimental area (11.6%), considered elevated when above 5%, allowing greater release of nutrients and lower risk of deficiency in high pH soils. However, the pH of the analysed soil has high acidity (4.3), which may explain the low levels of some nutrients obtained in the soil analysis of this experiment, unavailable due to the presence of Al. Trees of *I. paraguariensis* can be found naturally in soils of high acidity (Santin et al., 2013), presenting a strong tolerance to low pH and Al, growing even in these adverse conditions (Santin et al., 2013). This species seems to have some mechanism not yet evidenced of acidity tolerance, allowing its normal vegetative development (Santin et al., 2015).

As for nutrition, the N element is directly related to the synthesis and transport of cytokines, responsible for emitting new sprouts (Kiba et al., 2011; Hartmann et al., 2011). Although the positive influence of the nitrogen treatments in this work, the proportionality of the minimum quantities of these fertilizers, aiming to achieve similar results, is unknown. The green mass production of *I. paraguariensis* is influenced by fertilization in the form of urea, small doses (75 g) can reach 89.0% of the production obtained by larger doses (225 g) (Lourenço et al., 1997). This may indicate that it is possible to obtain the same gains from smaller dosages of the same fertilizer. This result could be observed in the long term (480 days) for the treatment with 250 g NPK, obtaining a significant increase of trees with sprouts emission (>35.0%), number of sprouts (2.9) and length (9.0 cm), when compared to the highest dosage of the same fertilizer (500 g NPK) for the same period.

The use of N and K-only doses in *I. paraguariensis* seedlings negatively affects their growth (Santin et al., 2008a; Santin et al., 2013). However, when incorporated P to the formulation, either at the same dosage or higher, the development appears very positive, being related to the processes of energy production and root growth (Santin et al., 2013). Although the need for P insertion in the soil analysed in this work, the trees of *I. paraguariensis* presented better vegetative development independent of the amounts of P added during the first evaluation (240 days). However, in the second evaluation, positive results were obtained for both with and without P. So far, few studies bring NPK fertilization in *I. paraguariensis*, which makes it difficult to understand the nutritional needs of this species (Santin et al., 2015), even in its natural environment.

Although the P and K elements also influence hormone synthesis, N appears to be the most important (Hartmann et al., 2011). There is a strong relationship between cytokine levels and the available N for plants (Kiba et al., 2011). The use of N for supplementation increases the amount of cytokine in the xylem, roots and shoot in agricultural species (Takei et al., 2001), indicating that the cytokines can function as a communicator of the presence of N, transmitting this information from the roots to the aerial part of the plant (Sakakibara, 2006).

High and low concentrations of N significantly altered the amounts of cytokines in both sprouts and roots of *Arabidopsis thaliana* (L.) Heynh (Kojima et al., 2009). This shows that cytokine is able to identify not only the presence of N, but also its quantity (Kiba et al., 2011). It is assumed that fertilizers with the highest N presence efficiently promote the synthesis and accumulation of cytokines, significantly increasing the sprout-related variables more rapidly (240 days). In long terms (480 days), these differences are diluted, even smaller amounts of N (12.5 g in the treatment of 250 g of NPK) are able to obtain equally good or even better results.

The relationship between nitrogen fertilization, the possible higher hormone production from this nutrient (Kiba et al., 2011; Kojima et al., 2009), the injury caused in the basal part of the trees (Santin et al., 2008b; Hartmann et al., 2011; Nascimento et al., 2018) and the accumulation of hormones - specifically cytokine - may have been the main factor for the production of more vigorous sprouts in *I. paraguariensis* mother trees.

A probable reason for the best vegetative response to urea fertilization at first (240 days), considering the high percentage of OM in the soil that would theoretically consume the N element, would be in the environmental conditions of the study site, which are unique, both in terms of the environment, with low temperatures and the forest typology (EMBRAPA, 1998; Martins-Ramos et al., 2011). Low temperatures, common in the region, may have hampered the degradation of N element by the soil fauna. The good results of the second evaluation of the sprouts can be summarized by the longest time for their development, the best interaction between N, P and K (Santin et al. 2008a) and the presence of P itself, more limiting for the growth of *I. paraguariensis* followed by N (Santin et al., 2013).

The treatment with 250 g of NPK with micronutrients obtained the lowest results among all fertilizations. There are few studies that provide information on possible levels of toxicity in relation to the micronutrients tested or other conditions that could affect the vegetative growth.

Considering the sprouts vegetative growth from cited works (Lourenço et al., 1997; Santin et al., 2008b; Santin et al., 2013) and comparing to those obtained by Nascimento et al., 2018 and this work (same study area), a significant difference can be noticed. The cited authors bring a higher percentage of sprouts emission, of greater number and length, even for the least representative treatments, when compared to this work. Also, those works were carried out in areas with higher temperatures and light incidence (Paraná state). Therefore, it is possible that the long term adaptation of this *I. paraguariensis* population to a low temperature and low light incidence may have naturally selected individuals, resulting in slow vegetative growth, considering even the best fertilization treatments.

Rooting of cuttings obtained from the fertilization treatments

Considering the number of cuttings produced, it is possible that some fertilization treatments allowed a faster sprouts development, resulting in longer sprouts but with fewer dormant buds. This hinders a higher production of propagules, which needs at least a leaf per cutting.

The possible low survival of cuttings found in some treatments during the first cuttings experiment (240 days after fertilization) may have been due to the momentary drop in relative humidity at 90 days (Figure 2). Relative humidity should always be kept as high as possible to conserve propagules (Xavier et al., 2013). During the second cuttings experiment (480 days after fertilization) there was a higher moisture stabilization (Figure 3), enabling the cuttings to remain in the rooting environment for a longer period before the evaluation (120 days).

The success of *I. paraguariensis* cuttings may be directly associated, among other factors, with the ontogenetic age of the plant material (Bitencourt et al., 2009, Stuepp et al., 2017; Nascimento et al., 2018). Cuttings from epicormic sprouts by complete girdling tend to easily exceed 50.0% of rooting even in aged mother trees (Bitencourt et al., 2009; Stuepp et al., 2017). Already verified by Nascimento et al. (2018), the *I. paraguariensis* population of this work has a low response to the complete girdling technique for reinvigoration and cuttings, with very similar rooting between epicormic sprouts (37.3%) and treetop sprouts (26.7%) (rooting occurring from April to July 2017, in the same rooting environment). Thus, the rooting results of cuttings obtained by combining complete girdling and fertilization are very positive, both for the first cuttings experiment (>60.0%) and for the second (>80.0%) experiment.

The N element is used for both the synthesis and the transport of plant hormones, mainly growth regulators such as auxins (Kiba et al., 2011), proving to be an important factor in the development of roots in cuttings of *I. paraguariensis*. The unique presence of N and K can stagnate or impair the development of seedlings, but when inserting P into the formulation there is a superior development (Santin et al., 2008a; 2013). This was not detected in the first cuttings experiment, as the best survival and rooting indices were observed in the treatment with 250 g of urea. In contrast, in the second evaluation (480 days), the best results, considering all the variables, were for treatments with 250 g of urea, 250 and 500 g of NPK.

Previous fertilization of *Paullinia cupana* var. sorbilis, (Mart.) Ducke mother trees with NPK presented a positive influence of cuttings survival (71.1%) and rooting success (66.3%), compared to non-fertilization (60.0% and 56.2%) (Albertino et al., 2012). The rooting of blackberry (*Rubus* spp.) cuttings is directly related to the fertilization of mother trees with urea (71.6%) compared to non-fertilization (52.5%) (Picolotto et al., 2015). This positive relation was observed for cuttings of *I. paraguariensis* from mother trees fertilized with urea and NPK for both cuttings experiments.

Fertilization of mother trees for cuttings production may increase the percentage of rooting, reduce the mortality (Albertino et al., 2012), and assist the initial process of radicle and leaf formation (Picolotto et al., 2015). A considerably good rooting percentage may indicate that the carbon and nitrogen ratios, hormone balance and substrate moisture were adequate for the root formation process (Moreira et al., 2010).

The N element positively influences root development by being directly linked to hormonal functions (Kiba et al., 2011) and carbohydrate metabolism, providing energy for its initial development (Hartmann et al., 2011). The fertilization of the mother trees can be considered an important factor for the predisposition for the cuttings rooting, promoting morphological alterations in the propagules, as formation of adventitious roots, determining also their quantity and length (Cunha et al., 2009).

The use of high and low doses of N did not significantly alter the total amount of auxin from aerial parts of *Arabidopsis* seedlings (Kojima et al., 2009). It is possible that the fertilization of *I. paraguariensis* mother trees has previously increased auxin levels and, in association with the hormonal imbalance caused by sprouts formation through girdling (Nogueira et al., 2017), favoured the rooting of the cuttings.

The use of reinvigorated sprouts (Santin et al., 2008b; Hartmann et al., 2011; Stuepp et al., 2017; Nascimento et al., 2018) associated with nutritional enhancement caused by different fertilizations (Santin et al., 2008a; Santin et al., 2013) may have been an important factor for the successful rooting of *I. paraguariensis* cuttings. The longer time before sprout collection (480 days) may have favoured its better development and better absorption of nutrients provided, associated with an improved environment conditions and longer period of acclimatization, resulting in better rooting.

CONCLUSIONS

The complete girdling and fertilization association can quickly increase the sprout emission in *I. paraguariensis*, specially with 250 g of urea or 500 g of NPK (5-20-10).

Longer periods can achieve better gains in epicormic sprout emission with smaller doses of NPK.

The cuttings technique was more successful from epicormic sprouts that were kept longer on the mother trees (480 days) than those collected in advance (240 days).

BIBLIOGRAPHY

ALBERTINO, S. M. F.; NASCIMENTO FILHO, M F. J.; SILVA, J. F.; ATROCH, A. L.; GALVÃO, A. K. L. Enraizamento de estacas de cultivares de guaranazeiro com adubação de plantas matrizes. **Pesquisa Agropecuária Brasileira**. v.47, n.10, p.1449-1454, 2012.

CUNHA, A. C. M.; PAIVA, H. N.; LEITE, H. G.; BARROS, N. F.; LEITE, F. P. Influência do estado nutricional de minicepas no enraizamento de miniestacas de eucalipto. **Revista Árvore**, v.33, p.607-615, 2009.

DARTORA, N.; SOUZA, L. M.; PAIVA, S. M.; SCOPARO, C. T.; IACOMINIA, M.; GORINA, P. A. J.; RATTMANN, Y. D.; SASSAKI, G. L. Rhamnogalacturonan from *Ilex paraguariensis*: A potential adjuvant in sepsis treatment. **PubMed. Carbohydrate Polymers**, v. 92, p. 1776-1782, 2013.

EMBRAPA. **Mapa Convenção cartográfica: escala 1:250.000**. Rio de Janeiro. 1998. 2 p.

FUKAKI, H.; TASAKA, M. Hormone interactions during lateral root formation. **Plant Molecular Biology**. v. 69, p.437-449, 2009.

GAIAD, S.; RAKOCEVIC, M.; REISSMANN, C. B. N. Sources affect growth, nutrient content, and net photosynthesis in maté (*Ilex paraguariensis* St. Hil.). **Brazilian Archives of Biology and Technology**. v. 49, n. 5, p.689-697, 2006.

HARTMANN, H. T; KESTER, D. E; DAVIES JR, F. T; GENEVE, R. L. **Hartmann and Kester's Plant propagation**: principles and practices. New Jersey: Prentice Hall, 8 ed. 2011, 915 p.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, **Sistema IBGE de Recuperação Automática – SIDRA. Produção da extração vegetal e da silvicultura de 2017**. Disponível em: https://sidra.ibge.gov.br/pesquisa/pevs/quadros/brasil/2015 Acesso em: 02 de outubro de 2018.

KIBA, T.; KUDO, T.; KOJIMA, M.; SAKAKIBARA, H. Hormonal control of nitrogen acquisition: roles of auxin, abscisic acid, and cytokine. **Journal of Experimental Botany**, v. 62, n. 4, p. 1399-1409, 2011.

KOJIMA, M.; KAMADA-NOBUSADA, T.; KOMATSU, H.; TAKEI, K.; HUROHA, T.; MIZUTANI, M.; ASHIKARI, M.; UEGUCHI-TANAKA, M.; MATSUOKA, M.; SUZUKI, K.; SAKAKIBARA, H. Highly sensitive and high-throughput analysis of plant hormones using MS-probe modification and liquid chromatography-tandem mass spectrometry: an application for hormone profiling in *Oryza sativa*. **Plant and Cell Physiology**. v. 50, p.1201-1214. 2009.

NASCIMENTO, B.; SÁ, A. C. S.; LEMOS, L. B.; ROSA, D. P.; PEREIRA, M. O.; NAVROSKI, M. C. Three epicormic shoot techniques in *I. paraguariensis* mother trees and its cutting according to the material rejuvenation degree. **CERNE**, v. 24, n. 3, p. 240-248, 2018.

LOURENÇO, R. S.; CURCIO, G. R.; RACHWAL, M. G.; MEDRADO, M. J. S. Avaliação de níveis de nitrogênio sobre a produção de erva-mate (*Ilex paraguariensis* St. Hil.) em Fernandes Pinheiro-PR, em latossolo vermelho escuro. **Boletim de Pesquisa Florestal**. n. 34, p. 75-98, 1997.

MARTINS-RAMOS, D.; CHAVES, C. L.; BORTOLUZZI, R. L. da C.; MANTOVANI, A. Florística de Floresta Ombrófila Mista Altomontana e de Campos em Urupema, Santa Catarina, Brasil. **Revista brasileira Biociências**, v. 9, n. 2, p. 156-166, 2011.

MOREIRA, R. A.; RAMOS, J. D.; CRUZ, M. C. M.; VILLAR, L.; HAFLE, O. M. Efeito de doses de polímero hidroabsorvente no enraizamento de estacas de amoreira. **Revista Agrarian**. v. 3, p. 133-139. 2010.

NOGUEIRA, G. S.; SILVA, F. A. C.; KUNZE, G.; FIGUEIRÓ, J. P. S.; KRUCHELSKI, S.; ZUFFELATO-RIBAS, K. C. Influência do número de folhas e da aplicação de IBA na estaquia caulinar de *Ficus benjamina* L. **Revista Agrarian**, v. 10, n. 36, p. 113-119, 2017.

PICOLOTTO, L.; VIGNOLO, G. K.; PEREIRA, I. S.; GONÇALVES, M. A.; ANTUNES, L. E. C. Enraizamento de estacas de amoreira-preta em função da adubação nitrogenada na planta matriz. **Revista Ceres**, v. 62, n.3, p. 294-300, 2015.

SAKAKIBARA, H. Cytokines: activity, biosynthesis, and translocation. **Annual Review of Plant Biology**. v. 57, p. 431-49, 2006.

SANTIN, D.; BENEDETTI, L.; BASTOS, M. C.; KASEKER, J. F.; REISSMANN, C. B.; BRONDANI, G. E.; BARROS, N. F. Crescimento e nutrição de erva-mate influenciados pela adubação NPK. **Ciência Florestal**. v. 23, n. 2, p. 363-375, 2013.

SANTIN, D.; BENEDETTI, L.; BRONDANI, G. E.; REISSMAN, C. B.; ORRUTÉA, A. G.; ROVEDA, L. F. Nitrogênio, fósforo e potássio no crescimento de mudas de erva-mate. **Scientia Agraria**. v. 9, n. 1, p. 59-66, 2008a.

SANTIN, D.; BENEDETTI, L.; REISSMAN, C. B. Nutrição e recomendação de adubação e calcário para cultura da erva-mate. In: WENDLING, I.; SANTIN, D. **Propagação e nutrição de erva-mate**. EMBRAPA. 21 ed. 2015.

SANTIN, D.; WENDLING, I.; BENEDETTI, E. L.; BRINDANI, G. E.; REISSMAN, C. B.; MORANDI, D.; ROVEDA, L. F. Poda e anelamento em erva-mate (*Ilex paraguariensis*) visando à indução de brotações basais. **Pesquisa Florestal Brasileira**, n.56, p.97-104, 2008b.

STUEPP, C. A.; BITENCOURT, J.; WENDLING, I.; KOEHLER, H. S.; ZUFFELLATO-RIBAS, K. C. Age of stock plants, seasons and iba effect on vegetative propagation of *Ilex paraguariensis*. **Revista Árvore**. v. 41, n. 2, p. 1-7, 2017.

TAKEI, K.; SAKAKIBARA, H.; TANIGUCHI, M.; SUGIYAMA, T. Nitrogen-dependent accumulation of cytokines in root and the translocation to leaf: implication of cytokine species that induces gene expression of maize response regulator. **Plant and Cell Physiology**, v. 42, p. 85-93, 2001.

XAVIER, A.; WENDLING, I.; SILVA, R. L. **Silvicultura clonal: princípios e técnicas**. UFV, Viçosa, 2 ed. 2013. 279 p.