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Nitrogen fertilization for ministrains of *Campomanesia aurea* O. Berg and its influence on productivity and rooting of minicuttings at different seasons of the year

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ABSTRACT: The objective of this work is to evaluate the effects of nitrogen doses on the ministrain of *C. aurea* conducted in minigarden system regarding productivity, rooting and root system quality of minicuttings at different seasons. The experiment was conducted at a greenhouse, and the treatments consisted of collection times (autumn, winter, spring and summer) and nitrogen doses (0, 0.3, 1.12, 1.95 and 3.60 g L^{-1}). We evaluated the survival of ministrains, productivity of minicuttings, percentage of rooting and root dry matter. The experiment comprised randomized blocks in a 5 x 4 factorial design. Data were subjected to analysis of variance and polynomial regression, and means were compared by Tukey test at 5%. The mean productivity was 10.0 and 18.9 minicuttings per ministrain in the spring and summer, respectively. Rooting was hindered by the increase in nitrogen doses. We concluded that the survival of ministrains is influenced by nitrogen fertilization, and that the N dose 1.12 g L^{-1} provides better results for productivity and rooting of minicuttings when collected during the spring and summer.

Key words: minigarden; native ornamental species; seedling production

Adubação nitrogenada em minicepas de Campomanesia aurea O. Berg e sua influência na produtividade e enraizamento de miniestacas em diferentes estações do ano

RESUMO: O objetivo deste trabalho foi avaliar o efeito de doses de nitrogênio em minicepas de *C. aurea* conduzidas em sistema de minijardim sobre a produtividade, enraizamento e qualidade do sistema radicular de miniestacas em diferentes estações do ano. O experimento foi conduzido em casa de vegetação e os tratamentos consistiram em épocas de coleta (outono, inverno, primavera e verão) e doses de nitrogênio (0, 0,3, 1,12, 1,95 e 3,60 g L^{-1}). Avaliou-se a sobrevivência das minicepas, a produtividade de miniestacas, porcentagem de enraizamento e matéria seca de raiz. O delineamento experimental foi em blocos casualizados em arranjo fatorial 5 x 4. Os dados foram submetidos à análise de variância regressão polinomial e teste de Tukey a 5% de probabilidade de erro. A produtividade média foi de 10,0 e 18,9 miniestacas por minicepa na primavera e verão, respectivamente. O enraizamento foi prejudicado pelo aumento das doses de nitrogênio. Conclui-se que, a sobrevivência de minicepas é influenciada pela aplicação de adubação nitrogenada, e que a dose de 1,12 g L^{-1} de nitrogênio apresenta resultados superiores na produtividade e enraizamento de miniestacas quando coletadas na primavera e verão.

Palavras-chave: minijardim; espécie ornamental nativa; produção de mudas

Introduction

Campomanesia aurea O. Berg is a species native to the Pampa Biome. It belongs to the Myrtaceae family, and is popularly known in Brazil as "guabirobinha-do-campo", "araçá-rasteiro" or "araçá-do-campo" (Lorenzi et al., 2006). It has an ornamental potential for gardens or pots due to its shrubby size, irregular shape, intense and aromatic flowering and production of edible fruits (Lorenzi et al., 2006; Stumpf, 2009).

The propagation of this species occurs naturally by seeds (Lorenzi et al., 2006), but vegetative propagation is frequently used in floriculture since it is a low-cost technique, easy to perform, and it often has a reduced seedling production time. However, the viability of cutting depends on the potential of the species to form adventitious roots and on the quality of the root system, which should be sufficient for the survival of seedlings (Fachinello et al., 2005).

Vegetative propagation, especially of forest species, has greatly improved due to the production of minicuttings in clonal minigarden systems. Minigardens are areas of vegetative multiplication formed by a set of ministrains. It provides shoots as propagules for the minicutting process (Xavier et al., 2013). The use of this technique allowed an increase in rooting index, root system quality and a decrease in or exemption of the use of growth regulators (Alfenas et al., 2004) due to a greater ability of juvenile materials to emit adventitious roots when compared to lignified materials (Fachinello et al., 2005).

Mineral nutrition influences the rooting of cuttings because of the vegetative vigor of the plants and, consequently, the nutritional status of the propagule collected (Xavier et al., 2013). Nitrogen is one of highest demanded nutrients by plants, forming part of the chemical composition of several cellular constituents, such as nucleotides and amino acids, which form nucleic acid and protein structures, respectively. They are absorbed mainly as ammonium (NH_4^+) and nitrate (NO_3^-) (Kerbauy, 2013). However, depending on the species, nitrogen fertilization affects differently the production and rooting of cuttings.

Previous research has shown that cuttings of *C. aurea* collected directly from the field present a low rooting (Emer et al., 2016; Emer et al., 2018). Therefore, the production of seedlings by cuttings collected from minigardens might be an alternative for the vegetative propagation of this species, since there is a control of nutritional management in matrix plants, besides obtaining juvenile material, which is more suitable to rooting. The objective of this work is to evaluate the effects of nitrogen doses on ministrains of *C. aurea* conducted in a minigarden system regarding productivity, rooting and root system quality of minicuttings at different seasons of the year.

Materials and Methods

Production of minicuttings

The experiment was conducted at a greenhouse without temperature control covered with polyethylene and an anti-aphid screen. The greenhouse belongs to the Department of Horticulture and Forestry of the Federal University of Rio Grande do Sul, located in the city of Porto Alegre, RS (30°04'26" S and 51°08'07" W). We used seedlings obtained from seeds collected at the municipality of Barão do Triunfo, RS (30º18'136" S and 51º50'282" W).

Seedlings with seven months of age and about 5-8 cm high were transplanted to 8-L pots containing medium sand as substrate. Four seedlings were planted per pot. Ten months after sowing, the treatments began. They consisted of different doses of nitrogen (Table 1). As nutrient sources, the commercial product Kristalon® and ammonium nitrate (NH_4NO_3) were used. According to the manufacturer's information, the Kristalon® fertilizer (formulation 6-12-36) contains 6% of Nitrogen (4.5% as nitric solutions and 1.5% as ammonia), 12% of Phosphorus (P_2O_5), 36% of Potassium (K_2O), 1.8% of Magnesium (Mg), 8% of Sulfur (S), 0.07% of Iron (Fe), 0.025% of Boron (B), 0.01% of Copper (Cu), 0.04% of Manganese (Mn), 0.004% of Molybdenum (Mo) and 0.025% of Zinc (Zn).

Fertilizations were carried out biweekly by fertigation with 50 mL of solution per pot. The control did not receive fertilization, only the same volume of water as in the other treatments was provided. Before each fertilization, the *Pour Thru* test (Cavins et al., 2000) was conducted to monitor the electrical conductivity (EC) and the hydrogenation potential (pH) of each treatment. The pH and EC of the irrigation water ranged from 4.45 to 6.71 and 0.091 to 0.128 mS cm^{-1}, respectively, throughout the experiment.

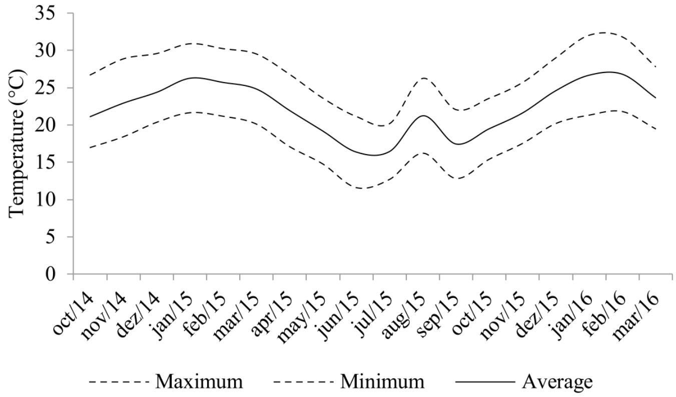
At twelve months, the plants sprouted and only the apical bud was removed to form ministrains and stimulate the growth of lateral shoots, keeping the plants about five centimeters high. The irrigation was performed by dripping and managed according to the needs of plants in function of climatic conditions throughout the year. The minimum, average and maximum temperatures during the conduction of the experiment were obtained from the Automatic Meteorological Station of the National Institute of Meteorology (INMET, 2017) for the city of Porto Alegre (Figure 1).

During the second half of August 2015, a weekly fertilization of ministrains began by using the same doses and volumes as the previous fertilization. In November 2015, due to an increase in electrical conductivity, the substrate was washed

Table 1. Fertilization doses (g L^{-1}) used for fertigation for the production of minicuttings of *Campomanesia aurea*.

Treatment	Fertilizer dose	Nitrogen dose
	(g L^{-1})	
T1	Control (without fertilization)	0
T2	5 g of Kristalon*	0.3
T3	5 g of Kristalon + 2.36 g of NH_4NO_3	1.12
T4	5 g of Kristalon + 4.72 g of NH_4NO_3	1.95
T5	5 g of Kristalon + 9.44 g of NH_4NO_3	3.60

*Kristalon: 0.3 g of N, 0.6 g of P, 1.8 g of K, 0.09 g of Mg, 0.4 g of S, 3.5 mg of Fe, 1.25 mg of Bo, 0.5 mg of Cu, 2 mg of Mn, 0.2 mg of Mo, 0.125 of B and 1.25 mg of Zn per liter of solution.



Data from BDMEP – INMET (2017).

Figure 1. Monthly averages for minimum, average and maximum temperatures in Porto Alegre, RS, during the conduction of the experiment.

three to four times by saturating and draining the pots using water until the electrical conductivity decreased. After this, the fertilization was again performed every fortnight.

Minicuttings were collected, without a fixed interval, during spring, summer, autumn and winter, adopting as criteria a height of 5-8 cm, four leaves and at least two fully expanded leaves. In the minigarden, the survival of ministrains and the productivity of minicuttings were evaluated.

Rooting of minicuttings

After each collection in the minigarden, the cuttings were standardized by a straight cut at the base and immediately treated with 200 mg L^{-1} of a hydroalcoholic solution of indolebutyric acid (IBA) for five seconds. After the IBA treatment, the cuttings were placed into multicellular trays made of expanded polystyrene containing 15 cm^3 cells filled with carbonized rice husks. They were then brought to an environment without temperature control under an intermittent nebulization irrigation system, where the relative air humidity remained above 90%.

After 90 days under nebulization, we evaluated rooting percentage and root dry matter of the minicuttings. All cuttings with visible roots (at least 1 mm) were considered rooted. The root dry mass was determined by placing the plant material in an oven at 65°C until constant weight, and then weighed using an analytical balance. The value found for root dry mass was divided by the number of rooted cuttings of each sample and expressed in mg.

Statistical analysis

The experiment comprised complete randomized blocks with a 5 x 4 factorial design. The factors were five fertilization solutions and four cutting seasons - autumn (April-June), winter (July-September), spring (October-December) and summer (January-March). There were four replications, comprising eight ministrains.

For all data, we performed analyses of variance (ANOVA) and then polynomial regressions or Tukey tests at 5% probability.

Results and Discussion

Production of minicuttings

Regarding the survival of ministrains, the interaction between the factors was observed. There was a decreasing quadratic response for winter, spring and summer. There were high mortality rates when the ministrains were fertilized using high nitrogen doses independent of the season (Figure 2). Less than 10% of the ministrains were alive until the summer, when we applied the highest nutritional dose (3.60 g L^{-1}).

An excess of salts in the culture substrate may have caused the high mortality of ministrains in the treatments using high nutritional doses. From June (autumn), there was an increase in plant mortality, when the electrical conductivity for the highest doses of fertilization applied was between 1.5 and 2.0 mS cm^{-1} (Figure 3).

The electrical conductivity provides an estimate of the amount of soluble salts in the substrate in function of the number of ions dissolved in water, which conduct electric currents proportionally to the amount of salts (Cavins et al., 2000). Thus, electrical conductivity values higher than 2 mS cm^{-1}, as observed in this study, were possibly responsible

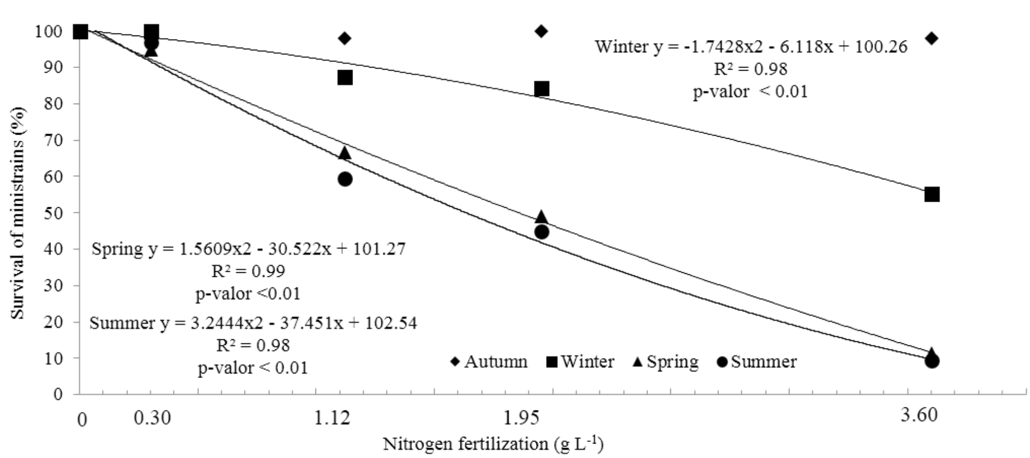


Figure 2. Survival of ministrains of *Campomanesia aurea* subjected to different nitrogen fertilization doses per season of the year. UFRGS, Porto Alegre, 2016.

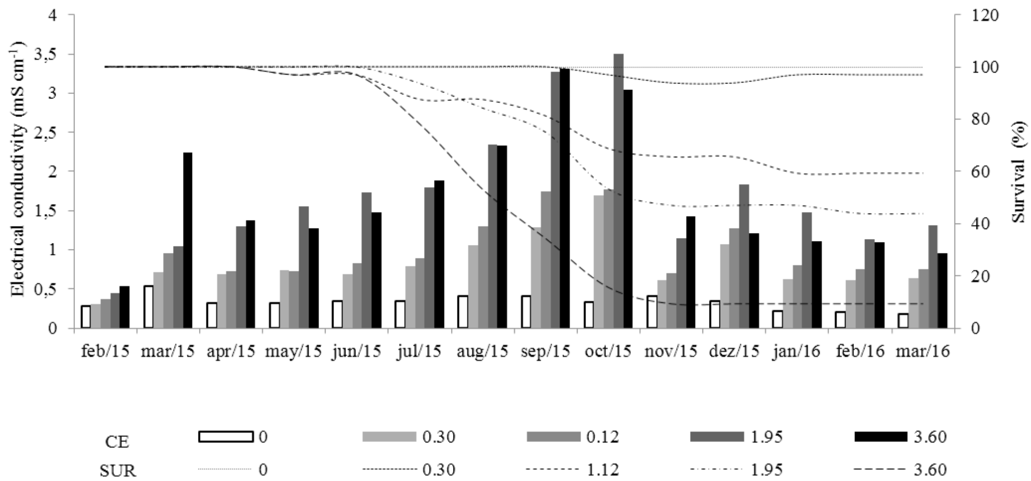


Figure 3. Electrical conductivity (mS cm^{-1}), determined by the method *Pour Thru*, of the substrate and survival of ministrains of *Campomanesia aurea* subjected to different nitrogen fertilization doses. UFRGS, Porto Alegre, RS, 2016.

for the high mortality of ministrains because of an excessive increase in the level of salts in the substrate.

Different species presented a variation in tolerance to osmotic stress caused by salinity levels. Electrical conductivity presents a linear relation with osmotic potential because, with the increase in the concentration of salts in the solution, there is a decrease in water potential, thus becoming less available to plants. The decrease in growth and biomass production is a good indicator for assessing the degree of stress and the ability of the plant to tolerate excess of salts (Larcher, 2004). In this sense, there was not only a stagnation of growth, but also death of matrix plants in this study, which reinforces the thesis of excess of salts in the substrate.

Thus, from November (spring), by washing the substrate for the removal of excess salts, we verified a decrease in the mortality of strains. It can be inferred that this is a species sensitive to salinity according to the classification of the *Pour Thru* method. This method provides an estimate of the need for fertilization and separates plants with low (1.0-2.6 mS cm^{-1}), medium (2.0-3.5 mS cm^{-1}) and high (2.6-4.6 mS cm^{-1}) nutritional needs (Cavins et al., 2000) into different groups. In other words, we can classify the species as having a low fertilization requirement since the maintenance of the EC below 2 mS cm^{-1} was favorable for the survival of ministrains and the productivity of minicuttings.

For *Eugenia uniflora*, a native species belonging to the same family, there is a moderate need for N for both emission and rooting of cuttings. The maintenance of the electrical conductivity at 0.5 mS cm^{-1} by the *Pour Thru* method was, according to the authors, suitable for the cultivation of this species (Lattuada et al., 2016). Such salinity levels were below those observed in this study.

The survival of ministrains was greater than 60% for the treatments fertilized with 0, 0.3 and 1.12 g L^{-1} of nitrogen even after one year of continuous collections. The survival and production of minicuttings in successive collections indicate the technical viability of the system for seedling production (Xavier et al., 2013), as well as the adequacy of the minigarden management and the nutrition for ministrains (Dias et al., 2012a).

Thirty three collections were performed during one year of production of *C. aurea* ministrains. The longest periods between collections (over 23 days) were in June, July and August (autumn and winter). In September, the collections were performed on average every 10 days. From October (spring), collections were weekly until the final evaluation period. The period between collections verified for *C. aurea* was shorter than that verified for clones of *Eucalyptus benthamii* × *Eucalyptus dunnii*, in which shorter intervals occurred during spring (9 to 11 days) and summer (8 to 15 days) (Brondani et al., 2012). This evidences a high shoot emission capacity by the species under study.

Regarding the productivity of minicuttings, there was also an interaction between collection season and nitrogen fertilization doses, with a quadratic behavior for autumn, spring and summer. The spring-summer period obtained the highest productivity of ministrains. There was an estimated production of 10.0 and 18.9 minicuttings per ministrain using the N dose 1.95 g L^{-1} for spring and summer, respectively (Figure 4). For treatments using 0 and 3.60 g L^{-1} of nitrogen, the lowest productivity occurred mainly in the absence of fertilization in spring-summer, which was otherwise more favorable to the emission of shoots. For the N dose 3.60 g L^{-1}, although there was an increased production during the seasons, there was a high mortality of ministrains, as already reported. Its use is not recommended.

Regardless of the nitrogen dose, the productivity of ministrains was low at the beginning of the crop (autumn-winter). These results are in part due to the low average temperatures observed during these seasons (Figure 2) and because the ministrains were still forming. A similar result was found in a minigarden with *Psidium guineense*, *P. cattleyanum* and *P. guajava*. There was a low production in the first collections associated to a low accumulation of reserves at the beginning of the cultivation (Altoé et al., 2011). The collections of propagules throughout the year are influenced

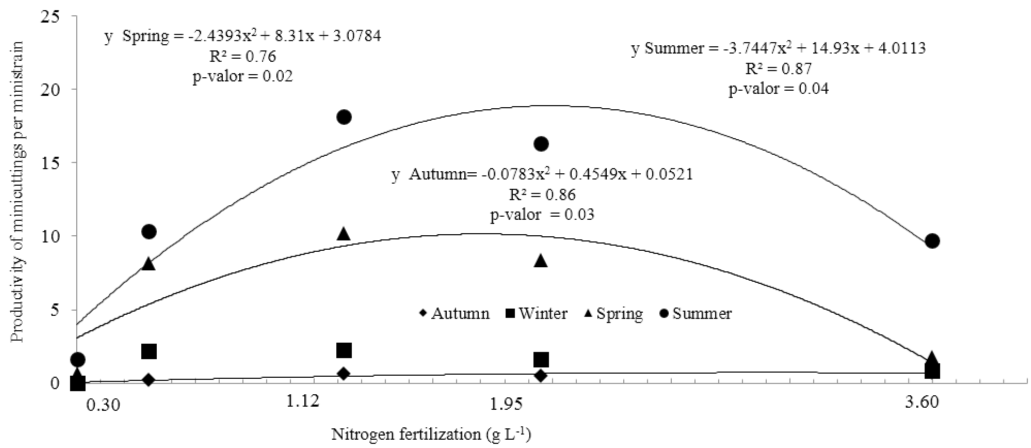


Figure 4. Productivity of minicuttings per ministrain of *Campomanesia aurea* subjected to different nitrogen fertilization doses per season of the year. UFRGS, Porto Alegre, 2016.

by temperature, light intensity and photoperiod (Alfenas et al., 2004). In this study, climatic factors were probably decisive since the production of cuttings was favored by the increase in temperature. This same behavior was also observed for *Eucalyptus dunnii* (Rosa et al., 2009), *Sapium glandulatum* (Ferreira et al., 2010), *Piptocarpha angustifolia* (Ferriani et al., 2011), clones of *Eucalyptus benthamii* × *Eucalyptus dunnii* (Brondani et al., 2012) and *Eugenia uniflora* (Peña Peña et al., 2015).

For *Psidium guineense, P. cattleyanum* and *P. guajava*, species belonging to the Myrtaceae family, the average yield of minicuttings per ministrain in each collection ranged from 1.94 to 3.99, 2.00 to 4.83 and 1.92 to 3.62, respectively, for the seven collections performed over a period of more than a year (Altoé et al., 2011). For *Eugenia uniflora*, an average production of 1.5 minicuttings per ministrain was verified every 10 days (Lattuada et al., 2016). For other native species, the mean productivity and the interval between minicutting collections varied according to species. For *Anadenanthera macrocarpa*, there was a productivity of 1.2-3.7 minicuttings per ministrain every 26 days (Dias et al., 2012b), and for *Erythrina falcata*, up to 2.9 every 15 days (Cunha et al., 2008).

Variations in productivity responses related to fertilization were also observed even within species of a same genus. The maximum yield of minicuttings (13.1 cutting strain^{-1} month^{-1}) of a clone of *Eucalyptus grandis* x *Eucalyptus urophylla* occurred by using the estimated N dose of 0.12 g L^{-1} (Rocha et al., 2015). However, for *Eucalyptus dunnii*, an increasing linear effect was observed for minicutting production using nitrogen doses of up to 0.6 g L^{-1} (Rosa et al., 2009).

The total productivity of minicuttings per ministrain during one year of production reached 35.3 cuttings at the dose 1.12 g L^{-1} of nitrogen. These values were similar to those verified for *Psidium guineense, P. cattleyanum* and *P. guajava* (35) (Altoé et al., 2011), and similar to the best results observed for *Eugenia uniflora* (38) (Lattuada et al., 2016). Considering the density of plants and the high productivity observed, an estimated production of 2,800 minicuttings per square meter per year was obtained. These values are below those observed, for example, for eucalyptus clones, where yields reached more than 20,000 cuttings per square meter per year (Brondani et al., 2008). However, the intense and long period of improvement of techniques and fertilization for eucalyptus crops should be taken into account when compared to native species. However, knowledge on native species is still incipient.

Rooting of cuttings

For rooting, there was no interaction between seasons and nitrogen fertilization doses. There were only effects of isolated factors. In relation to seasons, cuttings collected in the spring and summer showed high rooting percentages (59 and 56%, respectively), but were not statistically different from the winter (47%). This same behavior was observed for root dry mass (Table 2). The lowest rooting rate was observed in autumn (38%), as well as the lowest root dry mass. This result may been due to the absence of controlled conditions in the rooting bed of the greenhouse, so that cuttings collected in autumn were exposed to the climatic conditions of the subsequent period, in this case, winter.

The collection season also exerted a significant effect on the rooting of cuttings of *Eugenia uniflora*. This effect was attributed to several factors, among them juvenile propagules, physiological conditions, hormonal balance and nutritional conditions throughout the sprout collection periods. This species presented adaptability to different climatic conditions (Peña Peña et al., 2015). However, for *Psidium guineense, P. cattleyanum* and *P. guajava*, the percentage of rooted

Table 2. Rooting (%) and root dry mass (mg) of minicuttings of *Campomanesia aurea* collected at different seasons of the year. UFRGS, Porto Alegre, 2016.

Season	Rooted (%)	RDM (mg)
Autumn	38.63 b	07.84 b
Winter	47.37 ab	20.35 a
Spring	59.01 a	23.93 a
Summer	56.30 a	21.56 a
p-value	≤ 0.01	≤ 0.01

The means were compared by Tukey test at 5% probability.

minicuttings was not influenced by collection seasons, and presented 95.8%, 91.6% and 100% of rooting, respectively (Altoé et al., 2011).

One of the factors related to collection times that may have interfered with rooting is temperature. It has a complex interaction with photoperiods and endogenous levels of auxins and other hormones. High temperatures promote a faster sprout growth and a greater rooting (Hartmann et al., 2011). The results of rooting obtained in this study were higher than those found in previous experiments for cuttings of *C. aurea* collected directly from the field: 28, 20, 8 and 25%, in the summer, autumn, winter and spring, respectively (Emer et al., 2016; Emer et al., 2018). Among the factors that may have contributed to the results obtained by minicutting, there are better nutrition of matrix plants and the juvenile state of the propagules.

The fertilization of matrix plants is determinant for the nutritional condition of the propagules. It is responsible for the volume of carbohydrates, auxins and other metabolic compounds fundamental for the start and for the speed of root formation. Nutrients involved with the metabolic processes of dis-differentiation and meristem formation are essential for the formation of adventitious roots (Cunha et al., 2009). The juvenile state of matrix plants is an important factor especially for species with a difficult rooting. As the plant ages, there is an increase in the levels of inhibitors and a decrease in rooting co-factors (Fachinello et al., 2005).

The rooting percentage of *C. aurea* also varied in function of the dose of nitrogen applied to the ministrain. There was a linear decreasing behavior (Figure 5) different from that observed for *Eugenia uniflora*, in which nitrogen fertilization did not influence rooting (Lattuada et al., 2016). It was different from the behavior verified for clones of *Eucalyptus grandis* x *Eucalyptus urophylla*, where the rooting of minicuttings presented a linear behavior in function of the nitrogen doses applied, reaching a value higher than 90% by using the dose 0.24 g L^{-1}, 30% higher than the control (without nitrogen) (Rocha et al., 2015).

Excessive nitrogen fertilization may affect negatively rhizogenesis. Rooting can be improved by controlling nitrogen fertilization in matrix plants so that shoots are not stimulated by high levels of nitrogen, avoiding the formation of adventitious roots competing with a fast growth of shoots promoted by carbohydrates, nutrients and hormones (Hartmann et al., 2011). The decrease in rooting rates with the increase in nitrogen doses was observed in this study. The highest dose tested (3.60 g L^{-1}) obtained a 21% lower rhizogenesis than the treatment without this element.

Nitrogen is a component of proteins and nucleic acids and one of the most important nutrients for plant growth. It is readily absorbed as nitrate (NO_3^-) and ammonium (NH_4^+). After absorption, nitrogen is incorporated fast into growing tissues such as expanding leaves, root tips and meristems (Kerbauy, 2013). Thus, the use of balanced nutrient solutions is essential as they provide a nutritional balance for ministrains, which is important for obtaining satisfactory levels of rooting (Alfenas et al., 2004).

Although the results for rooting in this study were higher than results obtained by using cuttings collected directly from the field, some factors can still be improved, such as the selection of genotypes more suitable for rooting. We observed a great variability of rooting in cuttings subjected to a same fertilization treatment. They were morphologically similar, indicating that genetic factors may be acting on rooting since matrix plants had a seminal origin. In addition, the management of ministrains can also be improved, leaving only three to four initial branches after shooting in order to improve the architecture of the plant and increase the quality and the productivity of cuttings.

During the conduction of the experiment, we observed a capacity for regrowth of ministrains in two different moments. The first moment was during the experiment, when strains with apparently dead shoots (without leaves and branches) re-sprouted. The second moment was when, after the experiment was finished, we performed the cutting of ministrains at the substrate level. After approximately 15 days,

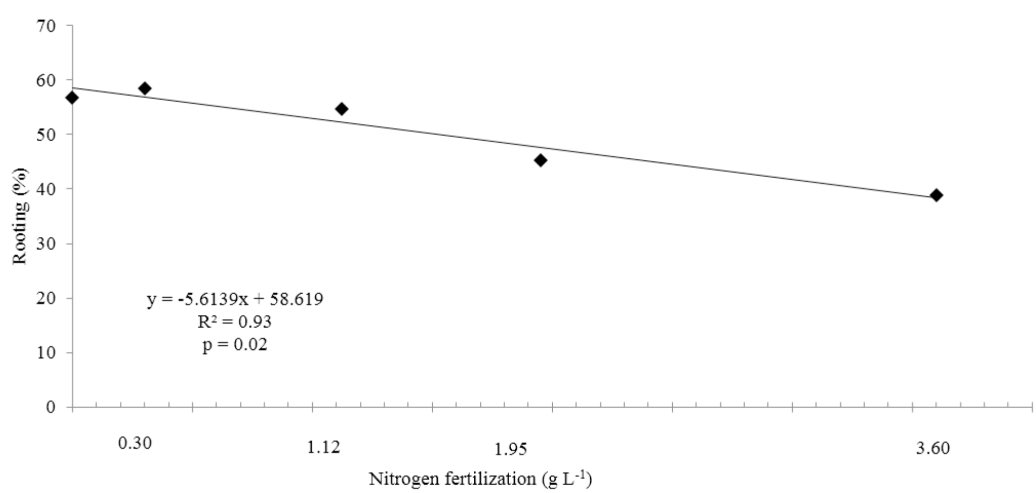


Figure 5. Rooting of ministrains of *Campomanesia aurea* subjected to different nitrogen fertilization doses. UFRGS, Porto Alegre, 2016.

they already presented an intense emission of shoots. This is probably related to the presence of underground xylopodium in this species (Legrand & Klein, 1977), and may constitute an interesting productive feature for the renewal of the strains of minigardens.

The use of materials with a seminal origin has proved to be effective for the production of minicuttings of *C. aurea*. It improved the vegetative propagation of the species, which may serve as a basis for future studies on the rejuvenation of selected genotypes using *in vitro* propagation, for example. In addition, there was also progress in the management of fertilization, noting that the maintenance of salinity levels up to 2 mS cm^{-1} (*Pour Thru* method) is effective to maintain an adequate productivity, avoid the death of ministrains and ensure higher levels of rooting.

Conclusion

The survival of ministrains is influenced by the application of nitrogen fertilization and that the N dose 1.12 g L^{-1} presents better results for the productivity and rooting of minicuttings, especially when collected during spring-summer.

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