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Successive mini-cuttings collection in *Piptocarpha angustifolia* mini-stumps: effects on maturation, adventitious root induction and root vigor

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ABSTRACT. The low percentage of seed germination and adventitious root induction in cuttings of *Piptocarpha angustifolia* has limited its silvicultural use. Thus, we studied the effect of tissue maturation on successive collections of mini-cuttings grown in a semi-hydroponic system. Likewise, we evaluated potential multiplication via the mini-cutting technique by assessing the survival and productivity of mini-stumps as well as the formation of adventitious roots and the root vigor. We made 32 sprout collections during two years in a clonal mini-garden. From these collections, mini-cutting experiments were installed throughout several seasons. Mini-cuttings of 8±1 cm long were prepared, planted in boxes with vermiculite and carbonized rice husk (1:1 v/v) and placed in a greenhouse. After 70 days, we evaluated the adventitious rooting percentage as well as the adventitious root and aerial vigor of the mini-cuttings. The appropriate survival of mini-stumps (68%) and the annual production of mini-cuttings per square meter (2,227) reflected the efficiency of the semi-hydroponic system for this species. The increasing rooting, multiplication rate and root vigor indicate no maturity in the course of the collection of mini-cuttings in *P. angustifolia* mini-stumps.

Keywords: juvenility, multiplication rate, ontogenetic age, root induction, vegetative propagation.

Coletas sucessivas de miniestacas em minicepas de *Piptocarpha angustifolia*: efeito sobre a maturação, enraizamento e vigor radicial

RESUMO. Os reduzidos percentuais de germinação de sementes e enraizamento de miniestacas de *Piptocarpha angustifolia* têm limitado sua evolução silvicultural. Objetivou-se estudar o efeito de sucessivas coletas de miniestacas cultivadas em sistema semi-hidropônico sobre a maturação e o potencial de multiplicação via miniestaquia, por meio da avaliação da sobrevivência, produtividade das minicepas e enraizamento e vigor radicial das miniestacas. Foram realizadas 32 coletas de brotações durante dois anos em minijardim clonal. A partir destas, foram instalados experimentos de miniestaquia em cada estação climática. Foram preparadas miniestacas de 8±1 cm, plantadas em caixas com vermiculita e casca de arroz carbonizada (1:1 v/v) e acondicionadas em casa de vegetação. Transcorridos 70 dias avaliaram-se o enraizamento e o vigor radicial e vegetativo das miniestacas. A boa sobrevivência de minicepas (68%) e produção anual de miniestacas por metro quadrado (2227) refletem a eficiência do sistema semi-hidropônico para a espécie. O aumento do enraizamento, taxa de multiplicação e vigor radicial indicam não haver maturação no decorrer das coletas de miniestacas em minicepas de *P. angustifolia*.

Palavras-chave: juvenilidade, taxa de multiplicação, idade ontogenética, rizogênese, propagação vegetativa.

Introduction

Piptocarpha angustifolia Dusén ex Malme, popularly known as “vassourão branco”, naturally grows in the Araucaria Forest as a pioneer species (Carvalho, 2003; Fossati & Nogueira, 2009). Its high vigor and phenotypic characteristics make this species attractive to the timber industry for potential forestry uses. However, it incipient sexual propagation, especially due its low germination rates, its slow growth rate and lack of uniformity (Fossati & Nogueira, 2009) indicate the need for improving vegetative propagation techniques.

Clonal forestry has generated significant advances in the timber production of Brazil and worldwide (Rosado, Rosado, Alves, Laviola, & Bhering, 2012). One of the challenges that this technique has to overcome is the effect of maturation in woody species, which are shown by physiological and biochemical alterations in the donor plants (Štefančič, Štampar, Veberic, & Osterc, 2007; Osterc, 2011). Likewise, one of the main consequences of applying this technique has been the loss of rooting capacity (Osterc, Štefančič, & Štampar, 2009; Osterc & Štampar, 2011; Husen, 2012; Wendling, Trueman, & Xavier, 2014a) and the reduction of root vigor (Bitencourt, Zuffellato-Ribas, Wendling, & Koehler, 2009) and vegetative vigor (McGranahan, Borralho, & Greaves, 1999), which often limit the expansion of clonal forestry (Wendling, Warburton, & Trueman, 2015).

The use of techniques, such as mini-cuttings, has subsidized the evolution of clonal forestry with increased productivity, standardization of crops and, especially, qualifying forest products (Wendling, Brondani, Dutra, & Hansel, 2010; Baccarin et al., 2015; Kratz, Stuepp, Pires, & Wendling, 2015). In *P. angustifolia,* this technique has proved to be promising. However, the results obtained thus far have not been technically feasible for commercial purposes. For example, it has been not possible to obtain a rooting percentage greater than 45% (Ferriani et al., 2011).

Despite the wide application of the mini-cutting technique in the forestry sector (Xavier, Wendling, & Silva, 2013), little has been studied about the effect of successive sprouts collections in mini-stumps. One of the envisioned hypotheses is the possibility of maturation over the collections, which can be a limiting factor for the rooting capacity and root vigor (Husen, 2012; Wendling, Trueman, & Xavier, 2014b; Rasmussen, Hosseini, Hajirezaei, Druege, & Geelen, 2015; Wendling et al., 2015). The maturation involves physiological and biochemical changes that alter the concentrations of hormones, such as indole-3-acetic acid (IAA), which is involved in the rooting process (Costa et al., 2013), and abscisic acid (ABA), which is linked to the root induction and elongation (Chen, Yang, Lur, Tsai, & Chang, 2006).

Similarly, the decline in the productivity and rooting of cuttings in mild temperatures have been evaluated as a key factor for the production of adventitious roots (Trueman & Richardson, 2008; Da Cunha, Paiva, Leite, Barros, & Leite, 2009). Temperature may affect the increase of endogenous levels of certain metabolites and hormones essential for the rooting process. Likewise, it influences the increase in metabolism and cell division (Hartmann, Kester, Davies Junior, & Geneve, 2011), which influences the maturation based on climate variations (Rasmussen et al., 2015). Little is known about the consequences of maturation in similar weather conditions and prolonged periods of evaluation.

The objective of this study was to understand the maturation effects of successive mini-cutting collections of the same mini-stumps across different seasons and the potential multiplication of the species via mini-cuttings by evaluating the survival and production of mini-stumps, rooting, root vigor and mini-cutting multiplication rate.

Material and methods

Experimental area characterization

The experiment was conducted between the winter (July) of 2013 and the autumn (June) of 2015 at the Forest Species Propagation Laboratory - Embrapa Forests in Colombo, Paraná State (25°20' S and 49°14' W, 950 m), Brazil. According to the Köppen classification, the climate of the region is temperate, Cfb type, with the coldest month temperature in the range of -3 and 18°C, always damp, with rains well distributed throughout the year and an average temperature of the warmest month below 22°C.

Original cuttings

Cuttings of *P. angustifolia* were obtained by traditional cutting techniques following the plant rescue methodology through epicormic shoots (Stuepp, Zuffellato-Ribas, Wendling, Koehler, & Bona, 2014). The cuttings came from coppice shoots of adult plants (approximately 12 years of age) collected in January, 2013 in a remnant of secondary, mixed ombrophyllous forests in the city of Petrolândia, Santa Catarina State (27°44' S and 50°02' W, 410 m), Brazil. After rooting in plastic tubes of 55 cm^3 filled with fine grained vermiculite and carbonized rice hull (1:1), the cuttings were placed in a shade house (50% of irradiance and irrigation with micro sprinkler irrigation three times a day for 10 minutes and a flow of 144 L $hour^{-1}$). A growth fertilization (4 g L^{-1} urea, 3 g L^{-1} superphosphate, 0.25 g L^{-1} FTE BR 10 [7% Zn, 4% Fe, 4% Mn, 0.1% Mo, 2.5% B, 0.8% Cu] and 3 g L^{-1} of potassium chloride) was performed every seven days for 60 days.

Management and nutrition of the mini-stumps

The plants were transferred to a semi-hydroponic system suspended in sand beds, spaced by 20 x 20 cm, in May, 2013. To adapt to the new nutritional management, the pruning of the apical budding was performed after 30 days for the homogenization of the mini-stumps. Pruning was performed 10 cm above the lap part of the mini-stumps, and at least two remaining leaves were kept. The mini-garden was established in a greenhouse covered with polyethylene, where the mini-stumps received drip fertigation three times a day at an average flow rate of 6 L m^{-2} day^{-1} with the adapted nutrient solution described in Wendling, Dutra, and Grossi (2007).

Samples were collected at intervals ranging from 18 days (summer) to 26 days (winter) in a selective way, i.e., shoots less than 15 cm in length and with less than three pairs of leaves were kept in the mini-stumps for subsequent collections. Thus, the mini-stumps were subjected to 32 successive collections over a two-year period, which included autumn, winter and spring of 2013, as well as summer, autumn, winter and spring of 2014 and summer of 2015.

The clonal mini-garden was implemented according to a completely randomized design in a model of split plot, with five replicates of 10 mini-stumps for each experimental unit.

Survival and mini-cutting production in the mini-stumps

During the successive collection of sprouts, the survival rate of mini-stumps (SM), the mini-cutting production per square meter per month (PPSM) and per mini-stump (PPMS) were recorded. These variables were evaluated for a period of 24 months (July, 2013 to June, 2015) that included spring (from September 23rd to December 21st), summer (from December 21st to March 21st), autumn (from March 21st to June 21st) and winter (from June 21st to September 23rd). For the purpose of transformation and data analysis, one month is considered equivalent to a period of 30 days.

Adventitious root induction in mini-cuttings

Mini-cuttings with 8±1 cm of length and an average diameter of approximately 0.5±0.1 cm were prepared, keeping up to two leaves with a 50% reduction of its original surface. The mini-cuttings were planted in plastic boxes (14.43 liters) filled with vermiculite and carbonized rice husk in a ratio of 1:1 (v/v) at approximately 2 cm of depth. These boxes were placed in a greenhouse with intermittent mist (average temperature of 24±2°C and 85% relative humidity; Figure 2).

After 70 days of installation, we evaluated the rooting percentage (live mini-cuttings with roots of at least 2 mm in length), the number of roots/mini-cuttings (NR), the length of the three major roots/mini-cuttings (LRL), the percentage of mini-cuttings with callus (survival of mini-cuttings without roots and with a mass of undifferentiated cells at the base), the percentage of callus and rooted mini-cuttings (survival mini-cuttings, with roots of at least 2 mm in length and that formed an undifferentiated cell mass at the base), the survival rate (survival of mini-cuttings that did not have roots induced or callus formation), the mortality rate (mini-cuttings with necrotic tissue), the sprout emission rate (percentage of mini-cuttings with new shoots of at least 2 mm of length) and the maintenance of the original leaves in the mini-cuttings (percentage of mini-cuttings that have retained the original leaves in the rooting bed). From these variables, we calculated the multiplication rate (MR) per square meter/month according to the Wendling et al. (2015) methodology.

$$MR = PPSMQ\ (m^2 month^{-1})\ .\ \%\ Rooting$$

where, MR represents the multiplication rate, and PPSM represents the mini-cutting production per square meter.

The adventitious rooting experiments were conducted in a completely randomized design with eight treatments (seasons) and four replicates of 20 mini-cuttings per experimental unit on the following dates: July 15th (winter, 2013); October 28th (spring, 2013); January 30th (summer, 2013); April 29th (autumn, 2014); July 28th (winter, 2014); November 29th (spring, 2014); February 2nd (summer, 2014); April 12th (autumn, 2015).

Statistical analysis

The data were analyzed with an analysis of variance (ANOVA), the treatment variances were evaluated for homogeneity with the Bartlett's test, and the variables with significant differences in the F test had their means compared with the Tukey test at 5% probability. To check the influence of minimum, maximum and average temperatures and productivity variables evaluated in the mini-garden and between the biometric variables in mini-cuttings, Pearson correlation analysis was applied ($p < 0.01$ and $p < 0.05$).

Results and discussion

Mini-stump productivity and survival

The survival of mini-stumps (SM) showed a downward trend over the 32 collections; the mortality started from the 6th collection and reached 68% at 24 months (Figure 1a). This result was expected considering the long period of shoot collection on the mini-stumps and the lack of replanting during the experiment. The mini-cutting productivity was similar to that reported in the literature and ranged from 4.5 (winter, 2013) to 12.1 (summer, 2013) mini-cuttings per mini-stump. Under similar conditions, the productivity of *P. angustifolia* reached 6.7 mini-cuttings per mini-stump in 30-day intervals in the spring (Ferriani et al., 2011).

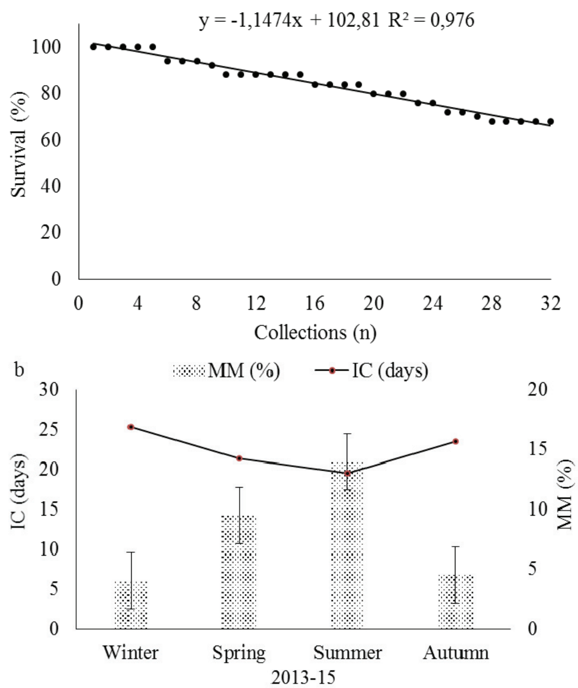

Figure 1. Survival of *P. angustifolia* mini-stumps in a clonal mini-garden according to the successive collections. (a) Relationship among mini-stumps mortality (MM) and intervals among data collection depending on the seasons. (b) Collections 1-4 (winter, 2013); collections 5-8 (spring, 2013); collections 9-12 (summer, 2013-14); collections 13-16 (autumn, 2014); collections 17-20 (winter, 2014); collections 21-24 (spring, 2014); collections 25-28 (summer, 2014-15); collections 29-32 (autumn, 2015). The bars indicate the standard deviation.

The seasonal effect on the mini-stumps mortality became evident, with a strong influence of the high temperatures of the summer and spring months (Figure 1b). Furthermore, the relationship with the mean interval between collections (IC) reflected the sensitivity of the mini-stumps to the adopted management conditions, which shows the effect of the minimum, maximum and average temperatures on the interval between collections. This result was evidenced by the negative correlation between these variables (Table 1).

The shortest interval between collections in the summer induced mini-stump death. This outcome might be the result of a greater stress generated in that period, which limited the recovery between successive collections. *P. angustifolia* shoots present an herbaceous characteristic, which allows the initiation of necrosis in the shoots cut points of the mini-stumps. This necrosis, in most cases, ceased as new sprouts grew; however, in certain extreme cases, the necrosis tended to expand and resulted the in mini-stump death.

A low initial productivity is commonly found in mini-cutting of forest species (Wendling et al., 2007; Stuepp, Zuffellato-Ribas, Wendling, & Koehler, 2015a). This characteristic may be related to a mini-stump adaptation to the semi-hydroponic system and seasonal effects, mainly of the temperatures (Da Cunha et al., 2009; Wendling et al., 2015). The increase in the average temperature of the summer favored the production of mini-cuttings per square meter (PPSM; Figure 2), with a positive correlation among these two factors (Table 1a), and a reduction in the intervals between samples (Figure 1b).

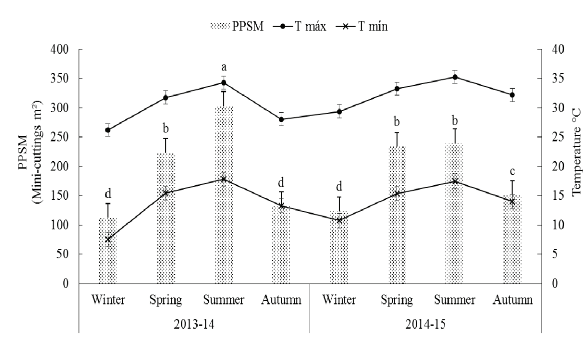

Figure 2. *P. angustifolia* mini-cutting production per square meter (PPSM) according to the seasons between winter, 2013 and autumn, 2015. The average numbers followed by the same letter did not differ from each other with the Tukey test at 5% probability. The bars indicate the standard deviation.

Table 1. Correlations between the minimum, maximum and average temperatures and the productivity variables evaluated in the clonal mini-garden (a) and among the biometric variables in *P. angustifolia* mini-cuttings after its rooting.

	Clonal mini-garden (a)			Greenhouse (b)					
	IC	MS	PPSM	RM	NR	ALR	S	C	LM
T Min.	- 0.88 **	- 0.45 ns	0.85 **	0.48 ns	0.71 ns	0.98 **	- 0.90 **	- 0.41 ns	- 0.84 **
T Max.	- 0.91 **	- 0.59 ns	0.85 **	0.49 ns	0.85 **	0.84 **	- 0.86 **	- 0.73 *	- 0.83 **
T Avg.	- 0.91 **	- 0.53 ns	0.87 **	0.50 ns	0.80 *	0.95 **	-0.91 **	- 0.58 ns	- 0.87**

* and ** represent significant differences at the 5 and 1% error probability, respectively; "ns" means no significant differences at 5% of probability in the F test. T Min, minimum temperature; T Max, maximum temperature; T Avg., average temperature; IC, interval between collects; MS, mini-stumps survival; PPSM, mini-cutting production per square meter; RM, rooted mini-cuttings; NR, number of roots; ALR, average root length; S, survival mini-cuttings; C, mini-cuttings with calluses; LM, mini-cuttings with maintained leaves.

The productivity of mini-stumps is a reflection of the adopted management and nutrition system. A semi-hydroponic system in sand, such as the one used in this study, was evaluated in previous studies with good results for *P. angustifolia* (Ferriani et al., 2011) and for several species of the *Eucalyptus* genus (Wendling et al., 2007; Da Cunha et al., 2009; Brondani et al., 2012a, 2012c). The methodology of selective collection and nutritional management of mini-stumps proved to be effective and had little variation among collections made in the same season. This system is a common variation in mini-cutting techniques (Brondani et al., 2012c) and has been linked to the physiological effect of mini-stumps, which indicates a greater or lesser recovery capacity under the influence of successive collections (Brondani et al., 2012a).

A higher production of mini-cuttings in the period of 2013-14 was evident, especially in the summer (23.5%) and spring (3.2%). In addition, it was higher if compared with the period 2014-15. This effect was not observed in the autumn and winter, with an increase in the period of 2014-15 of approximately 5.2% and 24.4%, respectively, compared with the period of 2013-14. These results indicate the effect of enlarging the root and air surface of the mini-stumps because the nutritional management was balanced and the change in average temperatures was not significantly different between two periods. These factors could influence the increase in productivity.

Likewise, the increase in productivity during the summer and spring was the result of higher average temperatures in these seasons, which is common in forest species mini-cuttings (Pires, Wendling, Auer, and Brondani, 2015; Peña Peña, Zanette, & Biasi, 2015). Temperature is a preponderant factor during mini-cutting production; it exerts an influence on nutrient absorption and metabolism of the mini-stumps, with a consequent increase in the cell division of plants (Hartmann et al., 2011; Rasmussen et al., 2015).

Research on the effects generated by successive collections in mini-stumps of forest species are still scarce (Mcmahon, Hung, & Trueman, 2013; McMahon, Hung, & Trueman, 2014; Wendling et al., 2015). Moreover, little is known about the efficiency in maintaining juvenility, which varies according to the species and the management methodology adopted (Hamann, 1998; Aimers-Halliday et al., 2003; Wendling et al., 2015). Maintaining the juvenile vigor of these plants is significantly important for the rooting process because the juvenile effect may be directly related to the endogenous concentrations of auxin and cofactors that are essential for the rooting process (Štefančič, Štampar, & Osterc, 2005; Ludwig-Müller, 2009; Kazan, 2013). After 32 collections, the surviving mini-stumps presented good phytosanitary and physiological conditions in terms of root systems and aerial parts and did not show symptoms of exhaustion.

The greatest rooting rate was found in spring, 2014 (71.3%), followed by those during spring 2013 (67.5%), winter 2014 (61.3%) and autumn 2014 (58.8%; Figure 3a). With the exception of summer, there was an increase in the percentage of rooting across the seasons in both evaluation periods (2013-14 and 2014-15). This outcome is partly due to a better technical adaptation to the species characteristics, and it is also a reflection of the proper management of the clonal mini-garden, which favored the rooting and mini-cutting root vigor.

The high productivity of mini-cuttings in the warmer seasons favored the multiplication rate, with the best results obtained in summer 2013 (175.1 plants m^2 $month^{-1}$), spring 2014 (166.1 plants m^2 $month^{-1}$), spring 2013 (162.5 plants m^2 $month^{-1}$) and summer 2014 (138.7 plants m^2 $month^{-1}$), which significantly differed from the rest of the seasons (Figure 3b).

The increase in the chronological age of the donor plants can cause severe losses in the rooting potential of woody species (Štefančič et al., 2007, Osterc & Štampar, 2011). One technique, suitable for maintaining the juvenility of these plants, is based on the continuous pruning of mini-stumps (Hamann, 1998; Aimers-Halliday et al., 2003; Wendling et al., 2015). Few reports evaluated this effect on woody species, and most research was performed with conifers (Wendling et al., 2014a, 2014b). In this study, the high levels of multiplication and rooting rates showed the efficiency of continuous pruning at 10 cm of height for maintaining the juvenility of *P. angustifolia* mini-stumps during a period of twenty-four months.

In general, the percentage of adventitious root induction and root vigor (48.8% and 71.3%, respectively; Figure 3a) were above the average reported in the literature (45.0%, 6.3 roots per mini-cutting and 8.9 cm per mini-cutting (Ferriani et al., 2011). These results indicate that it is possible to obtain consistent rooting percentages using appropriate plant materials and controlling key environmental factors in a greenhouse (temperature and humidity).

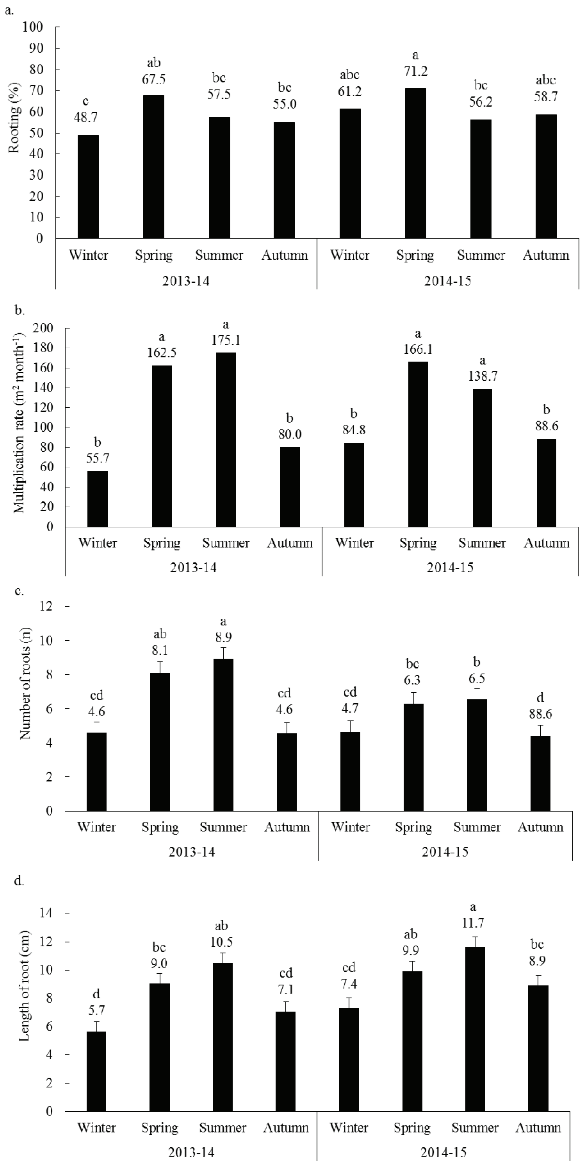

Figure 3. Rooting percentage (a), multiplication rate (b), number of roots (c), and average length of three major roots (d) in *P. angustifolia* mini-cuttings between winter 2013 and autumn 2015. The bars indicate the standard deviation.

Similarly, the number of roots and the longest root length were higher than those reported in the literature and reached 8.9 roots (summer 2013/14) and 11.7 cm (summer 2014/15), respectively. Both variables were positively influenced by the rising temperatures during summer and spring (Figure 3b,c), which is confirmed by the positive correlation between the average temperatures and NR (0.85) and LRL (0.84; Table 1b).

There was a clear reduction in the NR among seasons and between the two evaluation periods (2013-14 and 2014-15; Figure 3b) due to the higher emission of calluses in the cold seasons (Figure 4b). Another factor that may explain the reduction in the NR in both periods can be the maturation of mini-stumps.

Maturation in woody species has been the focus of several discussions (Greenwood, 1995; Aimers-Halliday et al., 2003; Wendling et al., 2014a,b); however, there is a clear need for further studies to elucidate its dependency on physiological effects. The influences of culture conditions may prevail in the catalyst vegetable ripening; for example, the exposure of plants to specific culture stress conditions can prolong the juvenile stage. In contrast, conditions that favor the vegetative vigor can maintain and accelerate this transition from the juvenile to adult phase (Bond, Czarnomski, Cooper, Day, & Greenwood, 2007).

In the case of woody species, long-term experiments are often impractical to determine these changes (Baccarin et al., 2015). However, ripening is not only a consequence of chronological age, and further studies are necessary to define these parameters in *P. angustifolia.*

The LRL showed an upward trend throughout the experiment, especially in summer 2014 (11.7 cm), summer 2013 (10.5 cm) and spring 2014 (9.9 cm), which were higher than the rest of the evaluated seasons. The increase in average temperatures in the summer and spring favored the metabolism of mini-cuttings, reduced the average time (days) of adventitious root induction and, consequently, increased the LRL. The temperature rise in the period of 2014-15 (Figure 2) was reflected in a reduced rooting in the summer 2014; however, it was favorable for the increase in LRL (Figure 3b).

The mini-cutting survival increased with lower temperatures, and it reached 25% in the winter 2013. These results are confirmed by the significant correlation between these variables (-0.86). There was also a trend toward a decrease the survival between the climatic periods of 2013-14 and 2014-15, which is a consequence of the higher percentage of mini-cuttings with callus and roots in the last period.

The high percentage of mortality in *P. angustifolia* has been justified by the oxidative stress generated by high temperatures in the warm seasons (Ferriani et al., 2011; Stuepp, Zuffellato-Ribas, Wendling, Koehler, & Bona, 2015b). Similarly, the mortality was significantly influenced by the high summer temperatures and reached 30% (summer 2013; Figure 4a). However, lower levels are reported in the literature for this species (50 and 42.5% for winter and spring, respectively; Ferriani et al., 2008; Ferriani et al., 2011).

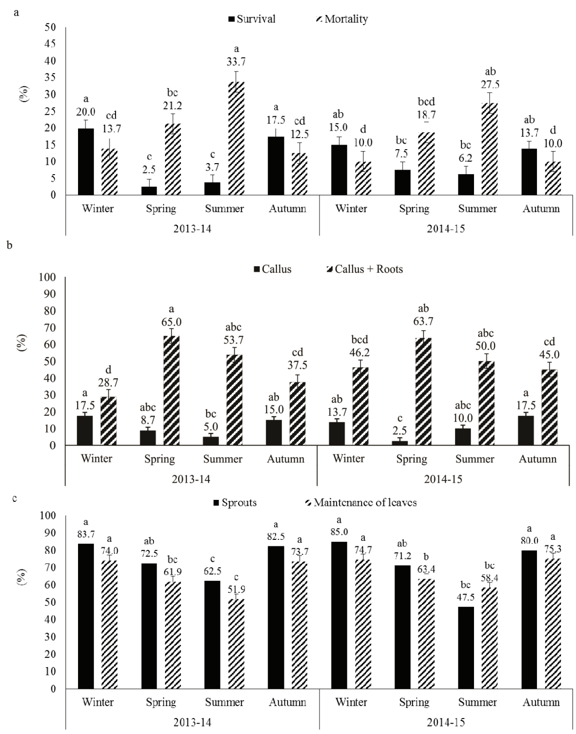

Figure 4. Overall averages of survival and mortality (a), callus emission and rooted mini-cuttings with callus (b), leaf maintenance and shoot emission (c) in *P. angustifolia* mini-cuttings between winter 2013 and autumn 2015. The bars indicate the standard deviation.

The ideal temperature for rooting cuttings is variable for different species, i.e., approximately 25-35°C for the *Eucalyptus* genus (Brondani et al., 2012a), 24±2°C for *Paulownia fortunei* (Stuepp et al., 2015a, b), and 20-30°C for *Anadenanthera macrocarpa* (Dias, Xavier, Oliveira, Paiva, & Correia, 2012). In addition, regardless of the species, humidity has been recommended to be above 80%. However, these conditions are not only ideal for rooting but also for the proliferation of pathogens (Wendling et al., 2010; Brondani, Wit Ondas, Baccarin, Gonçalves, & Almeida, 2012b; Shibuya, Taniguchi, Tsukuda, Shiozaki, & Itagaki, 2014), which might be one of the key factors in the increased mortality percentage during the summer.

The callus induction showed a slight upward trend between the two climatic periods; however, differences between the evaluated seasons were not verified. The highest value was recorded in the autumn of 2014 (17.5%). In general, the higher percentage of callus favored rooting because the indirect formation of roots is a characteristic of this species (revealed by the percentage of mini-cuttings with callus and roots in the spring 2013, 65%; Figure 4b).

The sprout emission was high in all the seasons, with an upward trend for the climatic period 2014-15 and followed the rooting trend. The high shoot emission may have been a major factor in the mini-cutting mortality in the warmer seasons. Higher temperatures considerably expand the respiratory rate of plants and deplete the carbohydrate reserves stored in mini-cuttings even before root initiation (Rasmussen, Smith, & Hunt, 2009).

Similarly, temperature has a strong influence on the leaf maintenance. Mild, cold seasons favored its maintenance on the mini-cuttings, which was evidenced by the negative correlation between maximum temperature and leaf maintenance (-0.87). The lower air temperatures during the rooting process can expand the availability of carbohydrates, thereby reducing the leaf senescence (Druege & Kadner, 2008; Shibuya et al., 2014; Schuler & McCarthy, 2015).

Vegetative propagation seems to be an excellent tool for the genetic improvement of forest species because it favors the breeding of genetically superior individuals, which results in a greater uniformity in grown plants (Wendling et al., 2010; 2015). In addition, it is an alternative for improving the productivity of forest species (Pijut, Woeste, & Michler, 2010). In this context, the production of viable mini-cuttings, their rooting potential and root vigor are considered as juvenility markers (Pijut, Woeste, & Michler, 2010; Husen, 2012; Wendling et al., 2015).

Conclusion

The juvenility in *P. angustifolia* clonal mini-gardens can be maintained through continuous pruning of the mini-stumps. Moreover, autumn and winter are the most favorable seasons for their survival. Regarding mini-cuttings, spring and summer are the recommended seasons as periods of higher productivity and better mini-cutting rooting.

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