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Propagation

Methods of application of indolebutyric acid and basal lesion on 'Woodard' blueberry cuttings in different seasons

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Abstract - Blueberry cuttings are difficult to root, so alternatives that maximize their rhizogenic potential are essential for the expansion of the crop. The objective of this work was to evaluate the effect of the basal lesion and different methods of indolebutyric acid (IBA) on the rooting of 'Woodard' herbaceous cuttings, collected in two seasons. The experimental design used was completely randomized in a 3x2x2 factorial arrangement, totaling 12 treatments and five replications. The factors consisted of different ways of IBA application (talc and alcohol in the concentration of 1,000 mg L^{-1}, and without IBA), season of collection (autumn and summer), and two types of cuttings (with and without lesion in the basal portion). Two hundred days after the beginning of the experiment, there was no significant effect of the basal lesion on the rooting of the cuttings. However, it was found that rooting is influenced by the season of collection, with greater leaf retention, dry weight, number of roots per cutting, length of roots and length of the largest root collected in summer. The application of IBA talc provided a higher percentage of rooted cuttings (61.0%) in relation to alcohol (31.0%) and control (41.0%) when collected in the autumn. There was no difference between seasons when IBA was applied with talc, however, the application with alcohol solution and the control resulted in higher percentages of rooted cuttings in the summer (70.0% and 67.0%, respectively). Summer was considered the best season to collect 'Woodard' blueberry cuttings, although the IBA applied with talc has increased the percentage of rooted cuttings in the autumn. The basal lesion did not promote an increase in rooting.
Index Terms: IBA, plant growth regulator, propagation, rooting, *Vaccinium* sp.

Métodos de aplicação de ácido indolbutírico e lesão basal em estacas de mirtilo 'Woodard' em diferentes épocas

Resumo - O mirtilo é uma espécie de difícil enraizamento; sendo assim, alternativas que maximizem o potencial rizogênico de suas estacas são fundamentais para a expansão da cultura. O objetivo deste trabalho foi avaliar o efeito da lesão basal e diferentes métodos de aplicação de ácido indolbutírico (AIB) no enraizamento de estacas herbáceas de mirtilo 'Woodard', coletadas em duas épocas do ano. O delineamento experimental utilizado foi inteiramente casualizado, em arranjo fatorial 3x2x2, totalizando 12 tratamentos e cinco repetições. Os fatores consistiram em diferentes formas de aplicação de AIB (em talco e em álcool, na concentração de 1.000 mg L^{-1}, e sem AIB), em épocas de coleta de estacas (outono e verão), e em dois tipos de estacas (com e sem lesão na porção basal). Após 200 dias do início do experimento, não foi observado efeito significativo da lesão basal no enraizamento das estacas. Porém, verificou-se que as estacas do mirtilo 'Woodard' são influenciadas pela época de coleta, com maior retenção foliar, massa seca, número de raízes por estaca, comprimento de raízes e comprimento da maior raiz, quando coletadas no verão. A aplicação de AIB em talco proporcionou maior porcentagem de estacas enraizadas (61,0%) em relação ao álcool (31,0%) e ao controle (41,0%), quando coletadas no outono. A aplicação em talco não diferiu entre as épocas, porém a aplicação em álcool e o controle resultaram em maiores porcentagens de estacas enraizadas no verão (70,0% e 67,0%, respectivamente). Conclui-se que o verão é a melhor época para a coleta de estacas de mirtilo 'Woodard', embora o AIB aplicado na forma de talco tenha aumentado a porcentagem de estacas enraizadas no outono. A lesão basal não promoveu incremento no enraizamento de estacas.
Termos para indexação: AIB, enraizamento, propagação, regulador vegetal, *Vaccinium* sp.

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Introduction

Blueberry (*Vaccinium* sp.) has economic importance within the group of small fruits due to its functional properties, flavor, color and wide possibility of processing, which results in expansion of the growing area worldwide (ZHANG et al., 2019a). This species can be propagated by various methods, such as seeds, grafting, tissue culture and cuttings, being the latter one the most used, because it allows maintaining the characteristics of the mother plant, ensuring high uniformity, with less costs and less time for the nursery production (FISCHER et al., 2014).

However, the difficulty of propagating the blueberry through cuttings is one of the main limiting factors for its expansion (SHAHAB et al., 2018). The formation of adventitious roots is a complex developmental process that reflects the plasticity of plants to adjust to stress conditions and regenerate plant tissues (ZHANG et al., 2019b).

The formation of adventitious roots is considered a prerequisite for successful cutting propagation. However, due to the special architecture of the blueberry root, which consists mainly of thin roots, its cultivation requires certain environmental conditions, such as moisture, permeability and soil pH, which makes the process difficult, usually leading to a lower percentage of rooting (AN et al., 2019).

For species that are difficult to root, such as blueberry, auxiliary techniques can be used using plant growth regulators, such as indolebutyric acid (IBA) to promote the emergence of adventitious roots (VIGNOLO et al., 2012). Although the natural auxin widely found in plants is indoleacetic acid (IAA), the exogenous application of synthetic auxins is more effective in promoting rooting quality, being often used in vegetative propagation by cuttings, as they act in a similar way to the natural plant growth regulator (BRAHA; RAMA, 2016).

Synthetic auxin can be applied by different methods, especially liquid (combined with alcohol) and powder (combined with talc). According to Hartmann and Kester (2002), the application of liquid IBA is, generally, more effective than the application in powder form, providing more uniform results. However, Fachinello et al. (2013) describe that the application via powder is easy to prepare and is more stable. In this context, experiments have been carried out with the application of auxins in both ways, liquid and powder, showing that the responses are different depending on the cultivar and the species (COLOMBO et al., 2018; KOYAMA et al., 2019; PEÑA et al., 2012; SHAHAB et al., 2018).

A technique that can also provide better results in fruit species that are difficult to root is to perform a lesion at the base of the cuttings. The exposure of the cambium and the cortex region provided by the incision in the tissue can facilitate the absorption of water and rooting-promoting substances (FACHINELLO et al., 2013), although the results of this technique are variable (KOYAMA et al., 2019; TREVISAN et al., 2008; WAGNER JÚNIOR et al., 2004).

Another factor conditioned to rooting is the season when cuttings are collected. Zem et al. (2015) observed that the season of the year in which the propagation material is collected influences the induction of rhizogenesis, which is related to tissue nutrient reserves and their activity, as well as the distribution of auxins throughout the seasons. Several authors have reported that summer is the best time to collect cuttings, because during this period of the year, it is most favorable for root emission and growth (KOYAMA et al., 2018, 2019; MARANGON; BIASI, 2013). However, Fischer et al. (2008), evaluating the effect of IBA on the rooting of woody cuttings of several blueberry cultivars, obtained 55.5% to 92.5% of rooting, depending on the cultivar, indicating that the woody cutting collected in winter season is a technique that presents viability of use for that species.

The factors that influence the rooting of cuttings are quite variable and their action can occur in isolation or by interaction with others (TREVISAN et al., 2008), and in the case of difficult rooting species, the study involving the combination of these factors becomes essential. Therefore, the objective of this study was to evaluate the effect of basal lesion and different methods of application of IBA, on the rooting of herbaceous cuttings of ‘Woodard’ blueberry, collected in two seasons of the year.

Material and methods

The trial was conducted from March to October 2015 and from January to August 2016, at the Fruit Sector of the Agricultural Research Center, State University of Londrina – PR, Brazil (latitude 23° 23’ S, longitude 51° 11’ W, 566 m asl). Herbaceous cuttings with 10 to 12 cm long were taken from the middle portion of the shoots of stock plants of nine-year-old ‘Woodard’ blueberry (*Vaccinium* sp.), grown in pots in a greenhouse, obtained from the collection of blueberry cultivars belonging to Embrapa Clima Temperado, Pelotas - RS, Brazil.

The experimental design used was completely randomized in a 3x2x2 factorial arrangement, with 12 treatments and five replications, and each plot consisted of 10 cuttings. The following factors were evaluated: forms of application of IBA (with powder talc and with alcohol at a concentration of 1,000 mg L^{-1}, and without IBA); season (autumn and summer); and types of cutting preparation (with and without lesions in the basal portion).

Before collecting the cuttings, a hydroalcoholic IBA solution was prepared by weighing 0.1 g of concentrated IBA (99.9% purity; Sigma-Aldrich®, St. Louis, MO, USA) on a semi-analytical scale, and dissolved in 50 mL of alcohol (PA). After complete dissolution of the IBA, the volume was adjusted to 100 mL with distilled water, obtaining a concentration of 1,000 mg L^{-1} of IBA. For the preparation of IBA in powder talc, 0.1 g of concentrated IBA was mixed with 100 g of industrial inert talc (Quimidrol®, Joinville, Brazil). For better homogenization, alcohol (P.A.) was added in sufficient quantity to form a paste and, then, it was transferred to an oven at 40°C, where it remained until complete evaporation of the solvent.

To prepare the cuttings, a bevel was performed at the bottom of the cuttings, just below the node, in order to increase the surface area of this part. The leaves from the basal portion were removed, keeping a pair of leaves cut in half at the top of the cutting. The lesion at the base of the cutting was performed with the aid of a sharp knife. For this, two superficial cuts were made in the basal part, on opposite sides, gently peeling a portion of the bark about 2 mm wide and 2 cm long. Cuttings were prepared in the morning and immediately placed on trays with water to prevent dehydration. After preparing the cuttings, the treatment with liquid IBA was applied in the basal part by rapid immersion for 10 s, and for the treatment with power talc was performed by touching the basal portion of the cutting in the powder solution.

The cuttings were placed in perforated plastic boxes (44x30x7 cm) containing the substrate carbonized rice husk, and subjected to an intermittent mist system controlled by a timer and solenoid valve. The valve was programmed to mist for 10 s every 6 min. The mist used (Model DAN-7755 Modular Greenhouse Sprinkler, Tel Aviv, Israel) has a flow rate of 35 L h^{-1}. The mist chamber was kept in an greenhouse covered with plastic film and black mash screen with 30% shading.

To control fungal diseases, the fungicide tebuconazole (1 mL L^{-1}) was sprayed weekly. Foliar fertilization was carried out every 15 days with the fertilizer Biofert Plus® (8-9-9 + micronutrients) at a concentration of 5 mL L^{-1}.

The experiments were evaluated identically at two seasons of the year, i.e., in mid-March (cuttings collected in autumn 2015) and in mid-January (cuttings collected in the summer of 2016).

Two hundred days after the installation of the experiments, the following variables were evaluated: cutting survival (% of live cuttings); rooted cuttings (% of cuttings that have emitted at least one root); leaf retention (% of cuttings that did not lose leaves); sprouted cuttings (% of cuttings with sprouts); number of roots per cutting; length of roots (cm); length of the longest root (cm) and root dry mass (g). Root dry mass was obtained by drying in an oven with forced air circulation at 78 °C for 24 h.

Data were subjected to fixed effects analysis of variance at 5% probability of error. The assumptions of normality of errors and homogeneity of variances were tested by Shapiro-Wilk and Bartlett, respectively. When one of the assumptions was not met, the data were transformed according to the Box-Cox method (1964). If a significant effect was found, the means were compared using the Tukey test ($p<0.05$). When there was a significant effect of simple factors with two levels, Tukey's multiple comparison test was not performed, only verified by the mean and by the F test.

To complement the data analysis, principal component analysis (PCA) was performed in order to reduce the dimensionality of the data, group and detect correlations between the evaluated parameters. The assumption of Bartlett's test of sphericity was used to assess whether the correlation matrix is not an identity matrix, that is, without correlation. PCA was performed by using FactoMineR (HUSSON et al., 2016) and Factoextra (KASSAMBARA; MUNDT, 2017) packages. All analyzes were performed by using R software (R CORE TEAM, 2020).

Results and discussion

According to the analysis of variance, there was no significant effect of factors and interactions for the percentages of survival and sprouted cuttings, with means of 71.83% and 37.8%, respectively. On the other hand, there was a significant effect of the season for the variables leaf retention, dry mass, number of roots, length of roots and length of the longest root, with a significant interaction between the forms of application of IBA and the season only for the percentage of rooted cuttings (Table 1), in which summer presented the best response for all these variables (Figure 1).

Cuttings collected in the summer showed 50.0% of leaf retention, while those collected in the autumn 13.0% (Figure 1a). Koyama et al. (2018) evaluated the influence of the length of 'Woodard' cuttings on different substrates, and reported that 5 cm-cuttings (minicuttings) rooted in carbonized rice husks, collected in the summer, resulted in 85.0% of leaf retention, while in the autumn only 15.0%, corroborating the results found in the present study. The higher values obtained by those authors may be related to the fact that minicuttings provide a smaller surface area exposed to the environment and, therefore, reduce the demand for water to keep tissues alive, compared to longer cuttings (LIMA et al., 2006).

According to Fachinello et al. (2013), the presence of leaves in herbaceous cuttings favors a more efficient rooting, as there is the production of rooting cofactors that act synergistically with auxins. Several authors reported that the higher percentage of cuttings with leaf retention may have contributed to the increase in rooting of blueberry cuttings (COLOMBO et al., 2018; KOYAMA et al., 2018; MARANGON; BIASI, 2013; PELIZZA et al., 2011).

Table 1. Sum of squares of variance analysis regarding the cutting survival (CS), leaf retention (LR), sprouted cuttings (SC), rooted cuttings (RC), dry mass of roots (DM), number of roots per cutting (NR), length of roots (LFR), length of the longest root (LL) of 'Woodard' blueberry subjected to different solutions of indolebutyric acid (IBA), with or without lesions at the base of the cuttings, and collected in different seasons.

Source of variation	df	CS (%)	LR (%)	SC (%)	RC (%)	DM[t] (%)	NR	LFR (cm)	LL (cm)
IBA (A)	2	253.4	0.90	3.4	730.0	0.02578	1.44	3.0	1.4
Lesion (B)	1	375.0	2.82	81.7	202.0	0.01553	0.71	9.1	5.4
Season (C)	1	1,401.7	205.35**	601.7	6,202.0**	2.79470**	105.48**	631.1**	1,205.6**
Repetition	4	1,390.0	7.56	2,510.0	1,360.0	0.19640	3.44	14.0	15.2
AxB	2	1,720.0	1.68	723.4	1,604.0	0.01794	1.44	6.8	8.2
AxC	2	1,293.4	3.90	1,143.4	4,864.0**	0.16032	2.56	1.8	1.6
BxC	1	15.0	2.02	1,041.7	135.0	0.00640	3.14	5.6	0.4
AxBxC	2	1,240.0	1.04	103.4	1,910.0	0.01340	0.02	3.8	1.4
Residual	44	17,010.4	87.12	18,009.2	16,676.0	1.32880	53.68	118.8	184.8
CV		27.37	44.70	53.47	35.73	59.33	29.20	21.23	20.86

df: degrees of freedom; **Significant by F test t 1% of probability. [t]Data transformed into $\sqrt{x}$. CV: coefficient of variation.

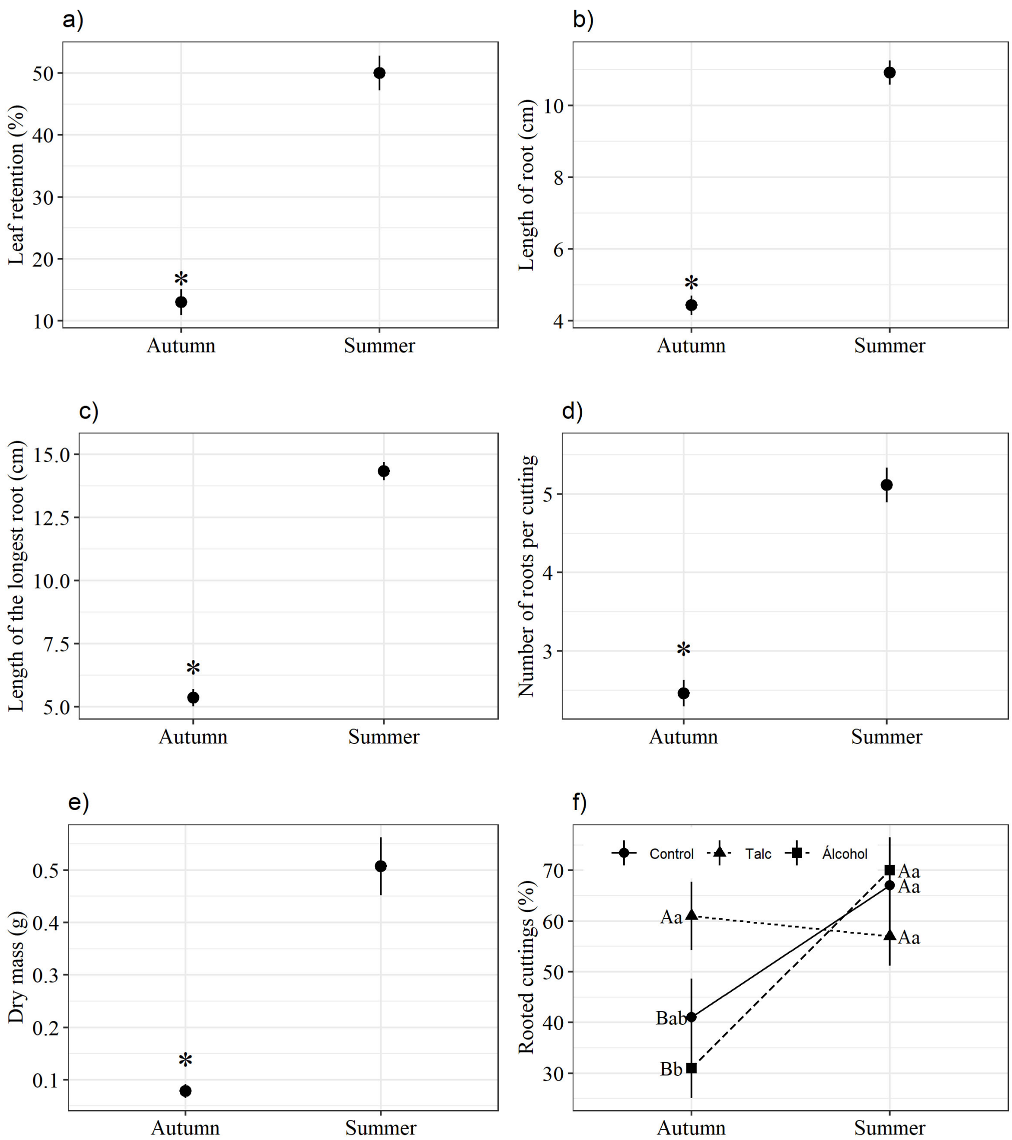

Figure 1. Leaf retention, length of root, length of the longest root, number of roots per cutting, dry mass and percentage of rooted cuttings of 'Woodard' blueberry cuttings subjected to different application methods of indolebutyric acid (IBA) (control, with powder talc and with alcohol), collected in the autumn of 2015 and in the summer of 2016.

Both the length of roots and the length of the longest root were higher in summer, 10.91 cm and 14.33 cm, respectively, in contrast to the lower averages obtained in autumn, 4.43 cm and 5.38 cm, respectively (Figures 1b and 1c). The greater length of roots obtained in summer can be attributed to favorable temperature, air humidity and longer day length, provided that higher temperatures and greater water availability can favor high root growth rates (HUSSAIN et al., 2017).

Koyama et al. (2018) also reported greater length of roots of ‘Woodard’ cuttings grown on carbonized rice husks when collected in summer compared to autumn, with 6.5 and 2.9 cm, respectively. In contrast to the observed results, Wagner Júnior et al. (2004) obtained for the same cultivar 1.21 cm in cuttings collected in the summer. According to these last authors, the use of gross sand as a substrate for a long period may have resulted in a lack of nutrients for greater root development. Campos et al. (2005) emphasize that foliar nutrient supply is necessary for multiplication of blueberry cuttings. Fisher et al. (2016) also reported that when foliar nutrients had been applied, there was an increase in rooting, as the substrate used was inert and the rooting period was relatively long. The length of the longest root in cuttings collected in autumn is in agreement with those found by Yamamoto et al. (2017), who observed from means from 3.5 cm to 7.1 cm. However, the means obtained in the summer were higher than that recorded by Nascimento et al. (2011), 3.79 cm.

The highest number of roots was also obtained in summer compared to autumn, with 5.12 and 2.46 roots per cutting, respectively (Figure 1d). The mean observed in summer was higher than those found by other authors for ‘Woodard’, which ranged from 2.30 to 4.43 (KOYAMA et al., 2018; WAGNER JÚNIOR et al., 2004). According to Campos et al. (2005), the number of roots per cutting is an important characteristic to determine the rooting potential in blueberry, provided that the vigor of the nurseries is not only related to the length of the root, but also to its quantity. Marangon and Biasi (2013) also reported that summer stimulates higher number of roots and the highest length of roots among the four seasons of the year.

Because the summer favored the emission and the root growth, there was also greater dry mass in cuttings collected at that season when compared to autumn, with 0.51 g and 0.08 g, respectively (Figure 1e). Koyama et al. (2018) and Shahab et al. (2018) also observed lower dry mass in ‘Woodard’ cuttings collected in autumn. All these rooting characteristics are essential for a well-formed root system, allowing a larger area of soil to be explored and favoring the absorption of water and nutrients, which ensures the development of the nursery and consequent reduction of mortality when taken to the field (COLOMBO et al., 2018).

Regarding the percentage of rooted cuttings, a significant interaction was verified between the forms of application of AIB and the season of collection of cuttings, thus, the statistical breakdown was performed. Only in autumn there was a significant difference observed, in which the application of IBA in powder talc provided a higher percentage of rooted cuttings (61.0%) when compared to alcohol (31.0%) and control (41.0%). There was no difference for the application with powder talc between seasons. However, the application in alcohol and the control resulted in higher percentages of rooted cuttings in the summer, 70.0% and 67.0%, respectively (Figure 1f).

Colombo et al. (2018) observed that ‘Powderblue’ blueberry minicuttings treated with IBA in powder talc rooted 10% more than those treated with alcohol. The same was reported by Shahab et al. (2018), who obtained 45.5% and 14.0% more rooting in ‘Woodard’ applied with powder talc and collected in autumn and spring, respectively. The efficiency of the application of AIB in powder talc may be due to the higher adherence of the plant growth regulator at the base of the cuttings, resulting in a longer exposure period (COLOMBO et al., 2018). Other advantages of this method are the ease of preparation and application, the lower risk of phytotoxicity to the cuttings and the long stability of the soluction, making this method more economical (FACHINELLO et al., 2013; SHAHAB et al., 2018).

On the other hand, in the experiment carried out by Peña et al. (2012), in which the effect of IBA concentrations and application methods on the rooting of semi-hardwood cuttings of ‘Florida’ and ‘Climax’ blueberry were assessed, the percentage of rooting when liquid IAB was applied (with alcohol) was higher than in powder talc. According to the authors, the lower rooting obtained in talc may have been due to the unevenness of the amount adhered to the cutting, which is affected by the humidity at the base of the cutting and by the texture of the bark (FACHINELLO et al., 2013).

The best performance observed in summer for most of the analyzed variables may be related to the fact that cuttings collected in late spring and early summer have intense vegetative growth. At that season, cuttings present a low degree of lignification, more herbaceous consistency, high exchange activity and a large number of young leaves, which are sites that produce auxins, carbohydrates and rooting cofactors (FACHINELLO et al., 2013). Also, in summer, the number of hours of sunlight is greater in subtropical areas, favoring a greater production of photosynthesized compounds (PAULA et al., 2009). In addition, in temperate perennial species, such as blueberry, endogenous auxin levels vary along the seasons, with higher concentrations in spring and summer than in autumn and winter (VÁLIO, 1986).

The lowest averages observed in autumn possibly occurred because in this season, temperate species prepare to go into dormancy, showing low activity in the young tissues of the secondary phloem, vascular bundles and cambium, in addition to a greater accumulation of phenols and inhibitors, resulting in slower formation of the root system (MARTINI et al., 2013).

There are reports of high rooting percentage of 'Woodard' blueberry cuttings collected during the growing season, reaching means of 80.0% and 82.5% in summer, mainly when multiplicated by minicuttings (KOYAMA et al., 2018; NASCIMENTO et al., 2011). On the other hand, when cuttings are collected in autumn and winter, the percentage of rooting is reduced, ranging from 29.5% to 66.7% (FISCHER et al., 2008; KOYAMA et al., 2018; SHAHAB et al., 2018; SHAHAB et al., al., 2018; YAMAMOTO et al., 2017). Thus, it is preferable that the collection of 'Woodard' herbaceous cuttings be carried out in the summer.

As discussed, the effect of the season of collection on rooting can also be attributed to climatic conditions, especially in relation to temperature (FACHINELLO et al., 2013). Cuttings collected in summer experienced higher temperatures in the first months (Figure 2), which may have contributed to greater leaf retention, stimulating emission and root growth.

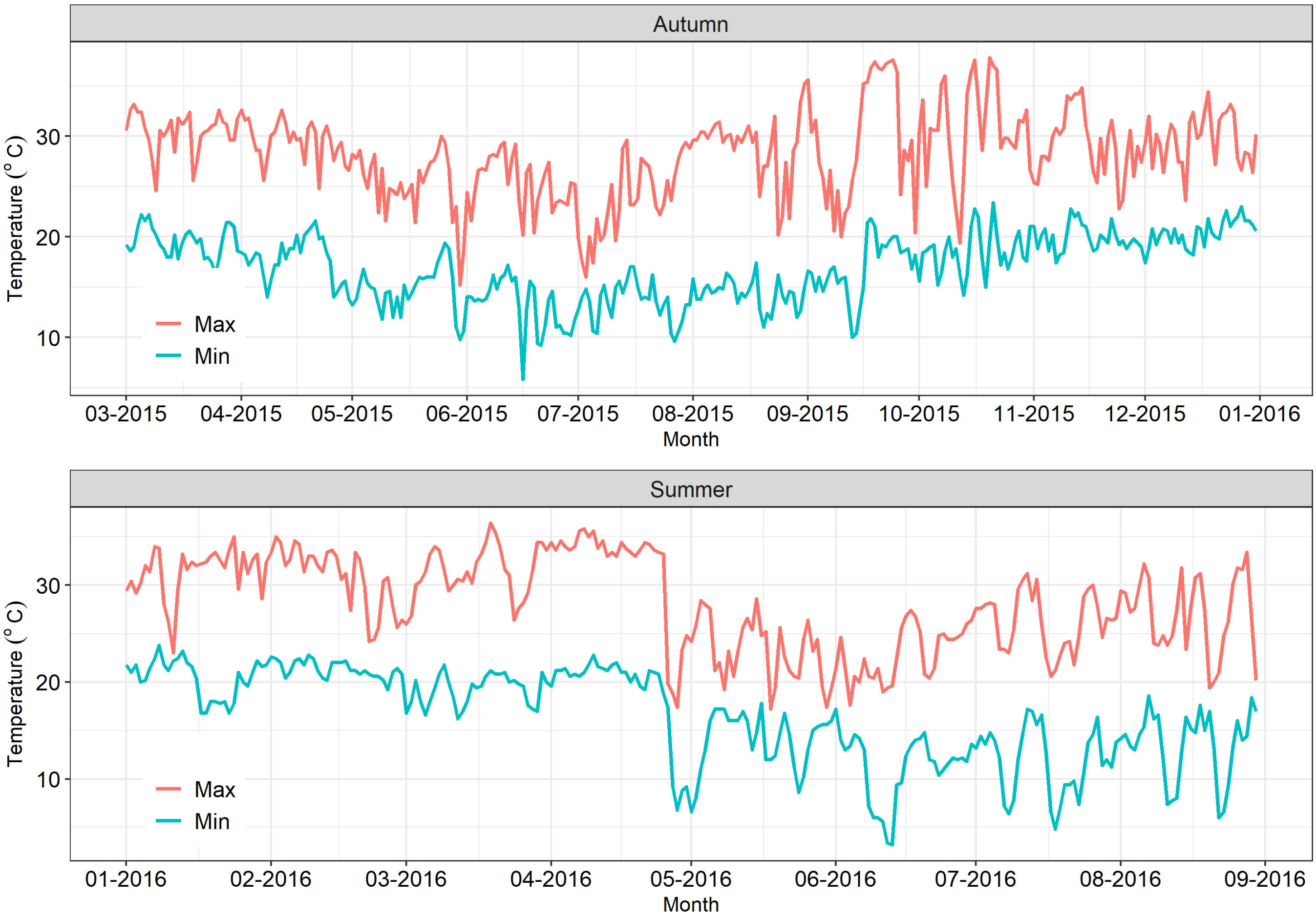

Figure 2. Maximum and minimum daily temperature (°C) in Londrina, PR, Brazil, during the autumn and the summer of 2015 and 2015.

The principal component analysis (PCA) allowed to reduce the dimensionality of the eight variables analyzed, in which the sum of the first two principal components resulted in the recovery of 84.3% of the total variability of the data. By means of PCA, the leaf retention and dry mass variables were more correlated with PCA 1, while the variable percentage of survival was more correlated with PCA 2. Through the analysis, the data were grouped only by the period factor. For all response vectors, summer showed better performance, with the exception of the survival of cuttings, which did not show a trend of higher values for any of the seasons evaluated, corroborating the univariate analyzes (Figure 3).

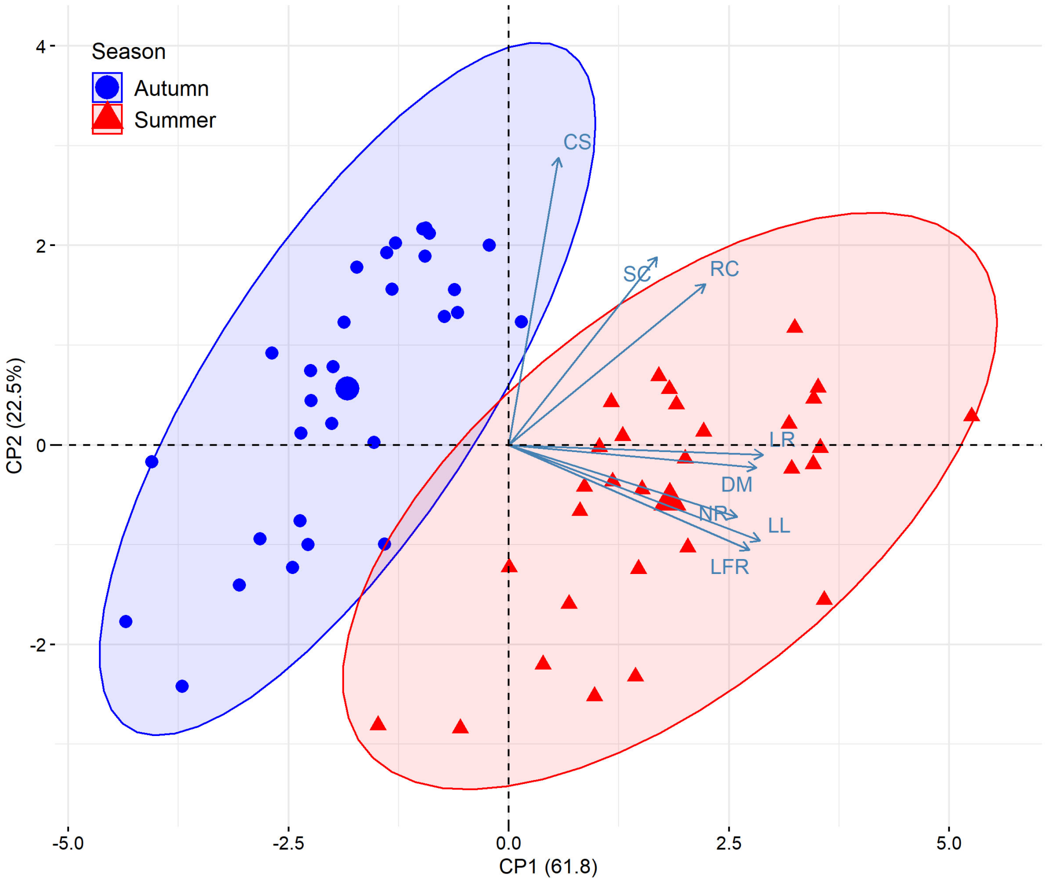

Figure 3. Biplot graph of the principal component analysis (PCA) considering the variables: cutting survival (CS), leaf retention (LR), sprouted cuttings (SC), rooted cuttings (RC), dry mass of roots (DM), number of roots per cutting (NR), length of roots (LFR), length of the longest root (LL) of 'Woodard' blueberry subjected to different solutions of indolebutyric acid (IBA), with or without lesions at the base of the cuttings, and collected in different seasons.

This result may be due to the fact that the higher temperature in summer favors cell division in root formation, however, in herbaceous cuttings, it stimulates a high rate of transpiration, either through leaves or shoots, inducing the wilting of the roots. Water loss is one of the main causes of cuttings death, especially when it comes to species that require a long time for root formation (FACHINELLO et al., 2013). Thus, the herbaceous consistency of the cuttings associated with higher temperatures in the summer (Figure 2), may have favored a greater loss of water by the cuttings, influencing their survival, although an average of 71.83% of live cuttings was observed.

Regarding the lesion performed at the base of the cuttings in order to stimulate cell division and promote the formation of root primordia (FACHINELLO et al., 2013), it was found that it did not contribute to increase the rooting of herbaceous cuttings 'Woodard', in the same way as observed in other cultivars (TREVISAN et al., 2008; WAGNER JÚNIOR et al., 2004). On the other hand, Koyama et al. (2019) reported that 'Brite Blue' blueberry cuttings with basal lesions, collected in the summer, presented 29% more rooting compared to cuttings without the lesion, collected at the same season, and 56% more than cuttings with lesions collected in the fall. According to the authors, the cuttings collection season was one of the factors that contributed to the higher rooting rate of cuttings with lesions at the base.

Conclusions

The multiplication of 'Woodard' blueberry is influenced by the season of cutting collection, with higher leaf retention, dry mass, number of roots per cutting, length of roots and length of the largest root in the summer. However, the application of 1,000 mg L^{-1} of IBA in the form of powder talc resulted in greater rooting in cuttings collected in the autumn. The presence of lesions at the base of the cuttings did not influence the rooting and development of blueberry 'Woodard' cuttings.

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