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ORIGINAL ARTICLE

Vegetative propagation of redberry using refrigeration, IBA and BAP

Propagação por estaquia e alporquia da amoreira-vermelha com uso de refrigeração, AIB e BAP

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ABSTRACT: Increasing interest in the cultivation of redberries has created a need to optimize seedling propagation. This work examines techniques for the vegetative propagation of redberry. Four sequential experiments were conducted. In the first two experiments, we collected stem and root cuttings. In the first experiment, the cuttings were treated with different IBA concentrations, 0, 1000, 2000, 3000 and 4000 mg L^{-1}, for 10 s. In the second experiment, the cuttings were separated into bundles of ten cuttings each and stored in a refrigerator (approximately 4 °C) for different time periods: 10, 20 and 30 days; the control was a portion of cuttings that was not subjected to refrigeration. In the third experiment we performed two methods of layering in semi-hardwood branches of redberry: tip and air layering. We also applied different IBA concentrations: 0, 1000, 2000, 3000 and 4000 mg L^{-1}. The fourth experiment evaluated only root cuttings stored for 14 days without being treated with IBA. After the storage period, the cuttings were treated with different concentrations of BAP, 0, 300, 600, 900, 1200 and 1500 mg L^{-1}, for 10 s. We concluded that root cuttings embed deeper than stem cuttings and do not require IBA to improve rooting or BAP to stimulate growth. Storage for 14 days was more effective in stimulating rooting and sprouting. Layering did not provide good results.

RESUMO: *O crescente interesse pelo cultivo da amoreira-vermelha fez surgir a necessidade de otimizar a produção de suas mudas. O objetivo deste trabalho foi estudar a técnica de propagação vegetativa da amoreira-vermelha. Foram realizados quatro experimentos sequenciais. Nos dois primeiros experimentos, foram coletadas estacas caulinares e radiculares. No primeiro experimento, as estacas foram tratadas com diferentes concentrações de AIB: 0, 1000, 2000, 3000 e 4000 mg L^{-1} por 10 s. Já no segundo experimento, as estacas foram separadas em feixes de dez estacas e armazenadas em geladeira (temperatura próxima a 4°C), por diferentes períodos: 10, 20 e 30 dias, além do controle, que foi constituído por uma parte de estacas que não passou pelo processo de armazenamento. No terceiro experimento, foram realizadas mergulhias em ramos semilenhosos da amoreira-vermelha por dois métodos: mergulhia de ponta e alporquia. Foram aplicadas diferentes concentrações de AIB: 0, 1000, 2000, 3000 e 4000 mg L^{-1}. O quarto experimento de enraizamento avaliou apenas estacas radiculares, sem serem tratadas com AIB, tendo sido armazenadas por 14 dias. Após o período de armazenamento, as estacas foram tratadas com diferentes concentrações de BAP: 0, 300, 600, 900, 1200 e 1500 mg L^{-1} por 10 s. Concluiu-se que estacas radiculares possuem maior enraizamento do que as estacas caulinares, não necessitando de AIB, na melhoria do enraizamento, e de BAP, para estimular a brotação. O armazenamento das estacas radiculares por 14 dias mostrou-se mais eficiente no estímulo do enraizamento e da brotação. A mergulhia não promoveu bons resultados.*

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1 Introduction

The redberries is an erect subshrub, prickly, with 0.4 to 2.5 m high. The fruits are aggregates, with fruitful hollow, receptacle fleshy, sweet and pleasant flavoured (CAMPAGNOLO; PIO, 2012b). In Serra da Mantiqueira, the red berry has been used as attractive for rural tourism, such as raspberry (MOURA et al., 2012). This fact has enabled greater knowledge by the tourist population of the unique and peculiar flavor of the fruits of this plant, which made agronomic interest came up for its cultivation.

In the case of the blackberry, there is the possibility to use both stem cuttings, from the reduction pruning done in the winter, or root cuttings, which have the advantage of the absence of spines on their stems (CAMPAGNOLO; PIO, 2012a), the same applies to redberries.

There are some techniques that are used in order to increase roots emission in stems and root cuttings. One of them is the cold storage of cuttings, which aims to maintain the propagation material in the dark and supply its necessity of cold. The storage assists in overcoming the dormancy of buds and provides increased emission of shoots on the cuttings (OHLAND et al., 2009; CELANT et al., 2010; SALIBE et al., 2010). In turn, the etiolation helps to induce inhibition of the enzyme IAA oxidase system, increasing the action of the natural auxin IAA in the cuttings, fostering rooting (BIASI, 1996).

Another technique is to treat the cuttings with growth regulators. The indol-butyric acid (IBA) is a synthetic auxin which aims to increase the emission and improve the quality of roots in stem cuttings (HAN; ZHANG; SUN, 2009). But in the case of root cuttings, starting from the presupposition that they have greater facility in root emission, as reported by Campagnolo and Pio (2012a), the application of cytokinins could help shoot emission. The benzylaminopurine (BAP) has been prominent among cytokinins for its efficiency in inducing the formation of large numbers of shoots and high rates of in vitro multiplication of micro raspberry seedlings (LEITZKE; DAMIANI; SCHUCH, 2010).

Layering, both tip and air, is also a technique of vegetative propagation of redberry. This technique has shown good results in black raspberry (*Rubus niveus*) (SILVA et al., 2012).

This work aimed to study different techniques of vegetative propagation of redberry, aiming the clonal seedlings production.

2 Materials and Methods

Four sequential experiments were performed from April to October at the Division of Fruit Crops, at Federal University of Lavras (UFLA), Lavras-MG. The seedlings used were coming from plants of redberry with an average of 40 cm tall and well branched, which were taken to the field in November 2009.

2.1 Experimental design and treatments

In the first two experiments, cuttings were collected in semi-hardwood branches and were standardized to 10 cm long and 7 mm diameter. Astraight cut was made on the apex cutting and another bevel at the base, removing the leaves. The root cuttings were standardized at 10 cm in length and 10 mm in diameter, with straight sections at both ends.

In the first experiment, cuttings were treated with different concentrations of IBA: 1000, 2000, 3000 and 4000 mg L^{-1} for 10 s, and the control composed only by water, following the recommendations of Villa et al. (2003). The experimental design was completely randomized using a 2 × 5 (type of cutting and IBA concentrations) factorial design with four replications and ten cuttings per experimental unit. In the second experiment, cuttings were separated into sheaves of ten cuttings and afterwards were wrapped in moistened paper and placed in sealed plastic bags and was stored in the refrigerator (4 °C) for different periods: 10, 20 and 30 days, and the control, which was constituted by a portion of cuttings (bundles of shoot and root cuttings) that did not pass through the storage process, according to the recommendations of Celant et al. (2010). The design was randomized using a 2 × 4 (type of cutting and storage periods) factorial design with four replications and ten cuttings per experimental unit.

In both experiments, the stem cuttings were buried two thirds of its length in the vertical position, and the root cuttings were totally immersed in the horizontal position, 3 cm deep in rooting beds filled with vermiculite, located inside the greenhouse with 50% shading. The cuttings were moistened daily and were evaluated 60 days after planting.

In the third experiment, layering was performed by two methods in semi-hardwood branches of redberry: the tip layering and in the air layering, according to the recommendations of Daneluz et al. (2009). In tip layering, bevel cuts were carried out at the end of semi-hardwood branches and in the air layering the side shoots on the central portion of branches were removed. Following the completion of bevel cuts and layers preparation, different concentrations of IBA was applied with a brush of fine bristles: 1000, 2000, 3000 and 4000 mg L^{-1} for 10 s, and control which consisted only of water.

In tip layering, was buried 15 cm of the end of the branches in plastic boxes filled with substrate prepared with pine bark that was moistened daily. On the air layering, a portion of 10 cm in length was involved with the same substrate, then wrapped up with transparent plastic and tied up the ends to prevent moisture loss. The experimental design was completely randomized in a 2 × 5 (type of layering and IBA concentrations) factorial design with four replications and ten branches of plants per experimental unit. At the end of 45 days were evaluated.

After the finalization of the three sequences experiments, in possession of statistical analyzes was carried out another seedlings experiment , but just using root cuttings, without being treated with IBA and stored for 14 days under refrigeration (at 4 °C) . After the storage period, the cuttings were treated with different concentrations of BAP: 300, 600, 900, 1200 and 1500 mg L^{-1} for 10 s, and the control composed only by water. The experimental design was completely randomized with six treatments (BAP concentrations), with four replications of ten cuttings per experimental unit. At the end of 60 days were evaluated.

For all experiments were measured the percentage of rooting and sprouting, the average number of roots and shoots and

the average length of roots and shoots. Data were subjected to analysis of variance and means subjected to regression analysis or comparison of means, at 5% probability by SISVAR program.

3 Results and Discussion

For the first experiment, Statistical analysis showed no interaction between the factors, only difference between the isolated factors. Root cuttings showed significant increase compared to stem cuttings for root emission, with an increase of 50.5% of rooted cuttings and root emission of 3.9 (Table 1). On the other hand, the sprouting percentage and the shoots length average were higher in stem cuttings.

Such results agree with Campagnolo and Pio (2012a), that verified that root cuttings of blackberry present higher emission compared the stem cuttings. Silva et al. (2012) also observed that the stem cuttings of black raspberry (*Rubus niveus* presented highest sprouting percentage compared the root cuttings.

Regarding the application of IBA (IBA) in cuttings, the results showed that rooting percentage without using IBA was 54.3%, while at a concentration of 4000 mg L^{-1} rooting percentage was 32.6%, 21.7% reduction in rooting (Figure 1A). The percentage of shoots also had a linear decrease with an increase in the IBA concentrations, going from 47.8% without application IBA to 13% with 4000 mg L^{-1} (Figure 1A). The roots and shoots emission also slightly decreased with the application of IBA concentrations. Greater emission of roots (3.74) and shoots (1.9) were obtained without the application of the plant regulator (Figure 1B).

Reduction in rooting and sprouting in stem cuttings treated with IBA concentrations was also observed by Tiberti et al. (2012) studing 'Boysenberry' (hybrid between 'Marionberry' and raspberry). Treating stem cuttings with IBA also reduced rooting percentage of blackberry 'Brazos', in an experiment carried out by Villa et al. (2003).

It is thought that the cuttings endogenous levels of auxin were at a level which would aid in the roots emission. Exogenous supplementation possibly caused phytotoxic effect, thus disadvantaging rooting and sprouting percentage.

In the second experiment to study the effect of the cold temperature on cuttings storage, statistical analysis showed an interaction between the factors for rooting and sprouting percentage and the average number of roots and shoots. Stem cuttings stored at cold temperature for 13 days and 12 days showed higher rooting percentage (58.56%) and average number of roots (3.7), respectively. On the other hand, root cuttings stored at cold for 11 days presented better results for rooting percentage (86.3%), whereas those stored for 13 days had higher average number of roots (7.4), as show in Figures 2A and 2C. Campagnolo and Pio (2012a) in a studying blackberry cuttings stored at cold temperature for 30 days, also found that the root cuttings had higher rooting compared the stem cuttings.

Regarding the percentage of sprouting, the results were almost similar, however without storage, stem cuttings (70%) and root cuttings stored for 14 days (71.5%) presented better results (Figure 2B). For number of shoots, stem cuttings stored for 13 days (1.82) and root cuttings without storage (2.34) showed superior results (Figure 2D). These results support that

Table 1. Rooting and sprouting percentage, average number of roots and shoots and average length of roots and shoots of stems and root cuttings of redberry *Rubus rosifolius.*

Cutting type	Variável analisada					
	% rooting	% sprouting	Nº roots	Nº shoots	Root length (cm)	Shoots length (cm)
Caulinar	29.5 b	48.5 a	0.9 b	1.4 a	1.4 a	1.9 a
Radicular	80.0 a	12.1 b	4.8 a	1.1 a	1.7 a	1.0 b
CV (%)	25.2	24.9	29.7	26.8	47.4	29.8

Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

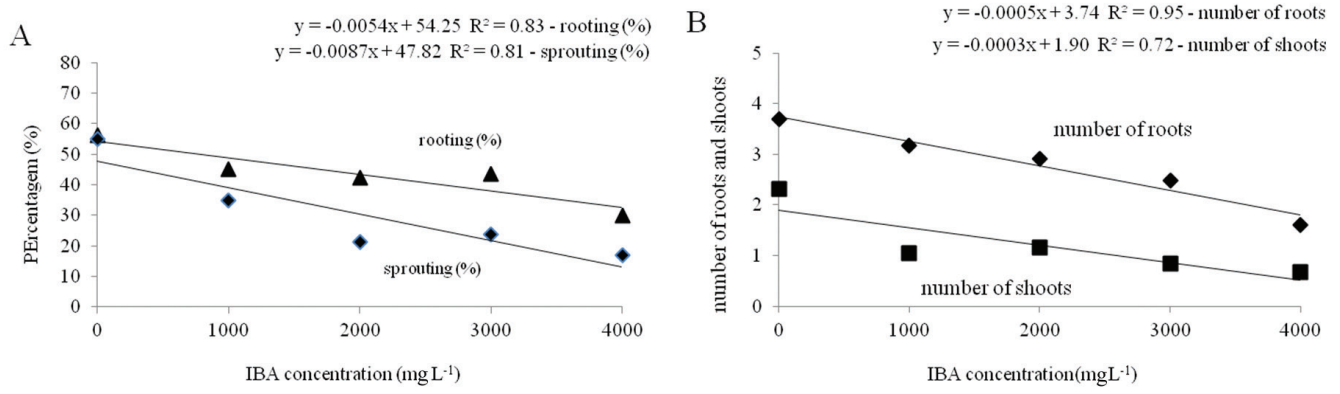

Figure 1. Rooting and sprouting percentage (A) and average number of roots and shoots (B) of redberry cuttings *Rubus rosifolius* treated with different concentrations of IBA.

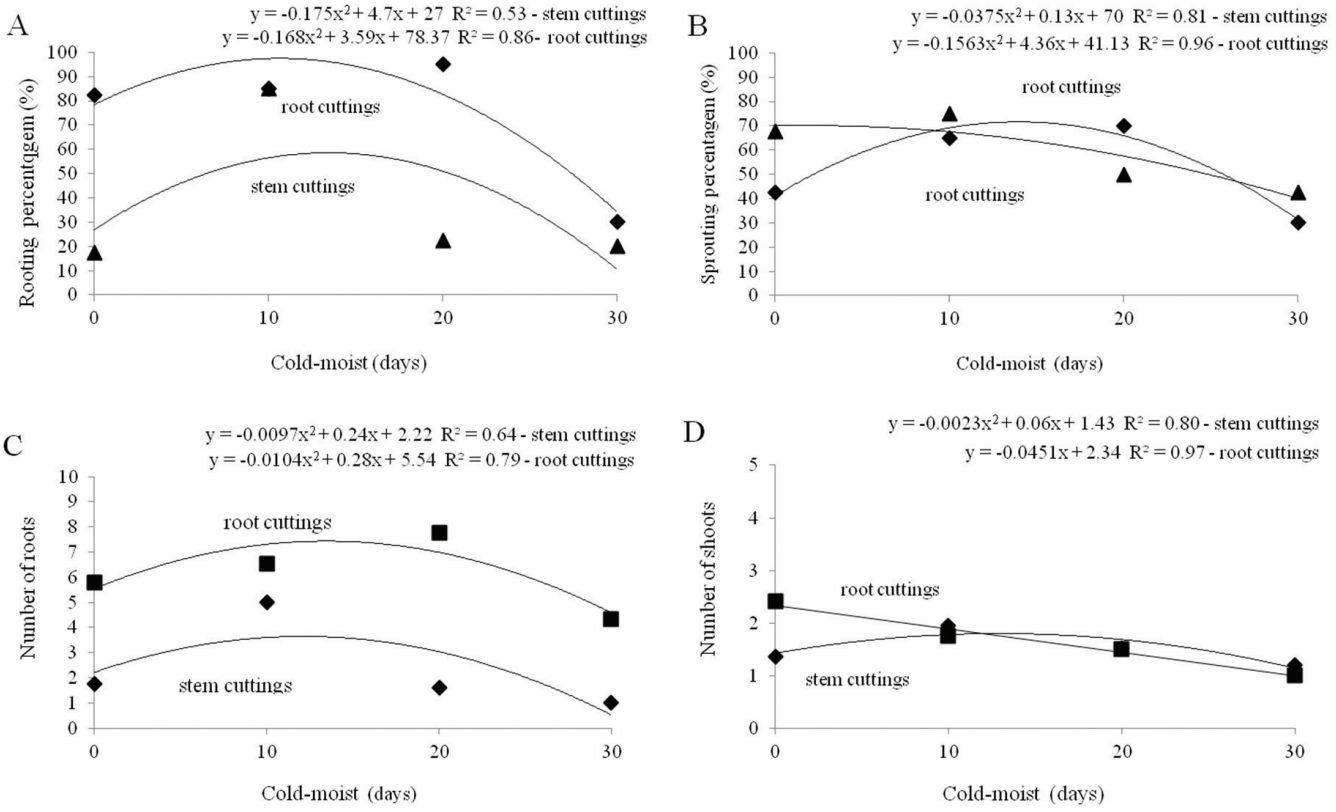

Figure 2. Rooting (A) and sprouting (B) percentage, average number of roots (C) and shoots (D) of redberry *Rubus rosifolius* cuttings stored at cold-moist for different periods.

root cuttings have greater facility to issue roots compared to cuttings. By storing the root cuttings at low temperature, there was an increase both in rooting as for the sprouting percentage. Campagnolo and Pio (2012a) also observed that the cold storage promoted an increase in rooting and sprouting in root cuttings of blackberry. Expose cuttings to low temperatures assist at sprouting and overcoming the dormancy of buds (CELANT et al., 2010). Storage under low temperatures also contributes to increasing roots emission since the shading increases phenolic compounds such as chlorogenic acid, phloroglucinol, catechol, caffeic acid and inhibitors of IAA oxidase synthesis, thus increasing the natural action of IAA in the cuttings (BIASI, 1996).

In the third experiment, none of the branches, which were used on layering, presented roots and shoots emission. In this case, it was held only the analysis of data regarding the tip layering. there was just statistical difference between the concentrations of IBA to the rooting and sprouting. Higher rooting percentage (32.5%) and sprouting (60.5%) were obtained without the application of IBA (Figure 3). Increasing concentrations applied at the apex of the branch linearly decreased rooting and sprouting. These results agree with Silva et al. (2012), that also found a decrease in the use of IBA on rooting and sprouting branches of black raspberry submitted layering tip.

However, the obtained results in rooting and sprouting of mulberry-red which went through the process of layering tip were lower than the results obtained with root cuttings.

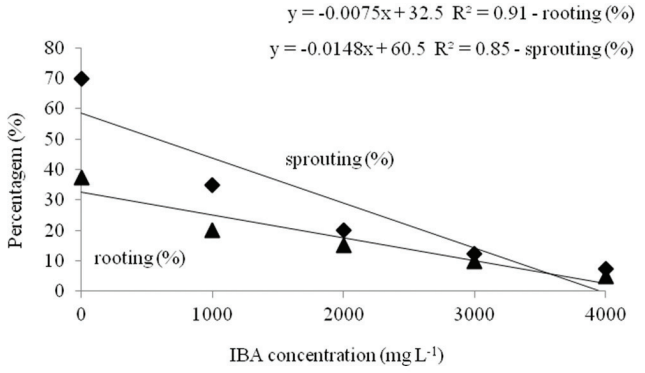

Figure 3. Percentage of rooting and sprouting of redberry *Rubus rosifolius* submitted layering tip and treated with different concentrations of IBA.

In the fourth experiment with the application of different concentrations of benzylaminopurine (BAP) on root cuttings of mulberry red, there was statistically difference only in the percentage of rooting and number of roots. The rooting percentage was 78.8% without using BAP, with a decrease of 52.6% when the cuttings were treated with 1500 mg L^{-1} BAP (Figure 4A). The same occurred for the average number of roots, which decrease 3.9 roots with 1500 mg L^{-1} BAP treatment (Figure 4B). These results were expected, since the plant regulator used has the purpose to stimulate shoot emission, and not rooting. This is due to the fact that cytokinins perfom opposite functions in regulation of cell proliferation in

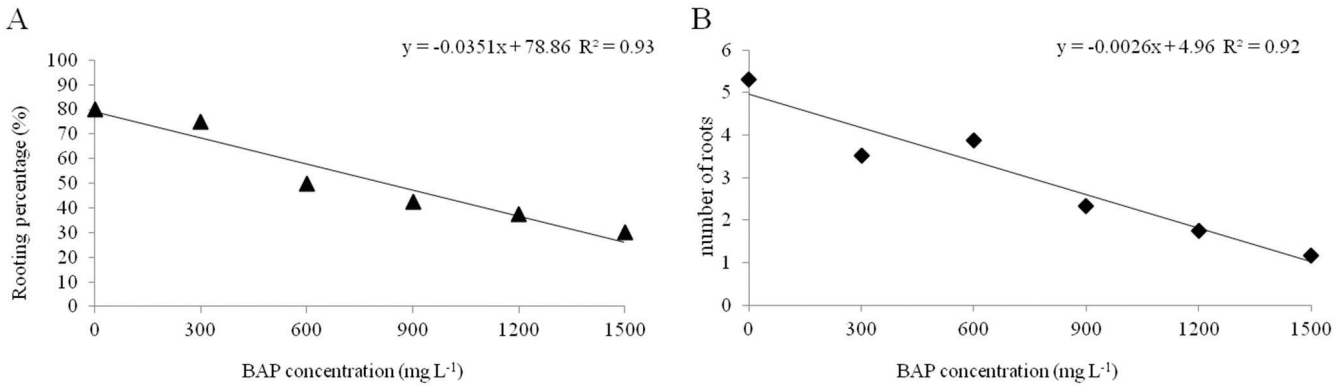

Figure 4. Rooting percentage (A) and average number of roots (B) of root cuttings of redberry *Rubus rosifolius* treated with different concentrations of BAP.

meristems of root and shoot. The auxin/cytokinin ratio directly affects the cell differentiation, whereas a high ratio stimulates root formation, low ratio stimulates shoots formation

4 Conclusions

According to the results, the red berry can be propagated by cuttings; root cuttings have greater rooting than stem cuttings, it's not necessary to use IBA to improve rooting and neither BAP to stimulate growth; root cuttings stored for 14 days showed significant increases in sprouting and rooting; layering did not provide good results.

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References

BIASI, L. A. Emprego do estiolamento da propagação de plantas. *Ciência Rural*, v. 26, n. 2, p. 309-314, 1996.

CAMPAGNOLO, M. A.; PIO, R. Enraizamento de estacas caulinares e radiculares de cultivares de amoreira-preta coletadas em diferentes épocas, armazenadas a frio e tratadas com AIB. *Ciência Rural*, v. 42, n. 2, p. 232-237, 2012a.

CAMPAGNOLO, M. A.; PIO, R. Phenological and yield performance of black and redberry cultivars in western Paraná State. *Acta Scientiarum. Agronomy*, v. 34, n. 4, p. 439-444, 2012b.

CELANT, V. M.; PIO, R.; CHAGAS, E. A.; ALVARENGA, A. A.; DALASTRA, I. M.; CAMPAGNOLO, M. A. Armazenamento a frio de ramos porta-borbulhas e métodos de enxertia de cultivares de marmeleiro. *Ciência Rural*, v. 40, n. 1, p. 20-24, 2010. http://dx.doi.org/10.1590/S0103-84782009005000223

DANELUZ, S.; PIO, R.; CHAGAS, E. A.; BARBOSA, W.; OHLAND, T.; KOTZ, T. E . Propagação da figueira 'Roxo-de-Valinhos' por alporquia. *Revista Brasileira de Fruticultura*, v. 31, n. 1, p. 285-290, 2009. http://dx.doi.org/10.1590/S0100-29452009000100041

HAN, H.; ZHANG, S.; SUN, X. A review on the molecular mechanism of plants rooting modulated by auxin. *African Journal of Biotechnology*, v. 8, n. 3, p. 348-353, 2009.

LEITZKE, L. N.; DAMIANI, C. R.; SCHUCH, M. W. Influência do meio de cultura, tipo e concentração de citocininas na multiplicação in vitro de amoreira-preta e framboeseira. *Ciência e Agrotecnologia*, v. 34, n. 2, p. 352-360, 2010. http://dx.doi.org/10.1590/S1413-70542010000200012

MOURA, P. H. A.; CAMPAGNOLO, M. A.; PIO, R.; CURI, P. N.; ASSIS, C. N.; SILVA, T. C. Fenologia e produção de cultivares de framboeseiras em regiões subtropicais no Brasil. *Pesquisa Agropecuária Brasileira*, v. 47, n. 12, p. 1714-1721, 2012. http://dx.doi.org/10.1590/S0100-204X2012001200006

OHLAND, T.; PIO, R.; CHAGAS, E. A.; BARBOSA, W.; DALASTRA, I. M.; KOTZ, T. E. Enraizamento de estacas apicais lenhosas de figueira 'Roxo de Valinhos' com a aplicação de AIB e cianamida hidrogenada. *Revista Brasileira de Fruticultura*, v. 31, n. 1, p. 273-279, 2009. http://dx.doi.org/10.1590/S0100-29452009000100039

SALIBE, A. B.; BRAGA, G. C.; PIO, R.; TSUTSUMI, C. Y.; JANDREY, P. E.; ROSSOL, C. D.; SILVA, J. R.; SILVA, T. P. Enraizamento de estacas do porta-enxerto de videira 'VR 043-43' submetidas a estratificação, ácido indolbutírico e ácido bórico. *Bragantia*, v. 69, n. 3, p. 617-622, 2010. http://dx.doi.org/10.1590/S0006-87052010000300013

SILVA, K. N.; PIO, R.; TADEU, M. H.; ASSIS, C. N.; CURI, P. N.; MOURA, P. H. A.; PATTO, L. S. Produção de mudas de framboeseira negra por diferentes métodos de propagação vegetativa. *Ciência Rural*, v. 42, n. 3, p. 418-422, 2012. http://dx.doi.org/10.1590/S0103-84782012000300006

TIBERTI, A. S.; PIO, R.; ASSIS, C. N.; SILVA, K. N.; TADEU, M. H. Propagação do 'Boysenberry' por estaquia e mergulhia. *Ciência Rural*, v. 42, n. 3, p. 423-428, 2012. http://dx.doi.org/10.1590/S0103-84782012000300007

VILLA, F.; PIO, R.; CHALFUN, N. N. J.; GONTIJO, T. C. A.; DUTRA, L. F. Propagação de amoreira-preta utilizando estacas lenhosas. *Ciência e Agrotecnologia*, v. 27, n. 4, p. 829-834, 2003. http://dx.doi.org/10.1590/S1413-70542003000400013