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Adaptação e crescimento inicial de clones de *Sequoia sempervirens* em Santa Catarina - Brasil

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10.37885/200801086

RESUMO

Sequoia sempervirens (sequoia) é uma espécie com madeira valiosa e potencial para plantio em locais fora se seu habitat natural. O sucesso na implantação de uma espécie como a sequoia pode representar uma alternativa florestal para o Brasil, especialmente para a metade Sul. O estudo objetivou investigar a implantação de testes clonais de sequoia, em duas regiões de Santa Catarina, buscando obter informações quanto a adaptação e crescimento dos diferentes clones. A implantação ocorreu em dois locais no Estado de Santa Catarina – Brasil: Campo Belo do Sul (local mais frio) e Arabutã (local mais quente) utilizando diferentes clones. Em Campo Belo do Sul a implantação foi realizada em dezembro de 2015 com três clones (SJ, A140 e A228), com avaliação de crescimento e danos por geada aos 6, 12 e 24 meses. Em Arabutã o plantio foi realizado com quatro clones (A127, A138, A140 e A228) em dezembro de 2016, sendo realizada avaliação de crescimento aos 6 e 12 meses. As mudas de sequoia implantadas na região mais fria (Campo Belo do Sul) apresentaram danos severos pela geada, sendo observada sobrevivência de apenas 2% após 24 meses de implantação. Em compensação, na área mais quente (Arabutã) a sobrevivência foi maior que 80% aos 12 meses, com crescimento das mudas superior a 50 cm de altura. Outras estratégias devem ser adotadas para buscar o estabelecimento da sequoia em áreas mais frias, buscando minimizar o efeito das geadas nas mudas. Antes de uma recomendação é necessário fazer mais avaliações, contudo, a espécie, mostra inicialmente potencial para áreas sem ocorrência de geadas.

Redwood: Estabelecimento em Campo, Teste Clonal, Danos por Geadas.

INTRODUÇÃO

As demandas produtivas envolvendo espécies florestais plantadas continuamente foram focadas em apenas algumas espécies, revelando falta de conhecimento para a incorporação de outras alternativas. Contudo, novas espécies podem ser uma excelente oportunidade para o aumento da produção e uma possível abertura para mercados inexplorados. Neste sentido surge *Sequoia sempervirens*, uma das espécies mais exploradas do mundo, com madeira que pode ser utilizada para inúmeras finalidades.

É uma espécie nativa da região costeira da Califórnia (EUA), estendendo-se quase que ininterruptamente até o sudoeste do estado do Oregon, em uma faixa de 8-56 km de largura. Na direção sul a espécie ocorre apenas em alguns lugares isolados. Quanto à altitude, a sequoia ocorre desde o nível do mar até quase 1.000 metros (LORIMER et al., 2009). Tem preferência por climas temperados úmidos, com influência oceânica, alcançando o máximo desenvolvimento em solos aluviais com depósitos de rochas sedimentares (KNOWLES; MILLER, 1993). As temperaturas médias anuais nas quais a espécie se desenvolve em seu local de origem variam entre 10 e 16 ºC e o desvio entre a média anual mínima e máxima não excede 5,6 ºC para as florestas costeiras e até 16,7 ºC para aqueles mais continentais (INFOR, 1998).

Em seu país de origem a sequoia foi submetida a intensa atividade exploratória, devido a fatores como elevada qualidade da sua madeira vermelha (daí o nome americano de redwood), seu rápido crescimento (entre 4 e 10 anos de idade pode crescer a uma taxa de 1,8 metros por ano) e o porte de grandes proporções, de onde podem ser extraídas peças de tamanhos consideráveis para diversos usos (COWN, 2008; ARENAS, 2004). A espécie se reproduz por meio de sementes (a capacidade germinativa das sementes é variável e geralmente muito baixa - 5-15%), transplantes de brotos que normalmente aparecem na base dos troncos após algum distúrbio ou ainda ser propagada por estacas (INFOR, 1998).

Antigamente acreditava-se que a sequóia crescia somente no cinturão de umidade (costa litorânea da Califórnia), em função da necessidade da névoa para sobreviver, porém há mais de 100 anos está crescendo com sucesso fora da Califórnia, em locais como: Seattle, Washington, Hawkinsville e Geórgia (EUA); Victoria e Colúmbia Britânica (Canadá) (KUSER, 1981). Sabe-se que a sequóia pode ser cultivada em muitas partes da Europa Ocidental, Turquia, Japão, Nova Zelândia (PALMER et al., 2012), Chile (UNDURRAGA, 1995), África do Sul (RAMÍREZ, 2002), Tasmânia e nos trópicos, com elevação suficientemente alta para proporcionar clima temperado.

À medida em que aumentam os esforços para plantar sequóias em muitas partes do mundo aumenta também a necessidade de se ter informações sobre diferenças entre

procedências. O uso de uma fonte adequada ou inadequada de sementes pode significar a diferença entre sucesso e falha nos plantios e condução de espécies exóticas (KUSER, 1996).

A taxa de crescimento desta espécie, tanto em altura como em diâmetro, excede a maioria das espécies madeireiras dos EUA. Na sua área de origem, em locais muito favoráveis e com manejo adequado, em rotações de 40 a 60 anos, os indivíduos obtêm 45 m de altura e 90 cm de diâmetro, com aumentos médios de volume anual de 21 $m^3\ ha^{-1}\ ano^{-1}$ (UNDURRAGA, 1995). Em ensaios localizados na África do Sul, obteve-se um aumento de volume de 30 $m^3\ ha^{-1}\ ano^{-1}$ (RAMÍREZ, 2002). Na Nova Zelândia, incrementos superiores a 40 $m^3\ ha^{-1}\ ano^{-1}$ foram relatados no extremo superior da ilha norte do país (PALMER et al., 2012). No Brasil, França, Inglaterra e em países asiáticos é encontrado um pequeno número de indivíduos da espécie, principamente na arborização de cidades e parques, o que prejudica a quantificação de crescimento.

Recentemente o interesse em pesquisas e no cultivo da espécie foi renovado (COWN; MCKINLEY, 2008), principalmente porque mais de 95% da sequoia das áreas naturais (EUA) está protegida em parques de conservação (STUART, 2007; COWN, 2008). Esta situação oferece uma oportunidade para a madeira cultivada em outros países, principalmente em regiões com históricos de elevados incrementos em espécies florestais, como no Brasil.

Como relatado na bibliografia, a propagação seminal da espécie é dificultada pela baixa germinação, viabilidade das plântulas e principalmente pelo longo tempo necessário para que a planta possa produzir sementes (BOE, 1974). Dessa forma, a propagação vegetativa assume papel de grande importância para a maximização de características de interesse, proporcionando em pouco tempo maiores e melhores resultados. Após a propagação vegetativa os clones devem ser testados a campo, buscando-se encontrar materiais mais adaptados aos diferentes locais. A implantação da espécie em diversos ambientes também é uma necessidade relacionada aos poucos estudos encontrados, objetivando-se conhecer a sobrevivência, crescimento e resistência em diversos ambientes utilizando-se diferentes clones para uma maior amplitude de conhecimento a fim de obter uma possível recomendação comercial.

A espécie pode encontrar condições adequadas para o desenvolvimento no país, principalmente na região Sul, em locais em que o cultivo de outras espécies, como *Eucalyptus,* por exemplo, é prejudicado em razão da ocorrência de fortes geadas. Este trabalho faz parte de um amplo estudo, visando resgatar, clonar e testar a espécie em diversos locais do Brasil. Desta forma, o objetivo do trabalho é avaliar a implantação de testes clonais de sequoia em duas regiões de Santa Catarina, buscando-se obter informações quanto à adaptação e crescimento dos diferentes clones nos locais de estudo.

MATERIAL E MÉTODOS

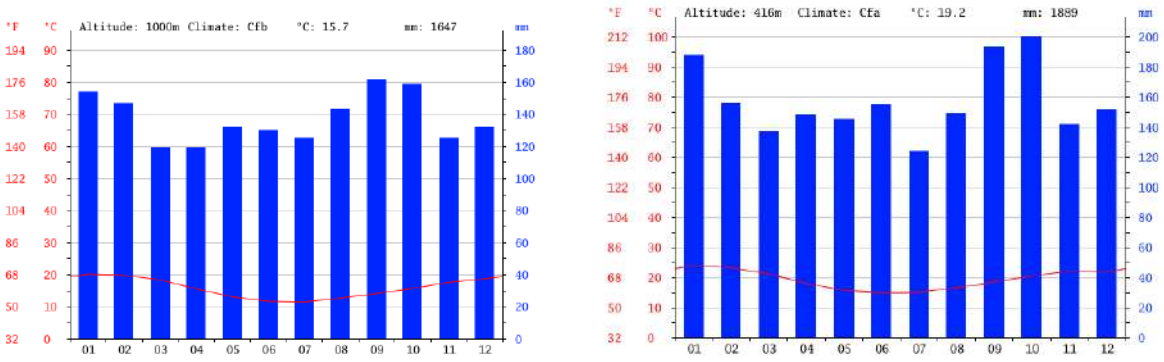
O teste clonal foi implantado em dois locais no estado de Santa Catarina: em Campo Belo do Sul, em área de uma Empresa Florestal, e em Arabutã, em uma propriedade particular. A análise da implantação foi realizada de forma separada, em função do plantio ter sido realizado em períodos distintos e com alguns clones diferentes.

Área 1 - Campo Belo do Sul (SC)

O local de implantação está situado nas coordenadas geográficas 27°55'12"S e 50°49'02"W. O tipo de relevo predominante no local é plano a levemente ondulado, com uma altitude média de 947 m. Os solos predominantes na região são nitossolos e latossolos (EMBRAPA, 1999). Conforme análise de solo (0-40 cm), o local apresenta a seguinte atribuição química: pH = 5,9; índice SMP = 4,5; Ca = 1,79 (cmolc/dm³); Mg = 3,93 (cmolc/dm³); Al = 3,66 (cmolc/dm³); H+Al = 25,6 (cmolc/dm³); CTC efetiva = 6,5 (cmolc/dm³); saturação de alumínio = 59,8%; saturação de bases = 10,8%; M.O. = 3,8%; C.O. = 3,2%; Argila = 68%; P (Mehlich) = 1,6 (mg/dm³); K = 23,7 (mg/dm³); CTC pH 7,0 = 29,2 (cmolc/dm³); Cu (Mehlich) = 3,6 (mg/dm³); Zn (Mehlich) = 1,0 (mg/dm³); Fe (Mehlich) =113,3 (mg/dm³) e Mn = 23,6 (mg/dm³).

O clima é típico do planalto meridional brasileiro, com temperatura média anual em torno de 16 ºC (Figura 1a). Pela classificação de Köppen, há predominância do clima Cfb, mesotérmico, subtropical úmido, com verões frescos e sem estação seca, mas com a ocorrência de geadas severas (FORMENTO et al., 2004). A precipitação média anual está entre 1650 (CLIMATE, 2017b) a 1800 mm (PANDOLFO et al., 2002).

FIGURA 1. DADOS CLIMÁTICOS DE CAMPO BELO DO SUL (A) E ARABUTÃ (B) – SC, BRASIL.

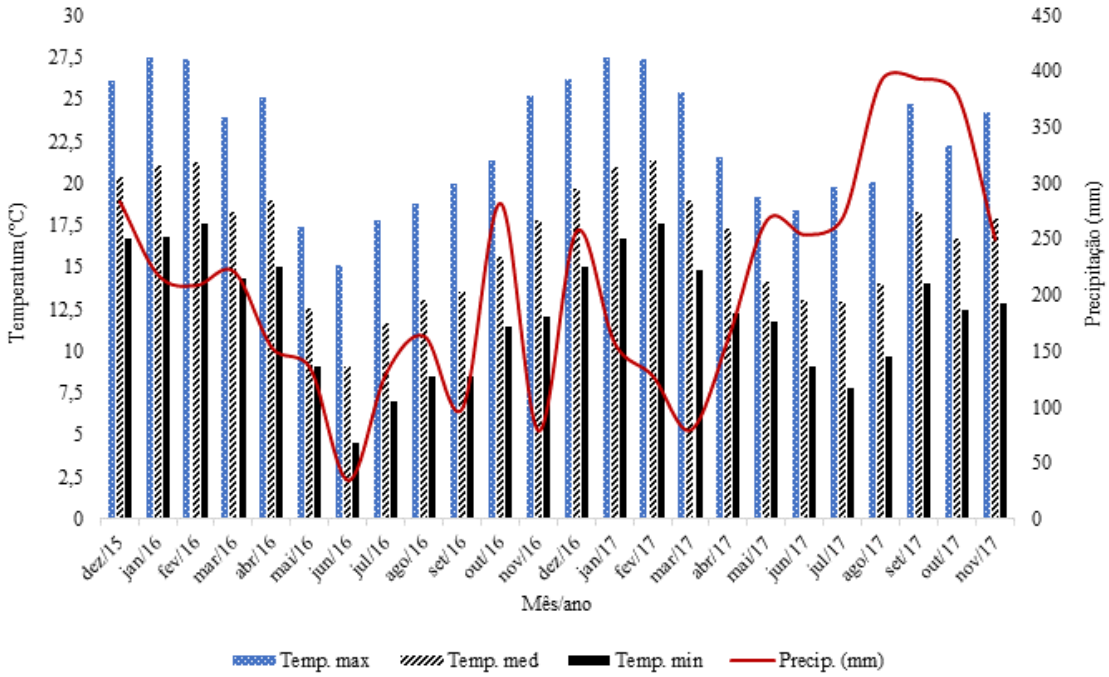


FONTE: climate-data.org. (2018).

Os dados médios mensais de temperatura máxima, média e mínima, e o total de precipitação mensal do período de plantio (dez 2015) até 24 meses após implantação (nov

2017), obtidos na estação meteorológica localizada a aproximadamente 1 km de distância do plantio estão na Figura 2. Durante o inverno de 2016 foram registradas 23 geadas, sendo 5 consecutivas entre 09 a 13/junho, com temperaturas mínimas entre -1,1 e -4,3 ºC e mais 4 geadas entre 17 a 20/julho, com mínimas entre -0,5 e -3,8ºC. No inverno de 2017 foram registradas 16 geadas, com 3 consecutivas entre 11 a 13/junho, com temperaturas mínimas entre -2,0 e -4,3ºC, e mais 3 entre 18 a 20/julho, com mínimas entre 0,3 e -3,9ºC.

FIGURA 2. DADOS MÉDIOS MENSAIS DE TEMPERATURA MÁXIMA, MÉDIA E MÍNIMA, E TOTAL DE PRECIPITAÇÃO MENSAL DO PERÍODO ENTRE DEZEMBRO DE 2015 ATÉ NOVEMBRO DE 2017, OBTIDAS NA ESTAÇÃO METEOROLÓGICA EM CAMPO BELO DO SUL – BAESA. DADOS FORNECIDOS PELA EPAGRI-CIRAM.



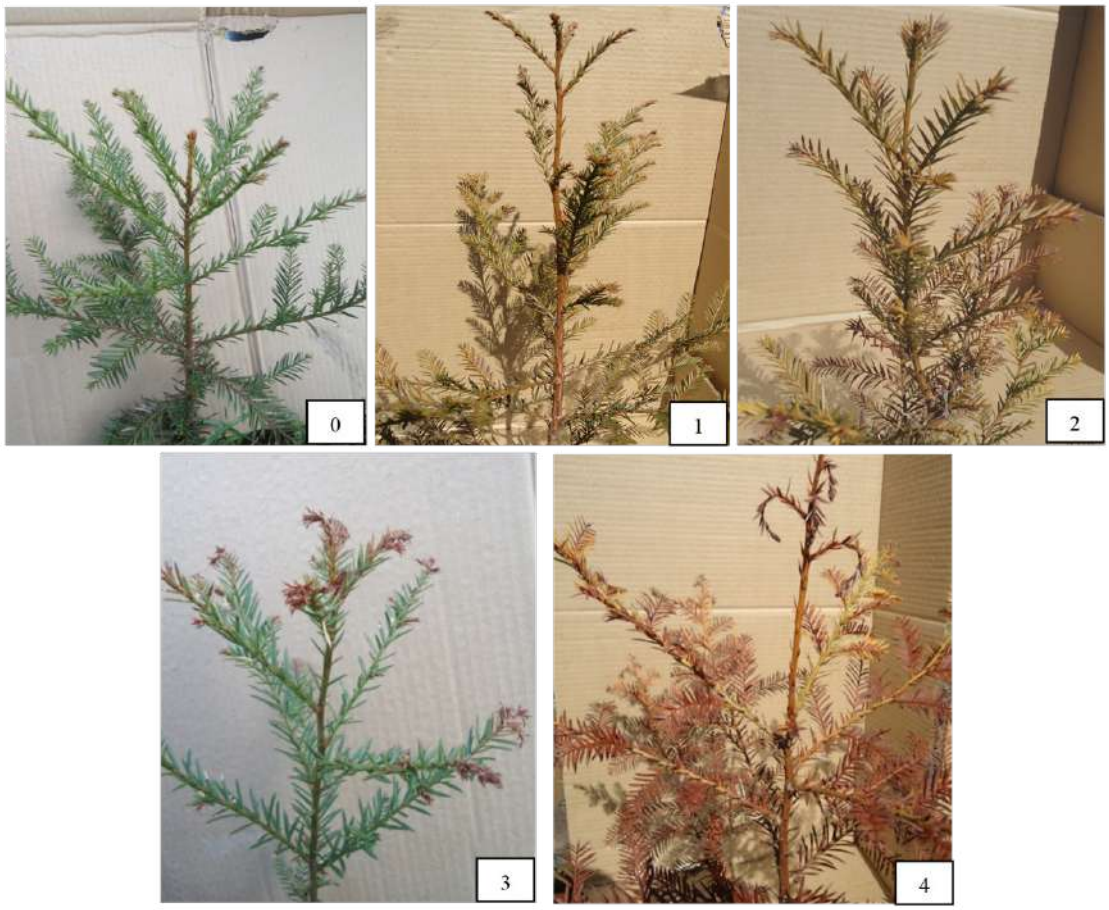
FONTE: PEREIRA, 2018.

Em Campo Belo do Sul a implantação aconteceu no início de dezembro de 2015. Trinta dias antes do plantio realizou-se uma subsolagem e após a passagem de enxada rotativa a fim de preparar o solo. As mudas utilizadas no plantio apresentavam altura de aproximadamente 30 cm e diâmetro do coleto maior que 4 mm. Quinze dias antes do plantio passaram por período de aclimatação, com redução da irrigação e manutenção a pleno sol. Quinze dias após o plantio foi realizada adubação com 150 gramas de NPK (5-20-20) por planta.

O teste foi implantado utilizando-se três clones (SJ, A140 e A228) em subparcelas lineares de 10 plantas e 30 repetições, totalizando-se 300 mudas por clone testado. O espaçamento de plantio utilizado foi de 2,5 x 2, 5 metros. O clone denominado SJ deriva de outro experimento conduzido no Viveiro Florestal da UDESC (NAVROSKI et al., 2015). Já os clones A140 e A228 foram provenientes de miniestacas do minijardim clonal, que apresentaram material suficiente para implantação do presente teste clonal a campo. (PEREIRA, 2018)

As avaliações foram realizadas aos 6, 12 e 24 meses após o estabelecimento do teste clonal, avaliando-se a sobrevivência (%), diâmetro a altura do colo (mm) e altura total (cm). Aos 6 meses também realizou-se avaliação de danos por geada, a qual foi conduzida por meio de notas, sendo estas: nota 0 - sem sintomas de geada, sem danos nas folhas e ápice; nota 1 - poucas folhas queimadas, sem danos no ápice; nota 2 - folhas queimadas na grande maioria, sem danos graves no ápice; nota 3 - danos graves nas folhas e no ápice e nota 4 - plantas secas, com as folhas e ápice totalmente queimados (Figura 3).

FIGURA 3. ESCALA DE NOTAS DE DANOS POR GEADA EM TESTE CLONAL DE *Sequoia sempervirens* APÓS SEIS MESES DE INSTALAÇÃO EM CAMPO BELO DO SUL – SC. NOTA 0 - SEM SINTOMAS DE GEADA, SEM DANOS NAS FOLHAS E ÁPICE; 1 - POUCAS FOLHAS QUEIMADAS, SEM DANOS NO ÁPICE; 2 - FOLHAS QUEIMADAS NA GRANDE MAIORIA, SEM DANOS GRAVES NO ÁPICE; 3 - DANOS GRAVES NAS FOLHAS E NO ÁPICE E 4 - PLANTAS SECAS, COM AS FOLHAS E ÁPICE TOTALMENTE QUEIMADOS.



Área 2- Arabutã (SC)

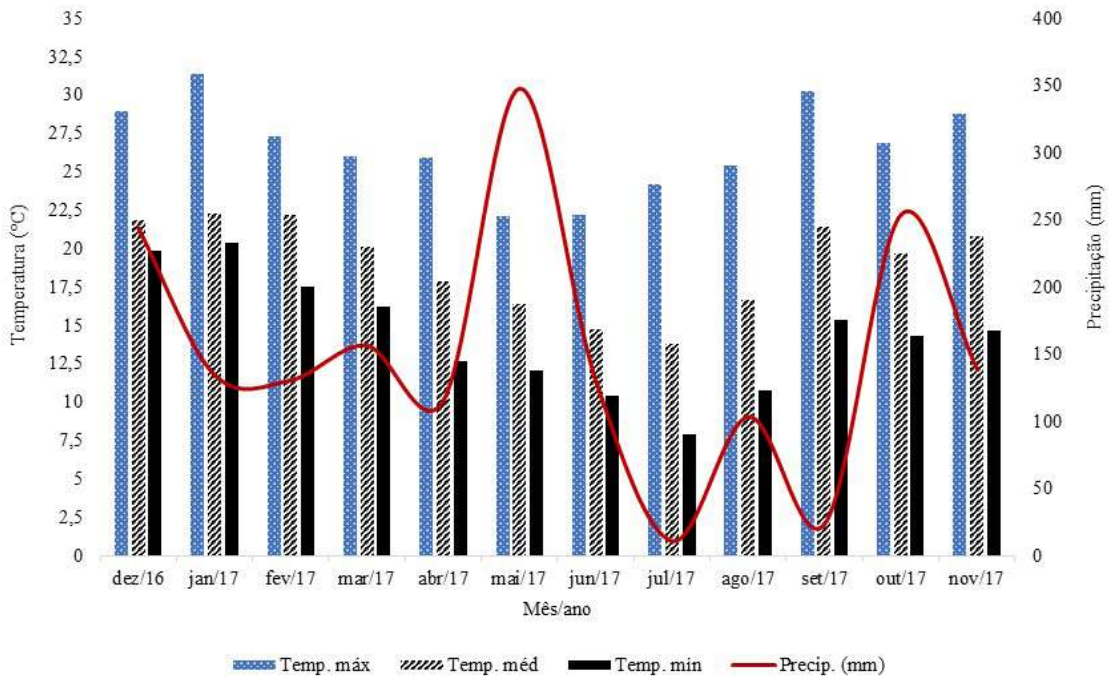
O local de implantação está situado nas coordenadas geográficas 27°11'50"S e 52°11'38"W. O tipo de relevo predominante no local é ondulado a fortemente ondulado, com uma altitude média de 415 m. Os solos predominantes na região são Nitossolos ou Cambissolos, sendo geralmente de origem basáltica (UBERTI, 2005). Conforme análise de solo (0-40 cm), o local apresenta a seguinte atribuição química: pH = 5,1; índice SMP = 5,7;

Ca = 4,95 (cmolc/dm³); Mg = 1,01 (cmolc/dm³); Al = 0,94 (cmolc/dm³); H+Al = 6,6 (cmolc/dm³); CTC efetiva = 7,2 (cmolc/dm³); saturação de alumínio = 14,1%; saturação de bases = 49,8%; M.O. = 2,0%; C.O. = 1,2%; Argila = 42,5%; P (Mehlich) = 1,25 (mg/dm³); K = 114,5 (mg/dm³); CTC pH 7,0 = 12,5 (cmolc/dm³); Cu (Mehlich) = 9,3 (mg/dm³); Zn (Mehlich) = 5,2 (mg/dm³); Fe (Mehlich) = 25,7 (mg/dm³) e Mn = 59,7 (mg/dm³).

Pela classificação de Köppen, o clima é Cfa, temperado úmido com verão quente. A temperatura média anual é de 19,2 ºC, sendo em janeiro a maior média de temperatura (24,0 ºC) e junho a menor (14,9 ºC). A pluviosidade média anual é aproximadamente 1900 mm (Figura 1b) (CLIMATE, 2017a).

Os dados médios mensais de temperatura máxima, média e mínima, e total de precipitação mensal do período de plantio (dez 2016) até 12 meses após (nov 2017) estão na Figura 4. Os dados de temperatura são originários da estação meteorológica localizada em Ouro (aproximadamente 60 km da área) e os dados de precipitação da estação localizada em Concórdia (situada a 16 km da área). Durante o período não foi observada a ocorrência de geadas, conforme dados da estação meteorológica, também não ocorrendo no local do experimento (comunicação pessoal do proprietário).

FIGURA 4. DADOS MÉDIOS MENSAIS DE TEMPERATURA MÁXIMA, MÉDIA E MÍNIMA (ESTAÇÃO METEOROLÓGICA DE OURO-SC) E TOTAL DE PRECIPITAÇÃO MENSAL (ESTAÇÃO METEOROLÓGICA DE CONCÓRDIA-SC) DO PERÍODO ENTRE DEZEMBRO DE 2016 ATÉ NOVEMBRO DE 2017. DADOS FORNECIDOS PELA EPAGRI-CIRAM.



FONTE: PEREIRA, 2018.

A implantação na área de Arabutã ocorreu no início de dezembro de 2016. Para o preparo da área, 30 dias antes do plantio, foi realizada aplicação de herbicida a base de glifosato. A abertura de covas foi realizada com motocoveadora, com aproximadamente 25

cm de profundidade e 20 cm de diâmetro em cada cova. Juntamente ao plantio foi realizada a adição de hidrogel nas covas, sendo utlizados 500 ml por planta de gel previamente hidratado a uma concentração de 3 g L^{-1} do polímero hidrorretentor.

As mudas utilizadas no plantio apresentavam altura de aproximadamente 25 cm e diâmetro do coleto médio de 4 mm. Quinze dias antes do plantio passaram por período de aclimatação, com redução da irrigação e manutenção a pleno sol. Dez dias após o plantio foi realizada adubação com 150 gramas de NPK (5-20-20) por planta. Após o plantio, em janeiro de 2017, foi efetivado o plantio de arroz de sequeiro e em outubro de 2017 a área contou com a adição de milho nas entrelinhas do talhão de sequoia.

O teste foi implantado utilizando-se quatro clones (A127, A138, A140 e A228) em subparcelas lineares de 8 plantas e 20 repetições, totalizando-se 160 mudas por clone testado. O espaçamento de plantio utilizado foi de 3,0 x 3,0 metros. Todos os clones foram oriundos de miniestacas do minijardim clonal, que apresentaram material suficiente para implantação do presente teste clonal a campo (PEREIRA, 2018). As avaliações foram realizadas aos 6 e 12 meses após o estabelecimento do teste clonal avaliando-se a sobrevivência (%), diâmetro a altura do colo (DAC - mm) e altura total (cm).

Análise estatística

Após a verificação da normalidade dos dados por meio do teste de Kolmogorov-Smirnov e da homogeneidade pelo teste de Bartlett realizou-se a análise de variância. Quando necessário, os dados foram transformados pela função $(x + 0,5)^{0,5}$ e as médias foram comparadas pelo teste de Scott-Knott, a 5% de probabilidade.

RESULTADOS

Área 1 – Campo Belo do Sul (SC)

O plantio de sequoia em Campo Belo do Sul apresentou susceptibilidade à geada, exibindo mortalidade superior a 60% após 6 meses (Tabela 1), período correspondente a avaliação realizada no inverno de 2016. Nesta mesma avaliação observou-se diferença entre os clones testados no local, sendo A228 e A140 superiores em relação ao clone SJ. O clone A140 apresentou superioridade também quanto ao DAC e menor dano por geadas. A maior altura e DAC obtida em uma planta aos 6 meses, foi 84 cm e 12,7 mm respectivamente.

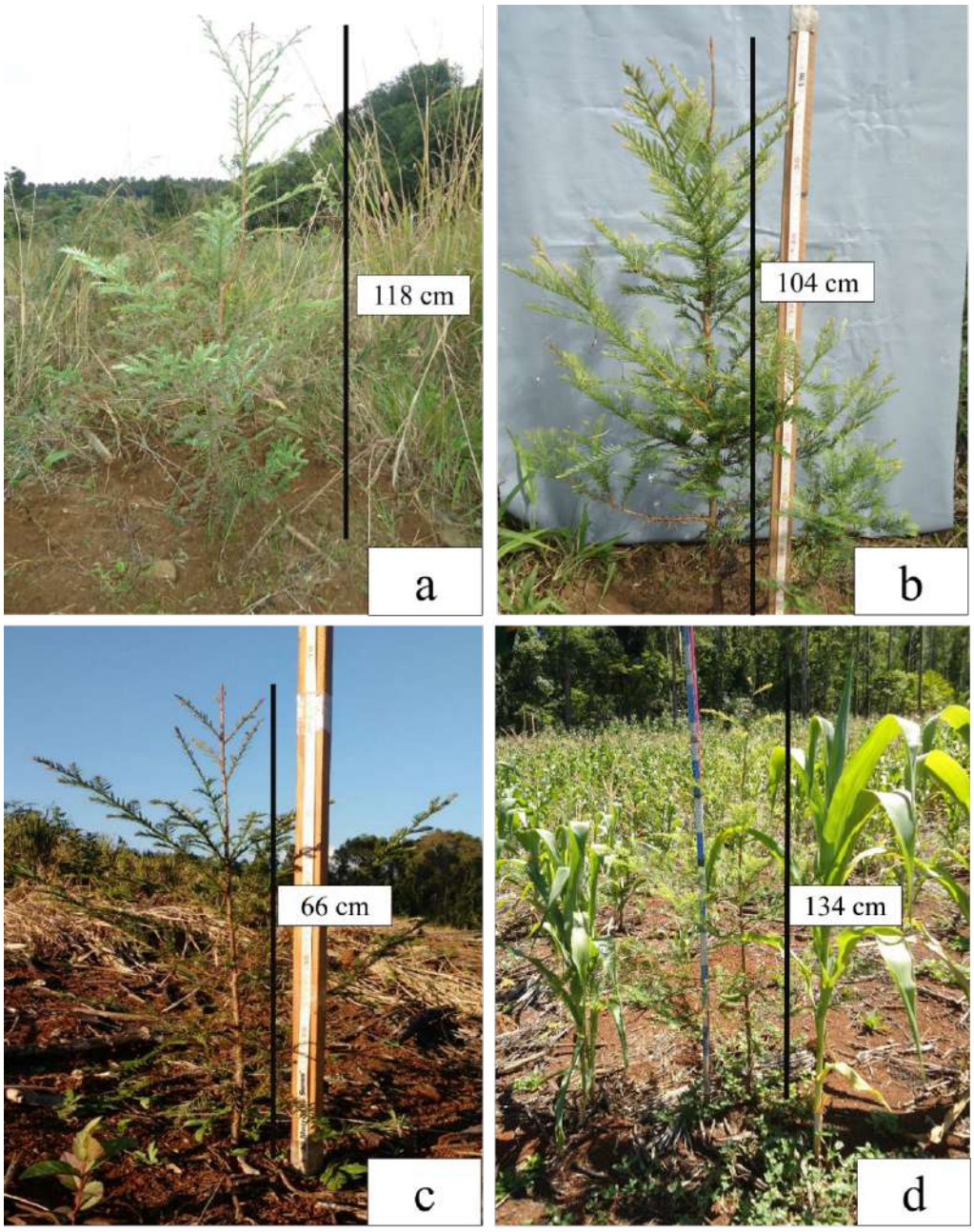
TABELA 1. SOBREVIVÊNCIA (%), DIÂMETRO A ALTURA DO COLO - DAC (MM), ALTURA (CM) E DANOS DE GEADA EM DIFERENTES CLONES DE *Sequoia sempervirens* APÓS 6, 12 E 24 MESES DE INSTALAÇÃO EM CAMPO BELO DO SUL – SC.

Clones	6 meses			
	Sobrevivência (%)	DAC (mm)	Altura (cm)	Danos de Geada
SJ	28,7 b*	6,3 b	47,3 a	3,1 b
A140	38,1 a	7,1 a	42,6 b	2,5 a
A228	42,7 a	6,1 b	40,1 b	3,2 b
Média	36,5	6,5	43,3	2,9
Máxima[1]	-	12,7	84,0	-
CV%	34,6	24,9	35,0	34,1
p-valor	0,008	0,048	0,013	0,041
Clones	**12 meses**			
	Sobrevivência (%)	**DAC (mm)**	**Altura (cm)**	
São Joaquim	2,3 b	6,5 b	48,8 b	
A140	17,7 a	12,2 a	67,7 a	
A228	9,8 b	9,3 b	45,6 b	
Média	9,9	9,3	54,0	
Máxima[1]	-	19,0	118,0	
CV%	46,7	31,5	26,8	
p-valor	0,004	0,031	0,034	
Clones	**24 meses**			
	Sobrevivência (%)	**DAC (mm)**	**Altura (cm)**	
São Joaquim	1,0	9,1 b	55,2 b	
A140	3,9	15,6 a	79 a	
A228	3,0	11,4 b	55,7 b	
Média	2,6	12,0	63,3	
Máxima[1]	-	27,5	115,0	
CV%	52,1	38,2	28,3	
p-valor	0,638	0,0142	0,009	

[1] Resposta atribuída ao valor de máxima resposta de um indivíduo.
* Médias seguidas por letras iguais não diferem entre si pelo teste de Scott-Knott a 5% de probabilidade.

Na avaliação realizada 12 meses após o plantio, a sobrevivência foi muito mais baixa (em relação a observada aos 6 meses). A média de sobrevivência entre todos os clones foi menor por 10%, com superioridade para o clone A140 que apresentou quase 20% de plantas vivas. Merece destaque pela baixa sobrevivência o clone SJ com somente 2,3%. Esta época de avaliação (12 meses) englobou todo o período de inverno, fato que não tinha ocorrido aos 6 meses. O clone A140, além de apresentar melhor sobrevivência, também apresentou superioridade em DAC e altura, sendo superior aos outros dois avaliados. A maior planta em altura aos 12 meses apresentou 118 cm (Figura 5a), representando um incremento de 34 cm em 6 meses. Já em DAC, o maior valor foi de 19 mm, resultando em 6,3 mm de incremento entre o intervalo das duas avaliações.

FIGURA 5. A) MAIOR INDIVIDUO DE SEQUOIA AOS 12 MESES EM CAMPO BELO DO SUL- SC; B) INDIVÍDUO DE SEQUOIA SEM BIFURCAÇÃO OU BROTAÇÕES BASAIS CAUSADAS PELA GEADA EM CAMPO BELO DO SUL- SC; C) MAIOR INDIVIDUO DE Sequoia AOS 6 MESES (C) E; D) AOS 12 MESES EM PLANTIO EXPERIMENTAL INSTALADO EM ARABUTÃ – SC.



Elevada mortalidade das mudas foi observada na avaliação aos 24 meses, sendo possível observar 2,6% de mudas vivas, sem diferença entre os clones. Apesar de não ter sido avaliado o dano por geada no inverno de 2017, percebeu-se elevada quantidade de mudas mortas, com somente poucos indivíduos sem bifurcação ou rebrota basal (Figura 5b). Diferenças entre os clones foram encontradas para altura e DAC, em que o clone A140 apresentou superioridade em ambas as variáveis. A maior altura observada foi de 115 cm e 27,5 mm para DAC. Em relação a diferença entre a avaliação de 24 e de 12 meses, houve redução da altura, provavelmente devido a geada, como será discutido adiante. Quanto ao incremento da altura média, observou-se quase 10 cm no intervalo de 12 meses.

Área 2- Arabutã (SC)

A sobrevivência das mudas de sequoia no plantio realizado em Arabutã (SC) foi superior a 80% após um ano de implantação (Tabela 2). A mortalidade entre os 6 e 12 meses foi de somente 3,4%. Houve diferença entre os clones avaliados para esta variável, sendo que, ao final dos 12 meses, os clones A127 e A140 foram superiores, apresentando sobrevivência superior a 85%.

Em relação ao DAC e altura das mudas, os clones não apresentaram diferenças significativas. Aos 12 meses após o plantio as mudas apresentaram DAC de 7,5 mm e altura pouco superior a 50%. O DAC apresentou incremento de 1,1 mm entre os 6 e 12 meses, já para altura observou-se um elevado incremento (16,5 cm). Aos 6 meses de avaliação a planta mais alta possuía 66 cm (Figura 5c) e aos 12 meses 134 cm (Figura 5d), representando quase 75 cm de incremento em 6 meses. Em DAC, o melhor indivíduo apresentava 11,3 mm aos 6 meses e 24,5 mm aos 12 meses, representando um incremento de 13,2 mm.

TABELA 2. SOBREVIVÊNCIA (%), DIÂMETRO A ALTURA DO COLO - DAC (MM), ALTURA (CM) EM DIFERENTES CLONES DE *Sequoia sempervirens* APÓS 6 E 12 MESES DE INSTALAÇÃO EM ARABUTÃ – SC.

Clones	06 meses		
	Sobrevivência (%)	DAC (mm)	Altura (cm)
A127	86,7 a	6,2	37,2
A138	74,4 b	6,0	33,7
A140	87,4 a	6,9	32,7
A228	88,2 a	6,7	34,2
Média	84,2	6,4	34,5
Máxima[1]	-	11,3	66,0
CV%	19,0	22,0	21,9
p-valor	0,0171	0,280	0,742
Clones	**12 meses**		
	Sobrevivência (%)	**DAC (mm)**	**Altura (cm)**
A127	86,7 a	7,5	53,9
A138	72,5 b	7,1	49,6
A140	86,1 a	7,9	48,8
A228	77,8 b	7,5	51,6
Média	80,8	7,5	51,0
Máxima[1]	-	24,5	134,0
CV%	26,0	19,9	19,2
p-valor	0,0173	0,4334	0,6763

[1] Resposta atribuída ao valor de máxima resposta de um indivíduo.
* Médias seguidas por letras iguais não diferem entre si pelo teste de Scott-Knott a 5% de probabilidade.

FONTE: O autor (2018).

DISCUSSÃO

A adaptação de uma nova espécie, principalmente quando se trata de uma espécie com enorme potencial madeireiro como a sequoia, pode representar um marco na silvicultura, podendo-se traduzir em incremento financeiro para produtores e empresas, novas opções para o mercado consumidor e diminuição da pressão sobre espécies nativas. A sequoia é uma espécie que apresenta potencial para uso comercial, sendo testada com sucesso em países como Chile (UNDURRAGA, 1995), África do Sul (RAMÍREZ, 2002) e principalmente na Nova Zelândia (PALMER et al., 2012). No Brasil, a espécie é encontrada de forma isolada em praças, parques e unidades de conservação como as Florestas Nacionais (FLONA's), principalmente no Sul do Brasil. Isso pode indicar uma possível adaptação da espécie às condições climáticas mais frias do país.

Na Nova Zelândia, principal país com sequoia fora do ambiente nativo, a espécie foi introduzida pela primeira vez na década de 1860. No entanto, a maioria dessas plantações falhou e a espécie deixou de ter interesse comercial (KNOWLES; MILLER, 1993). Na década de 1990, o interesse em estabelecer uma indústria florestal com sequoia na Nova Zelândia foi reativado (BROWN et al., 2008). O clima do país é geralmente adequado para a espécie, com as falhas de plantio no início do século XX podendo ser atribuídas à falta de localização apropriada, ausência de controle de ervas daninhas e desconhecimento sobre micorrizas adequadas (MEASON et al., 2016). Uma vez estabelecidos, os plantios da Nova Zelândia são capazes de atingir mais de 50 $m^3\ ha^{-1}\ ano^{-1}$ aos 40 anos em locais ótimos (NICHOLAS; WATT, 2011, PALMER et al., 2012). O Sul do Brasil possui similaridade climática com a Nova Zelândia e o sucesso da sequoia neste país nos leva a acreditar que o teste clonal de material já implantado na região pode gerar bons resultados. Contudo, variações genéticas e geográficas podem determinar o sucesso ou não da implantação.

A literatura é bastante contrastante em relação a sobrevivência e adaptação da espécie em locais mais frios e com ocorrência de geadas. Donnet (1984) comentou que a sequoia é severamente prejudicada em temperaturas inferiores a 0 °C. Neste mesmo sentido, algumas referências mais antigas já abordavam que sequoias jovens são facilmente feridas por geadas, especialmente na primavera, provocando muitas vezes a mortalidade (DALLIMORE 1931; FRITZ, 1958). Os mesmos autores colocam também que mudas jovens são facilmente danificadas por ventos fortes. Quando expostas aos ventos predominantes as sequoias geralmente têm ápices deformados e o crescimento em altura é lento.

O principal fator responsável pela elevada mortalidade em Campo Belo do Sul foi a ocorrência de fortes geadas. As geadas também foram responsáveis por perdas de áreas implantadas em áreas mais frias da Nova Zelândia (DEAN, 2007). O efeito das agressões pelas geadas nas plantas é variável com as espécies. Para cada espécie vegetal há uma

temperatura em que o congelamento do protoplasma ocorrerá, sendo possível que ocorra antes da temperatura chegar a zero grau. Algumas espécies podem apresentar uma maior resistência ao congelamento, continuando vivas mesmo após o fenômeno ter ocorrido. Quando a geada se deposita na planta ocasiona o congelamento do protoplasma das células, destruindo o tecido vegetal e, possivelmente, matando a mesma (PERISSATO et al., 2013.).

Kreyling et al. (2015) testando a tolerância a frio por método relativo de filtração eletrolítica (LT50) de 27 espécies do Hemisfério Norte, observaram uma tolerância ao frio de até -9,2 °C para *Sequoia sempervirens*. Ainda no mesmo estudo, foi possível observar que a amplitude térmica é uma das principais responsáveis por elevados danos às mudas. Essa constatação pode explicar em grande parte os elevados danos pela geada na área de Campo Belo do Sul. Um exemplo da grande amplitude térmica na região foi a temperatura de -4,3 (mínima) e 14,3 (máxima) obtida no mesmo dia.

Outro fator que contribuiu para o aumento dos danos pela geada e, consequentemente, a mortalidade das plantas em Campo Belo do Sul foi o grande intervalo entre as geadas. No inverno de 2016 (mais severo), somente 9 geadas ocorreram de forma consecutiva em número superior a 3 eventos e as 14 restantes ocorreram em dias isolados ou em dois dias sequenciais. Geadas que ocorrem de forma mais esporádica são mais danosas às plantas do que várias geadas sequenciais. Isto porque em geadas periódicas as plantas podem se "preparar" para a sua ocorrência através de uma gradual aclimatação dos seus processos vegetativos e dessa forma não sofrer danos (SAKAI; LARCHER, 1987).

Os danos pelas geadas no plantio em Campo Belo do Sul (avaliados 6 meses após implantação) mostram grande heterogeneidade no impacto às plantas. Foram encontradas plantas sem sintomas de geada, com todas as folhas aciculares verdes (Figura 3a) e outras totalmente secas (Figura 3e). Esta variação ocorreu sem influência clonal, ou seja, os clones apresentaram qualquer um dos sintomas, apesar do clone A140 apresentar danos menores. Essas variações podem ser atribuídas ao "efeito c" da clonagem. O "efeito c" da clonagem, refere-se aos efeitos não-genéticos decorrentes das interações de ações no processo de produção de mudas e posterior crescimento, interações com o meio ambiente, interação nutricional e fisiológica, o qual modifica a resposta esperada, ou seja, crescimento e comportamento por igual das plantas originárias de um mesmo clone (XAVIER et al., 2013).

A ausência de geadas na área da Arabutã, pode ser observada pela elevada sobrevivência das mudas (>80% aos 12 meses). A temperatura mínima observada na região foi de 0 ºC em junho de 2017, contudo sem ocorrência de geada. Esse resultado mostra uma melhor adaptação da espécie ao local mais quente, em relação aos dois ambientes testados, ou pelo menos sem ocorrência forte de geada. Outro fator que contribuiu para a boa sobrevivência das mudas foi a elevada precipitação após o plantio – 250 mm (dez/2016) e

em todo o período dos 6 meses iniciais (Figura 4), apesar das elevadas temperaturas nos meses de dez/2016 e jan/2017. Os primeiros meses após implantação são os mais críticos à sobrevivência das mudas (SANTIN et al., 2015). A mortalidade das mudas (20%) possivelmente seja decorrência da adaptação ao plantio. O potencial de estabelecimento de espécies florestais é expresso pela capacidade de adaptação e o vigor das mudas, frente "s" reais condições ecológicas observadas no campo (MACEDO et al., 2002).

Ao longo do tempo, o vigor mostra o desenvolvimento adequado das mudas e sua qualidade, podendo ser afetado por estresse hídrico, ataque de pragas ou doenças e danos físicos ocorridos. No plantio em Arabutã, o ataque de pragas não chegou a provocar mortalidade de mudas, contudo o ataque de gafanhoto-soldado (*Chromacris speciosa*) causou a diminuição da área foliar e quebra de dominância apical de alguns indivíduos (não foi realizada avaliação do ataque). Este ataque ocorreu nos meses de setembro e outubro de 2017, desaparecendo na sequência. Após este período as mudas atacadas apesentaram recuperação com emissão de novas brotações. Outro fato que não apresentou avaliação quantitativa, mas que pode apresentar uma linha para novos trabalhos, foi o maior crescimento das mudas cultivadas sob sombra parcial do milho. Esta comparação foi realizada com plantas que não estavam rodeadas pelo milho, as quais apresentavam menor desenvolvimento. Esse comportamento é contrário do relatado por Olson et al. (1990) na região natural da espécie. Os autores citam que o crescimento juvenil de sequoias é melhor em plena luz solar, embora as mudas possam suportar sombra pesada.

No geral, as mudas plantadas em ambos os locais apresentaram bom crescimento, com altura superior a 50 cm em 12 meses de cultivo. Além disso, foram observadas árvores dominantes com mais de um metro de altura em Arabutã (Figura 5d), além de incremento superior a 70 cm de altura entre os 6 e 12 meses. Estes resultados são similares aos encontrados em plantio na Nova Zelândia, onde as árvores alcançaram 1,4 metros entre o primeiro e segundo ano (DEAN, 2007). Olson et al (1990) afirma que a sequoia pode crescer cerca de 46 cm na primeira estação e entre 0,6 a 2,0 m ano^{-1} entre os 4 e 10 anos. Os mesmos autores destacam ainda que em muitos casos o crescimento pode ser lento até os 10 anos, necessitando-se de um período de adaptação. Em um teste de procedências de sequoia instalado em vários locais, as maiores árvores apresentaram 2,70 metros de altura na Califórnia - EUA aos 4 anos (KUSER, 1996).

Os resultados obtidos no presente trabalho mostram o potencial da espécie para áreas sem ocorrência severa de geadas. Contudo, assim como ocorreu em outros países, alguns “erros” na implantação não devem estancar novas pesquisas, principalmente em áreas mais frias. Sugestões para novos trabalhos podem ser oferecidas, como testar a espécie em sub-bosque ou em plantios mistos e ainda, avaliar procedências de vários locais. Conforme

Kuser (1996), procedências mais ao Norte da Califórnia apresentaram melhor adaptação em áreas da França com ocorrência de geadas, enquanto procedências mais aos Sul da Califórnia obtiveram melhor adaptação e crescimento em locais mais quentes. Além disso, novos plantios devem ser estabelecidos em outros locais buscando obter mais informações sobre a adaptação e crescimento da sequoia na Brasil.

CONCLUSÕES

Mudas de sequoia implantadas em Campo Belo do Sul (região mais fria) apresentaram elevado dano por geada, sendo observada sobrevivência de apenas 2% do material após dois anos de implantação. Em contrapartida, na área de Arabutã (área mais quente) a sobrevivência foi superior a 80% aos 12 meses, com bom crescimento das mudas.

Novas estratégias devem ser adotadas para buscar o estabelecimento da sequoia em áreas mais frias. Nas áreas sem ocorrência de geada novas pesquisas podem ser desenvolvidas para maximizar o crescimento.

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Influência da variação luminosa em *Cedrela fissilis*

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10.37885/200901193

RESUMO

Umas das condições essenciais para o bom ou não desenvolvimento das plântulas é a exposição de luz, contudo, muitas espécies florestais necessitam de certos níveis de sombreamento na fase inicial de desenvolvimento. Dessa forma, o objetivo do estudo foi avaliar como mudas de cedro (*Cedrela fissilis*) reagem a diferentes níveis de intensidade luminosa. Foram utilizadas mudas de cedro de diferentes matrizes de aproximadamente 25 cm de altura as quais foram submetidas a diferentes níveis de sombreamento. Os tratamentos utilizados para o nível de incidência luminosa foram compostos por T1: cobertura simples de sombreamento da casa de sombra; T2: camada simples de sombrite; T3: camada dupla de sombrite. Após 90 dias de aplicação dos tratamentos de sombreamento foram avaliados diâmetro do coleto, altura e variáveis fisiológicas: taxa fotossintética líquida (*A*), condutância estomática (*Gs*), transpiração (*E*), concentração intercelular de CO_2 no mesofilo (*Ci*) e a relações de concentração intercelular de CO_2 no mesofilo sobre a concentração de CO_2 externa atual (Ci/Ca). Em relação "s" variáveis fisiológicas as mudas de cedro apresentaram diferenças de fotossíntese e transpiração, onde mudas mantidas em maiores níveis de sombreamento apresentaram menores médias. Quanto "s" variáveis de crescimento, a altura das mudas foi influenciada pelos níveis de sombreamento e matrizes. No geral, mudas cultivadas em maior quantidade de luz apresentaram maior crescimento. Pode-se concluir que para a produção de mudas de *Cedrela fissilis* indicada a manutenção das mudas em casa de sombra simples com intensidade luminosa próxima a 1100 lux.

Palavras-chave: Cedro, Produção de Mudas, Sombreamento, Variáveis Fisiológicas.

INTRODUÇÃO

A preocupação mundial com relação à qualidade ambiental tem se mostrado cada vez mais frequente, gerando uma demanda crescente de serviços e produtos florestais, em especial, na produção de mudas para a restauração de áreas degradadas, reflorestamentos para fins econômicos e arborização urbana (KELER et al., 2009).

Diante do uso do cedro (*Cedrela fissilis*), surge a necessidade de conhecer exigências específicas para o desenvolvimento desta espécie florestal visando seus diversos usos, cujo êxito depende da qualidade das mudas produzidas. Estas além de resistirem às condições adversas encontradas no campo, devem desenvolver-se produzindo árvores com crescimento volumétrico desejável (GOMES et al., 1991).

Umas das condições essenciais para o bom ou não desenvolvimento das plântulas é a exposição de luz. A radiação incidente nas folhas próximas ao chão, devido à atenuação da radiação através dos vários estratos da cobertura vegetal, pode ser de cerca de 1% a 2% da radiação incidente nas folhas do dossel. Em clareiras, provocadas pela queda ou retirada de árvores, a quantidade de luz pode ser similar à incidente no dossel (LEE et al., 1996) e variar da borda para o centro desta, ou mesmo variar com o decorrer do tempo, devido ao crescimento de plantas adjacentes (KITAJIMA, 1996).

Quando as plântulas experimentam uma mudança nas condições de luz, a maioria delas é capaz, em maior ou menor grau, de aclimatar-se à mudança ocorrida (KITAJIMA, 1996). Através desse conhecimento prévio, o objetivo do estudo foi avaliar como mudas de cedro (*Cedrela fissilis*) reagem a diferentes níveis de intensidade luminosa. Nossa hipótese é que mudas cultivadas em sombreamento mais leve sejam favorecidas no crescimento inicial e promovam maiores trocas fotossintéticas.

MATERIAIS E MÉTODOS

O experimento foi conduzido entre agosto e novembro de 2019, em casa de vegetação no viveiro florestal do Centro de Ciências Agroveterinárias – CAV (UDESC), localizado no município de Lages, SC. De acordo com a classificação de Köppen o clima do município é mesotérmico úmido com verão ameno (Cfb – temperado). A temperatura média anual de 15 ºC e a precipitação média anual é de 1300 mm a 1500 mm.

Sementes de *Cedrela fissilis* foram coletadas em quatro árvores matrizes (denominadas de M64; M47; M37 e M63) localizadas nos três estados da região Sul do Brasil, ao Programa de Resgate e Formação do Banco de Germoplasma, executado como medida mitigadora no processo de licenciamento ambiental entre o empreendedor Transmissora Sul Brasileira

de Energia S.A. (TSBE). Os frutos coletados foram secos ao sol para sua abertura, e após as sementes que foram utilizadas para a semeadura.

A semeadura foi realizada em tubetes de polietileno rígido de 180 cm^3, preenchidos com substrato comercial composto por turfa, vermiculita expandida, casca de pinus e carvão vegetal, apresentando as seguintes, conforme o fabricante: pH = 6,0 (± 0,5); condutividade elétrica = 0,7 (± 0,3) mS cm^{-1}; densidade = 500 kg m^{-3}; capacidade de retenção de água – CRA10 (p/p) = 150% e umidade máxima (p/p) = 50%. Ao substrato adicionou-se fertilizante de liberação controlada (FLC) na formulação 19-6-10 (NPK), caracterizado pela liberação lenta de 4 a 5 meses.

A semeadura foi efetuada colocando-se duas sementes em cada recipiente. Para a cobertura das sementes foi utilizada uma camada de 1 cm de vermiculita de granulometria média, visando cobertura homogênea sobre as sementes da espécie. Após a semeadura as bandejas foram levadas para casa de vegetação, onde permaneceram por 60 dias. Com 40 dias, procedeu-se o raleio das mudas, permanecendo a mais vigorosa e centralizada no recipiente. Aos 60 dias efetuou-se a diminuição da densidade das mudas na bandeja em 50%, passando da densidade inicial de 400 plantas/m^2 para 200 plantas/m^2. Neste momento, as mudas foram colocadas em casa de sombra, permanecendo por mais 30 dias para a realização do experimento. Na casa de sombra foram realizadas quatro irrigações diárias (09h00min; 12h00min; 15h00min; 18h00min) de 10 minutos cada, utilizando-se o sistema de microaspersão. Esta casa possui cobertura do tipo nylon, com sombreamento aproximado de 30-33%. No início do experimento foi realizada medição das variáveis dendrométricas, sendo que as mudas apresentaram médias de altura de 25,5 cm e diâmetro do coleto de 5,6 mm.

O delineamento experimental foi inteiramente casualizado, em esquema bifatorial 3 x 4, sendo os níveis do fator A compostos por três diferentes níveis de sombreamento e os níveis do fator D por quatro matrizes (M64; M47; M37 e M63). Foram utilizadas oito repetições de seis plantas cada.

Os tratamentos utilizados para o nível de incidência luminosa foram compostos por diferentes sombreamentos, sendo T1: cobertura simples de sombreamento da casa de sombra; T2: camada simples de sombrite; T3: camada dupla de sombrite. Os tratamentos T2 e T3 foram mantidos dentro da casa de sombra, ao lado do tratamento T1. Para melhor quantificação de luz foi realizada a medição de intensidade luminosa utilizando-se um luxímetro. Essa medição foi realizada no interior de cada estrutura de cultivo das mudas, pouco acima das folhas. Realizaram-se 10 avaliações de 3 repetições cada, sendo encontradas as seguintes médias de luminosidade: T1: 1100 lux, T2: 800 lux e T3:500 lux.

As avaliações de trocas gasosas foram realizadas utilizando-se cinco repetições (uma planta por repetição). Estas avaliações foram realizadas com o auxílio de um medidor portátil

de fotossíntese (modelo Li-6400xt), determinando-se os valores de taxa fotossintética líquida (*A* - µmol CO_2 $m^{-2}s^{-1}$), condutância estomática (*Gs* - mol m^{-2} s^{-1}), transpiração (*E* - mmol H_2O m^{-2} s^{-1}), concentração intercelular de CO_2 no mesofilo (*Ci* - µmol CO_2) e concentração de CO_2 externa atual (*Ca* - µmol CO_2). Estas variáveis permitiram posteriormente calcular as relações de concentração intercelular de CO_2 no mesofilo sobre a concentração de CO_2 externa atual (Ci/Ca - µmol CO_2). Dentro da casa de vegetação, as plantas receberam, durante a mensuração, irradiância de aproximadamente 900 mmol de fótons m^{-2} s^{-1}. Estas avaliações foram realizadas três vezes durante o experimento, aproximadamente a cada 30 dias de intervalo. Os valores apresentados são as médias das avaliações.

Ao final do experimento, 120 dias de sombreamento e 210 após semeadura foi realizada a medição de altura das mudas (cm) e diâmetro (mm) com paquímetro digital com precisão de 0,01 mm. A altura foi medida com régua graduada, do nível do substrato até o último par de folhas. As variâncias dos tratamentos foram testadas quanto à homogeneidade pelo teste de Bartlett. Quando houve homogeneidade das variâncias os dados foram submetidos à análise de variância e quando houve diferença significativa pelo teste F os tratamentos comparados através do teste de Tukey ao nível de 5% de significância por meio do software estatístico SISVAR (FERREIRA, 2019).

RESULTADOS E DISCUSSÕES

Em relação a taxa fotossintética (*A*), não foi observada interação entre os fatores (p-valor: 0,3204). Também não houve diferença entre as matrizes (p-valor: 0,0945). Houve efeito significativo entre os tratamentos de sombreamento. Obteve-se uma menor taxa fotossintética (*A* - µmol CO_2 $m^{-2}s^{-1}$) no tratamento 3 (T3), onde a incidência de luz foi de 500 lux, (Tabela 1). No segundo tratamento (T2), a intensidade luminosa subiu para uma média de 800 lux consequentemente o valor medido da fotossíntese subiu para 2,93. O maior valor registrado foi o de 4,49 de fotossíntese no primeiro tratamento (T1) sendo que esse foi exposto a cerca de 1100 lux médios.

Diante dessas informações, é necessário relatar a importância da luz para as plantas no seu desenvolvimento como um todo, pois, com a restrição luminosa, a tendência demonstrada é que as mudas de *Cedrela fissilis* sejam afetadas negativamente. Quando a planta está no escuro, ela irá consumir somente oxigênio, uma vez que está apenas respirando e não realizando a fotossíntese. Quando são expostas à luz e aumentada gradualmente sua intensidade, em determinado momento, todo o oxigênio produzido na fotossíntese irá ser utilizado na própria fotossíntese (FREITAS et al., 2016). Este ponto é chamado de ponto de compensação fótica ou luminosa**.** A partir deste ponto, a quantidade de luz cada vez maior faz com que haja uma reação em cadeia e eleve as taxas fotossintéticas (BARROS et al., 2019).

A fotossíntese depende da luz, o principal fator que influência este processo é a quantidade e a qualidade de energia luminosa (BARROS et al., 2019). É notável a importância da luz para a fotossíntese e sua interação com a clorofila (OLIVEIRA; GUALTIERI, 2017). Os resultados obtidos em plantas de cedro corroboram os de Piel et al. (2002). Esses autores verificaram que plantas de *Juglans regia* cultivadas em maior taxa luminosa apresentaram maiores taxas de fotossíntese e condutância estomática.

Tabela 1. Medias de Fotossíntese (*A* - µmol CO_2 $m^{-2}s^{-1}$), Condutância (*Gs* – mol m^{-2} s^{-1}), *Ci* (µmol CO_2); Transpiração (*E* - mmol H_2O m^{-2} s^{-1}) e *Ci/Ca* (µmol CO_2) em mudas de *Cedrela fissilis* em função de matrizes e tratamentos de sombreamento.

Variável	Matriz	Tratamento sombreamento			
		T1 (1100 lux)	T2 (800 lux)	T3 (500 lux)	Média
Fotossíntese *A* - µmol CO_2 $m^{-2}s^{-1}$	M64	3,58	2,88	2,37	2,94
	M47	3,99	2,33	1,90	2,74
	M37	4,38	3,43	2,04	3,48
	M63	6,02	8,10	2,25	3,79
Média		4,49 A*	2,93 B	2,15 B	
Condutância *Gs* – mol m^{-2} s^{-1}	M64	0,06	0,06	0,06	0,06
	M47	0,10	0,21	0,06	0,12
	M37	0,09	0,09	0,05	0,08
	M63	0,15	0,38	0,05	0,19
Média		0,10	0,19	0,05	
Ci µmol $_cO2$	M64	296,5	326,9	329,9	317,7
	M47	329,3	325,8	350,8	335,3
	M37	328,8	344,6	353,9	340,4
	M63	326,3	330,4	328,0	328,2
Média		320,7	331,9	339,8	
Transpiração *E* - mmol H_2O m^{-2} s^{-1}	M64	0,89	1,06	0,93	0,96
	M47	1,47	0,72	0,88	1,03
	M37	1,28	1,24	0,67	1,11
	M63	1,87	1,04	0,73	1,21
Média		1,37 A	1,02 AB	0,81 B	
Ci/Ca µmol CO_2	M64	0,74	0,81	0,82	0,79
	M47	0,81	0,80	0,87	0,82
	M37	0,79	0,82	0,83	0,81
	M63	0,80	0,81	0,81	0,80
Média		0,78	0,81	0,83	

* Tratamentos com letras diferentes e maiúsculas na linha refere-se a diferença significativa pelo teste de Tukey a 5% de erro.

A segunda variável observadas no experimento foi a condutância estomática (*Gs* – mol m^{-2} s^{-1}). Essa variável representa a taxa de passagem de dióxido de carbono (CO_2) ou vapor de água que sai através dos estomas de uma folha. Conforme demostrado na tabela 1 não houve interação (p-valor: 0,231) e nem diferenças significativas nos valores de condutância

observados entre os tratamentos de sombreamento e matrizes. A condutância estomática média foi de 0,11 mol m^{-2} s^{-1}. A intensidade luminosa é um dos principais fatores responsáveis por alterações em condutância estomática, e estas refletem nas taxas assimilatória líquida e na eficiência de carboxilação (SCHOCK et al. 2014). Os maiores valores ocorreriam quando a radiação solar é máxima, permanecendo altos até que o potencial hídrico da folha seja reduzido, induzindo o fechamento dos estômatos (FERREIRA et al., 1999).

Quanto a concentração interna de carbono (*Ci* - µmol CO_2) também não houve interação entre os fatores sombreamento e matrizes (p-valor: 0,493). Também não foi observada diferença para os fatores de forma isolada. Apesar de não haver diferença entre os tratamentos de sombreamento, o valor máximo foi observado no tratamento em que a incidência luminosa era a menor (T3). Isso pode ser explicado devido a menor taxa fotossintética (A) observada nas plantas desse tratamento. Quanto menor é a taxa fotossintética, maior é o consumo de oxigênio interno ocasionando uma maior concentração de carbono nas plantas. O valor médio encontrado para concentração interna de carbono foi de 330,8 µmol m^{-2} s^{-1}.

Já para a taxa de transpiração (*E*), foi possível observar uma diferença entre os três tratamentos de sombreamento (p-valor: 0,0321), não havendo interação entre os fatores (p-valor: 0,0956). Nota-se que foi apresentado maior valor de transpiração celular no primeiro tratamento com um valor médio de 1,37 mmol H_2O m^{-2} s^{-1}. Esse fato tem relação direta com a maior incidência de luz que o mesmo (T1) foi exposto. Com uma maior quantidade de luz recebida a planta tem a tendência de aumentar sua temperatura interna e consequentemente elevar a sua transpiração foliar (GONÇALVES et al., 2012). Constata-se também a diminuição gradativa dessa transpiração conforme houve uma diminuição na exposição a luz nos outros dois tratamentos (T2 e T3). Ressalta-se ainda que a transpiração é o principal mecanismo envolvido na regulação da temperatura foliar devido às menores aberturas estomáticas e, por consequência, ocorrem diminuição da transpiração foliar e aumento da temperatura da folha, por conta da redução da dissipação do calor latente sinalizando que a capacidade de refrigeração das plantas diminui via transpiração (VIEIRA JÚNIOR et al., 2007; KUMAR; PORTIS JÚNIOR, 2009).

Outra variável observada foi a relação entre carbono na câmara subestomática e carbono externo (Ci/Ca), notando-se que não houve diferenças significativas entre os três tratamentos (p-valor: 0,0854). Também não foi observada interação entre os fatores (p-valor: 0,0943). O tratamento T3 foi o que apresentou o maior valor médio, de 0,83 µmol CO_2. Considerando-se que a concentração de CO_2 externa (Ca) mantêm-se constante, o aumento na relação Ci/Ca deve-se apenas a variações na concentração interna (Ci). Se Ci está aumentando significa que o CO_2 que está chegando às células não está sendo fixado totalmente reduzindo então a taxa fotossintética (TATAGIBA et al., 2014).

As reduções em alguns parâmetros de trocas gasosas nos tratamentos, de maior sombreamento podem ter ocorrido em virtude da baixa disponibilidade de irradiância e da redução de temperatura influenciadas pela sazonalidade, reduzindo as taxas fotossintéticas e, em consequência, o ganho de carbono (VALLADARES; PEARCY, 1998), o que justifica o fato desses parâmetros serem mais elevados no tratamento com maior luminosidade.

Em relação às variáveis dendrométricas, o DAC não apresentou interação (p-valor: 0,262) nem diferenças isoladas para os fatores (Tabela 2). Já para a altura, houve interação entre os fatores (p-valor: 0,0421). O DAC médio encontrado para as mudas foi de 10,3 mm.

Tabela 2. Médias de diâmetro a altura do coleto – DAC (mm) e altura (cm) em mudas de *Cedrela fissilis* em função de matrizes e tratamentos de sombreamento.

Variável	Matriz	Tratamento sombreamento			Média
		T1 (1100 lux)	T2 (800 lux)	T3 (500 lux)	
DAC (mm)	M64	10,5	10,4	11,2	10,7
	M47	9,7	11,3	10,2	10,4
	M37	10,1	10,4	8,9	9,9
	M63	10,2	10,1	10,4	10,3
Média		10,1	10,6	10,3	
Altura (cm)	M64	40,7 a A	39,2 a A	35,1 ab B	38,3
	M47	38,1 ab A	38,0 a A	42,2 a A	39,4
	M37	42,3 a A	34,3 a A	33,3 b B	36,7
	M63	35,6 b A	33,4 b A	33,6 b A	34,2
Média		39,2	36,1	36,2	

* Médoas seguidas pela mesma letra minúscula na coluna e maiúscula na linha no diferem entre si pelo teste de Tukey, a 5% de probabilidade de erro.

Quanto à altura das mudas, as matrizes M64 e M47 apresentaram no geral as maiores médias, independente do tratamento de sombreamento. A matriz M64 apresentou médias superiores para os tratamentos com maior nível de luminosidade (T1 e T2), assim como a matriz M37. Já as mudas das matrizes M47 e M63 não apresentaram diferença estatística entre os níveis de sombreamento. Esse resultado de maior altura para mudas sob maior intensidade luminosa em cedro é contrário ao observado para a maioria das espécies florestais. A maioria das espécies demostram maior crescimento em altura e menor crescimento em diâmetro sob sombreamento, visto que a capacidade em crescer rapidamente quando sombreadas é um mecanismo de adaptação das plantas, compreendendo uma valiosa estratégia para escapar do sombreamento (NERY et al., 2016).

Como foram demonstradas anteriormente, todas as atividades fisiológicas que dependem de uma fonte luminosa para ter um melhor desempenho obtiveram valores maiores, isso afeta o crescimento da muda, fazendo com que as alturas médias sejam maiores.

CONCLUSÃO

Em relação s variáveis fisiológicas as mudas de *Cedrela fissilis* apresentaram diferenças de fotossíntese e transpiração, onde mudas mantidas em maiores níveis de sombreamento apresentaram menores médias. Quanto s variáveis de crescimento, a altura das mudas foi influenciada pelos níveis de sombreamento e matrizes. No geral, mudas cultivadas em maior quantidade de luz apresentaram maior crescimento.

Pode-se concluir que para a produção de mudas de *Cedrela fissilis* indicada a manutenção das mudas em casa de sombra simples com intensidade luminosa próxima a 1100 lux.

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“Serragem e fertilizante de liberação controlada na produção de mudas de *Mimosa scabrella* Benth.

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doi 10.37885/200801171

RESUMO

O objetivo do estudo foi avaliar a influência de diferentes proporções de serragem e fertilizante de liberação controlada na produção de mudas de *Mimosa scabrella* Benth. As sementes foram coletadas na Fazenda Experimental do Centro de Ciências Agroveterinárias (CAV), da Universidade do Estado de Santa Catarina (UDESC). As sementes foram submetidas a quebra de dormência através da imersão em água e semeadas em tubetes (180 cm^3). O delineamento utilizado foi o inteiramente casualizado, com quatro tratamentos e cinco repetições. Os tratamentos utilizados foram: T1: Substrato comercial + adição de 6 g L^{-1} de fertilizante de liberação controlada; T2: Substrato comercial + 20% de serragem + adição de 6 g L^{-1} de fertilizante de liberação controlada; T3: Substrato comercial + 40% de serragem + adição de 9 g L^{-1} de fertilizante de liberação controlada; T4: Substrato comercial + 60% de serragem + adição de 12 g L^{-1} de fertilizante de liberação controlada. Foi realizada a avaliação aos 90 dias, avaliando-se altura (cm), diâmetro de coleto (mm) e número de folhas. De modo geral, a compensação da adição de serragem ao substrato comercial pela suplementação do fertilizante de liberação controlada não apresentou boa resposta no crescimento das mudas. Desta forma, adição de serragem, mesmo na dosagem de 20%, não é indicada para a bracatinga.

Palavras-chave: Bracatinga, Substrato Alternativo, Produção de Mudas, Espécies Florestais.

INTRODUÇÃO

Mimosa scabrella Benth., conhecida popularmente como bracatinga, é uma espécie florestal pioneira e de rápido crescimento (MAZUCHOWSKI et al., 2014) cuja ocorrência geográfica natural abrange desde o sul do estado de Minas Gerais até o norte do estado do Rio Grande do Sul, com presença predominante nos estados do Paraná e Santa Catarina (IWAKIRI, 2019). A bracatinga apresenta relevante importância para a região sul do Brasil devido as várias alternativas de uso dos seus multiprodutos, pois além de ser amplamente utilizada na geração de energia, possui potencial ornamental, forrageiro, medicinal e na construção civil (SAUERESSIG, 2014).

A espécie é amplamente utilizada na recuperação de áreas degradadas e, para tanto, a produção de mudas de qualidade é um processo fundamental para aumentar as chances de sucesso do plantio (VIEIRA et al., 2019). Nesse processo, além da escolha do recipiente, a escolha do substrato adequado tem grande influência, devendo possuir características físico-químicas que favoreçam o desenvolvimento das raízes, retenção de água, boa aeração, alta disponibilidade de nutrientes, ausência de fitopatógenos, baixo custo e longa durabilidade (SILVA; QUEIROZ, 2014).

Atualmente, existem diversos substratos comerciais disponíveis no mercado, no entanto, busca-se, através da adição de componentes alternativos e da diminuição do uso de insumos químicos, reduzir os custos sem comprometer o rendimento e qualidade no processo de produção das mudas, como é o caso do fertilizante de liberação lenta ou do fertilizante de liberação controlada (CAMARGO; RUFANO, 2019; GONÇALVES et al., 2016; NADAI et al., 2015). Além disso, este pode ainda ser combinado a um material alternativo de origem vegetal, que geralmente são resíduos gerados pelas indústrias madeireiras da região (SODRE; CORA, 2007), como a serragem ou pó de madeira.

O uso de resíduos orgânicos na produção de mudas de espécies florestais é uma alternativa promissora, haja vista que possuem altas concentrações de nutrientes, contribuindo para o desenvolvimento das plantas, além de serem produzidas em grande volume e, muitas vezes, não possuírem destinação pelas indústrias, sendo comumente descartados (QUINTANA et al., 2009).

Outro fator relevante para a produção de mudas é a realização de uma adequada fertilização. A fertilização do substrato é uma das fases mais importantes em um programa de produção de mudas de espécies arbóreas. O substrato utilizado para enchimento dos recipientes deve conter os nutrientes necessários para o crescimento das mudas e para os perdidos por lixiviação, por ocasião da irrigação. Assim, o volume do recipiente, associado às características físico-químicas do substrato, frequência e a intensidade de irrigação irão determinar o tempo de residência do nutriente no sistema (JOSÉ et al., 2009).

Os fertilizantes comumente usados na produção de mudas florestais são fontes de nitrogênio, fósforo e potássio em fórmulas solúveis ou de liberação controlada. A utilização de adubos de liberação controlada têm se mostrado como uma alternativa para a produção de mudas, devido à eliminação da adubação de cobertura, pois são adicionados somente no momento de preparo do substrato, resultando em mudas de melhor qualidade (BARBIZAN et al., 2002; MENDONÇA et al., 2008).

Nesse contexto, o presente trabalho teve como objetivo avaliar o crescimento inicial de mudas de bracatinga sob diferentes proporções de serragem combinado com fertilizante de liberação controlada.

MATERIAL E MÉTODOS

O estudo foi conduzido no viveiro experimental da Universidade do Estado de Santa Catarina (UDESC), no município de Lages, situado nas coordenadas geográficas 27º19'0'' S e 50º19'35'' W e altitude de 900 m, onde o clima é classificado, segundo Köppen, como mesotérmico úmido (Cfb), com precipitação anual de 1.809 milímetros e média de temperatura de 15,9 °C (INMET, 2019).

As sementes utilizadas no experimento foram coletadas na Fazenda Experimental da UDESC em março de 2019. As sementes foram submetidas ao processo de quebra de dormência através da imersão em água quente (80°C) seguido de resfriamento por um período de 12h. Posteriormente, foram semeadas três sementes em cada tubete (180 cm^3) contendo substrato comercial e diferentes proporções de serragem e fertilizante de liberação controlada. Segundo o fabricante, o substrato tem na sua composição vermiculita expandida e casca de pinus, e apresenta características como capacidade de retenção de água - CRA10 55%, condutividade elétrica de 0,326 μs/cm, densidade seca de 264,0 (kg m^{-3}) pH de 5,5 e umidade máxima de 52%.

O delineamento utilizado foi o inteiramente casualizado, com quatro tratamentos e cinco repetições. Os tratamentos utilizados foram: T1: Substrato comercial + adição de 6 g L^{-1} de fertilizante de liberação controlada; T2: Substrato comercial + 20% de serragem + adição de 6 g L^{-1} de fertilizante de liberação controlada; T3: Substrato comercial + 40% de serragem + adição de 9 g L^{-1} de fertilizante de liberação controlada; T4: Substrato comercial + 60% de serragem + adição de 12 g L^{-1} de fertilizante de liberação controlada. A serragem utilizada foi obtida em serraria da região, sendo uma mescla do processo de serra de eucalipto e pinus.

A mistura de substrato comercial e serragem foi homogeneizada com as devidas doses do fertilizante de liberação controlada e em seguida os tubetes foram distribuídos em bandejas plásticas de 54 células. A semeadura foi efetuada colocando-se duas sementes em cada recipiente. Para a cobertura das sementes foi utilizada uma camada de 1 cm de

vermiculita de granulometria média, visando cobertura homogênea sobre as sementes da espécie. Após a semeadura, as bandejas foram levadas à casa de vegetação, onde permaneceram por 60 dias. Com 40 dias, procedeu-se o raleio das mudas, permanecendo a mais vigorosa e centralizada no recipiente. Aos 60 dias procedeu-se a diminuição da densidade das mudas na bandeja em 50%, passando da densidade inicial de 400 plantas/m^2 para 200 plantas/m^2. Neste momento, as mudas foram colocadas em área de aclimatação a pleno sol, até o final do experimento.

A medição foi realizada aos 90 dias considerando a altura (cm) e contagem de número de folhas das mudas, sendo avaliado também o diâmetro do coleto (mm). As variâncias dos tratamentos foram testadas quanto à homogeneidade pelo teste de Bartlett. Quando houve homogeneidade das variâncias os dados foram submetidos à análise de variância e quando houve diferença significativa pelo teste de F os tratamentos comparados através do teste de Tukey ao nível de 5% de significância por meio do software estatístico SISVAR (FERREIRA, 2011).

RESULTADOS E DISCUSSÕES

Houve diferença significativa (p-valor <0,05) para todas as variáveis analisadas (Tabela 1). De forma geral, o tratamento 1 (controle), o qual não apresentou incorporação de serragem ao substrato comercial apresentou maior eficiência, com maiores valores em relação aos tratamentos com adição de serragem.

A maior altura das mudas de bracatinga foi obtida para o tratamento T1, em que não houve adição de serragem. Este tratamento apresentou média 22% superior em relação ao valor médio dos demais tratamentos. Não houve diferença entre os tratamentos em que houve a incorporação da serragem ao substrato comercial. Mesmo com a suplementação de adubação por meio do aumento da dosagem de FLC, não foi suficiente para que a mudas de bracatinga obtivessem qualidade equivalente ao uso do substrato comercial.

Essa diminuição do crescimento das mudas de bracatinga deve estar relacionada às propriedades da serragem e a não adaptação da espécie para o cultivo com este produto, principalmente pelas características relacionadas à acidez. A qualidade da serragem depende do tipo da madeira, do tempo e da condição de armazenamento e do teor de tanino presente (BURÉS, 1997). Esse autor cita que, dependendo do tempo de armazenamento, a serragem pode ser usada como substrato sem a necessidade de realizar compostagem; entretanto, ressalta que serragens, ainda que envelhecidas e naturalmente compostadas, podem apresentar fermentação ácida e prejudicar o crescimento das plantas. Além disso, Fernandes (2005) afirma que a presença de partículas finas causa problemas relacionados a

retenção excessiva de umidade e a diminuição da aeração, sendo esses dois dos principais entraves quanto à utilização da serragem na composição de um substrato.

A altura da muda é considerada um dos parâmetros mais antigos na classificação e seleção de mudas e, ainda, continua apresentando contribuição importante, podendo ser indicada como parâmetro para essa avaliação (NAVROSKI et al., 2016).

Tabela 1. Altura (cm), diâmetro do coleto (mm) e número de folhas de mudas de *Mimosa scabrella* sob diferentes concentrações de serragem e fertilizante de liberação controlada.

Tratamentos	Altura (cm)	Diâmetro do coleto (mm)	Número de folhas
T1	22,24 a	2,08 a	12,02 a*
T2	18,3 b	1,86 ab	12,02 a
T3	17,1 b	1,69 b	12,71 a
T4	16,1 b	1,58 b	9,27 b

* Tratamentos com letras iguais não diferem entre si no teste de Tukey a 5% de significância.

Quanto ao diâmetro de coleto, houve uma resposta semelhante observada na altura das mudas. O tratamento sem adição de serragem (T1) apresentou a maior média, mas neste caso, não diferenciou-se do tratamento T2, que apresentou na sua constituição além do substrato comercial, 20% de serragem + adição de 6 g L^{-1} de FLC. Conforme aumentou-se a porcentagem de serragem no substrato, parece haver uma diminuição no valor do diâmetro do coleto. O resultado obtido no presente estudo, corrobora com Gondin et al. (2015), que estudando mudas de paricá (*Schizolobium amazonicum* Huber ex Ducke), observaram que o desenvolvimento da espécie foi prejudicado quando a serragem foi adicinada ao substrato.

Diversos estudos (GOMES et al., 2002; GOMES e PAIVA, 2004) têm apontado que o diâmetro do colo é a variável que melhor prediz o desempenho no pós-plantio, indicando a qualidade das mudas, porém ocorrem variações para cada espécie e condições de plantio (RITCHIE et al., 2010). O diâmetro do colo é, dentre as variáveis de mais fácil mensuração, a mais promissora para indicar a qualidade das mudas e possui estreita correlação não somente com a sobrevivência, mas, sobretudo, com o ritmo de crescimento das mudas após o plantio (BINOTTO et al., 2010).

Em relação ao número de folhas, o tratamento com maior dosagem de serragem e FLC - T4 (substrato comercial + 60% de serragem + adição de 12 g L^{-1} de FLC) apresentou a menor média de folhas, diferenciando-se dos demais. Os tratamentos T1, T2 e T3 apresentaram médias muito próximas (12). Para o número de folhas, a espécie apresenta menor influencia negativa com a incorporação da serragem.

Luz et al. (2013) em seu estudo sobre o uso de diferentes doses de serragem como fonte de substrato para a produção de mudas de quiabo (*Abelmoschus esculentus* L. Moench), avaliaram a altura e diâmetro do coleto por meio de 6 tratamentos com diferentes quantidades de serragem (0%, 20%, 40%, 60%, 80% e 100%) e obtiveram maiores médias de altura

e diâmetro na utilização de substratos com 20% de serragem e menores médias ao utilizar 100% de serragem. Dessa forma, tendo em vista que os demais tratamentos do presente estudo apresentaram resultados gradativamente inferiores, sugere-se que a incorporação da serragem possivelmente tenha reduzido a drenagem de água nos recipientes, causando excesso de umidade para as plantas.

Estudos envolvendo materiais alternativos com potencial de utilização no processo de produção de mudas florestais são essenciais não somente para reduzir os custos desse processo, mas também para garantir o equilíbrio ambiental através da redução do uso de produtos químicos sem que haja interferência negativa na qualidade das mudas, e, consequentemente, no estabelecimento das plantas no campo.

De modo geral, houve uma redução da altura e do diâmetro do coleto das mudas de bracatinga com a adição de serragem. Contudo, os valores destes parâmetros com a adição de 20% de serragem ainda ficam dentro do considerado ideal para a espécie, ou pode-se esperar um maior tempo para expedição das mudas. Esse resultado pode inicialmente indicar uma diminuição nos custos, porém, é necessário realizar um estudo de viabilidade econômica, visto que há um aumento na dose de FLC juntamente com a serragem. Novos estudos podem ser elaborados para determinar a resposta da espécie ao uso da serragem decomposta.

CONCLUSÕES

Houve diferença no crescimento inicial das mudas de bracatinga em relação a diferentes proporções de serragem, sendo que o tratamento (T1), no qual não se utilizou esse componente, apresentou melhores resultados, indicando que a uso de serragem no substrato interfere negativamente na produção de mudas dessa espécie.

O uso do fertilizante de liberação controlada, na dose 6 g L^{-1}, propiciou bom crescimento quando utilizado em conjunto somente com o substrato comercial, ou seja, sem a adição de serragem.

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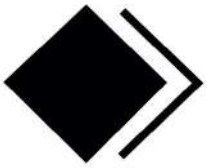
E57 Engenharia florestal [recurso eletrônico] : desafios, limites e potencialidade / Robson José de Oliveira. – Guarujá, SP: Editora Científica Digital, 2020.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-87196-43-5
DOI 10.37885/978-65-87196-43-5

1. Engenharia florestal. I. Oliveira, Robson José de.
CDD 634.9

Elaborado por Maurício Amormino Júnior – CRB6/2422

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DOI: 10.5965/2238117118e2019020

Revista de Ciências Agroveterinárias - EDIÇÃO ESPECIAL
XII SIMPÓSIO FLORESTAL CATARINENSE

Ágar e carvão ativado influenciam no desenvolvimento *in vitro* da parte aérea e radicular de sequoia

Agar and activated charcoal influence the in vitro development of shoot and root sequoia

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RESUMO

Plantios experimentais com *Sequoia sempervirens* (Lamb), espécie conhecida mundialmente pelo grande porte, longevidade e qualidade madeireira, estão iniciando no Brasil, tornando necessário a disponibilidade de mudas de qualidade. Assim, a técnica de cultura de tecidos, via micropropagação, se torna uma grande aliada pela garantia de superioridade genética, produção em grande escala e tempo reduzido de propagação. Atualmente há inúmeros protocolos para diversas espécies, porém, é necessário verificar todos os agentes influentes no sucesso do desenvolvimento *in vitro*. O objetivo deste trabalho foi determinar a influência do carvão ativado e de concentrações de ágar no desenvolvimento *in vitro* aéreo e de raízes de *S. sempervirens*. Os tratamentos realizados envolveram a presença/ausência do carvão ativado (1 g L^{-1}), combinados com concentrações de ágar (4, 5 e 6 g L^{-1}) em meio MS 50%. Para o número e comprimento de brotos e de raízes, apenas o carvão ativado foi significativo, sendo que a presença beneficiou o desenvolvimento dos explantes. Para porcentagem de calo, houve interação dos fatores, sendo que maiores concentrações de ágar (5 e 6 g L^{-1}) na presença do carvão foram prejudiciais, pois apresentaram maior calosidade, o que minimiza as taxas de enraizamento. Indica-se a adição de carvão ativado (1 g L^{-1}) e de menor concentração de ágar (4 g L^{-1}) em meio de cultura MS 50% para um melhor desenvolvimento *in vitro* de sequoia.

PALAVRAS-CHAVE: *Sequoia sempervirens* (Lamb), espécie lenhosa, micropropagação, enraizamento *in vitro*.

ABSTRACT

Experimental seedlings with *Sequoia sempervirens* (Lamb), a species known worldwide for its large size, longevity and quality of timber, are beginning in Brazil, making the availability of quality seedlings necessary. Thus, the technique of tissue culture, via micropropagation, becomes a great ally for the guarantee of genetic superiority, large-scale production and reduced propagation time. Currently there are numerous protocols for several species, however, it is necessary to verify all influential agents in the success of *in vitro* development. The objective of this work was to determine the influence of activated charcoal and agar concentrations on aerial and root development of *S. sempervirens*. The treatments involved the presence / absence of activated carbon (1 g L^{-1}), combined with concentrations of agar (4, 5 and 6 g L^{-1}) in 50% MS medium. For the number and length of shoots and roots, only activated carbon was significant, and the presence benefited the development of the explants. For the percentage of callus, there was interaction of the factors, and higher concentrations of agar (5 and 6 g L^{-1}) in the presence of medium were harmful, since they presented higher callosity, which minimizes the rooting rates. It is indicated the addition of activated charcoal (1 g L^{-1}) and lower concentration of agar (4 g L^{-1}) in 50% MS culture medium for a better *in vitro* development of sequoia.

KEYWORDS: *Sequoia sempervirens* (Lamb), woody species, micropropagation, rooting *in vitro*.

INTRODUÇÃO

A espécie *Sequoia sempervirens* (Lamb), também conhecida como redwood, nativa da América do Norte, tem destaque mundial pelo seu grande porte, longevidade e qualidade madeireira e já é cultivada com sucesso em muitos países (LIU et al. 2006). No Brasil, a sequoia foi plantada apenas para fins de arborização. Por ser uma espécie de clima temperado, NAVROSKI et al. (2015) destacam a importância de testes silviculturais no sul do país, especialmente em regiões de maior altitude, já que o

desenvolvimento de algumas espécies de *Eucalyptus*, por exemplo, é prejudicado em razão da ocorrência de fortes geadas.

Assim, a produção de mudas de sequoias, com qualidade, já adaptadas ao Brasil é essencial para realizar pesquisas sobre o crescimento e desenvolvimento desta espécie em plantios comerciais. Entretanto, a principal forma de propagação, via sementes, apresenta entraves como baixa taxa de germinação (média de 10%) e baixa sobrevivência de plântulas (BOE 1974). Além disso, segundo estudos, plantas jovens, que é o caso das matrizes no Brasil, produzem sementes com menor viabilidade. Segundo OLSON et al. (1990) a maior viabilidade de sementes de sequoia foi obtida em árvores com 250 anos de idade.

Com isso, a cultura de tecidos, via a técnica de micropropagação, surge como uma alternativa altamente viável, pois possibilita a produção de um grande número de plantas assépticas em um curto espaço de tempo (GRATTAPAGLIA & MACHADO 1998), além de manutenção da fidelidade genética em relação às árvores matrizes.

O processo de elaboração de um protocolo de micropropagação depende do controle de inúmeras variáveis. Para isso, vários fatores (físicos, fisiológicos, genéticos, etc) devem ser testados, a fim de se encontrar um protocolo com eficiência, praticidade e custo compatível. Uma das maiores limitações ao uso da micropropagação têm sido o baixo enraizamento das partes regeneradas *in vitro*, onde diferentemente das plantas herbáceas, na maioria das espécies lenhosas, o processo de rizogênese não foi ainda elucidado (OLIVEIRA et al. 2013).

Dentre os fatores que interferem no desenvolvimento *in vitro* das raízes e na sobrevivência deste material quando transplantado para o meio *ex vitro*, está o uso do carvão ativado no meio de cultura, durante a fase de enraizamento. Este agente é conhecido por favorecer o crescimento de raízes, adsorver substâncias inibitórias ao desenvolvimento e de liberar substâncias naturais que beneficiam o crescimento das culturas (GUSON et al. 2012). Além disto, o estado físico do meio de cultivo é notadamente conhecido por influenciar o crescimento de plantas *in vitro*. O ágar, polímero utilizado para solidificar o meio de cultura, pode interferir nas características das raízes formadas e consequentemente no sucesso do transplante e sobrevivência das mudas micropropagadas (TIBOLA et al. 2004).

O objetivo deste trabalho foi testar a presença do carvão ativado e de diferentes concentrações de ágar no desenvolvimento aéreo e de raízes *in vitro* de sequoia.

MATERIAL E MÉTODOS

Como fontes de propágulos para a micropropagação foram utilizadas minicepas de matrizes selecionadas de sequoia, de aproximadamente 40 anos de idade, localizadas na Floresta Nacional (Flona) de São Francisco de Paula, RS, as quais foram propagadas pelo processo de estaquia convencional após 120 dias do anelamento parcial ou completo da árvore matriz. Para tanto, as estacas foram coletadas das árvores matrizes e transportadas até o Viveiro Florestal do Centro de Ciências Agroveterinárias (CAV) da Universidade do Estado de Santa Catarina (UDESC). Cinco meses após a estaquia, as plantas enraizadas foram aclimatadas e conduzidas para o estabelecimento do minijardim clonal. Mensalmente, durante quatro meses, realizou-se a poda de formação das minicepas.

Brotações juvenis (herbáceas) provenientes das minicepas do jardim clonal de sequoia foram utilizadas como material vegetal para o estabelecimento *in vitro.* Este material foi individualizado em segmentos nodais de aproximadamente 2 cm de comprimento e previamente limpos em água corrente (por 10 minutos) e detergente neutro. Em câmara de fluxo laminar ocorreu a assepsia com imersão em álcool 70% por um minuto, seguido de solução de hipoclorito de sódio (2,5% de cloro ativo) durante 15 minutos, por fim, lavados três vezes em água destilada autoclavada.

Em todas as etapas o meio de cultivo utilizado foi MS (MURASHIGE & SOOG 1962) com a metade da concentração original de sais (MS 50%) e, além dos sais e vitaminas característicos deste meio de cultivo, foram adicionados 30 g L^{-1} de sacarose e 0,1 g L^{-1} de mio-inositol e o pH ajustado para 5,8 antes da inclusão do ágar. Como protocolo, antes da utilização, os frascos de 200 mL de capacidade de volume, com 30 mL meio de cultura, foram autoclavados a 121 ºC e 1,5 atm de pressão, por 20 minutos.

Após a inoculação dos segmentos nodais de sequoia em meio de cultura, o material foi armazenado no escuro por sete dias e depois permaneceram em sala de crescimento, com condições controladas de 42 µmol m^{-2} s^{-1} de intensidade luminosa, fotoperíodo de 16 horas e temperatura de 25 ± 2 ºC. Com 30 dias de cultivo estes segmentos nodais apresentaram brotações que foram multiplicadas em novos frascos contendo o mesmo meio de cultura (MS 50%) adicionado de 1 mg L^{-1} de BAP (6 benzilaminopurina). Posteriormente, estes materiais com novas brotações foram utilizados para a realização deste

experimento.

Os explantes de sequoia foram padronizados em tamanho aproximado de 2 cm. Os tratamentos estudados foram dois fatores: presença/ausência do carvão ativado (1 g L^{-1}) e concentrações de ágar (4, 5 e 6 g L^{-1}) em meio de cultura MS 50%. O delineamento foi inteiramente casualizado em fatorial 2x3, com seis repetições de cinco explantes por tratamento.

Após 60 dias de cultivo, as variáveis avaliadas foram: número médio de brotos por explante; comprimento médio de brotos (cm); número de raízes; comprimento médio das raízes (cm) e a presença de calo. Os dados obtidos foram submetidos à análise de variância, e quando significativos pelo teste de F ($p<0,05$) foram submetidos ao teste de Tukey a 5% de probabilidade de erro, empregando o programa estatístico SISVAR (FERREIRA 2011).

RESULTADOS E DISCUSSÃO

Não houve interação dos fatores testados (ágar x carvão ativado) para número e comprimento (cm) dos brotos e das raízes, sendo que para estas variáveis somente a presença do carvão ativado no meio de cultura influenciou ($p<0,05$) no desenvolvimento *in vitro* dos explantes (Tabela 1).

Tabela 1. Número e comprimento de brotos (cm) e de raízes de explantes de sequoia *in vitro* cultivados em meio MS 50% adicionado ou não de carvão ativado (1 g L^{-1}).

Table 1. Number and length of shoot (cm) and roots of in vitro seed explants cultivated in 50% MS medium added or not of activated charcoal (1 g L^{-1}).

Carvão ativado (1 g L^{-1})	Nº de brotos	Comp. brotos (cm)	Nº de raízes	Comp. raízes (cm)
Presença	4,74 a*	6,27 a	0,78 a	0,76 a
Ausência	3,69 b	2,92 b	0,24 b	0,13 b

*Médias seguidas por letras diferentes na coluna diferem entre si pelo Teste de Tukey ($p<0,05$).

O carvão ativado normalmente é adicionado ao meio de cultura em concentrações que variam de 0,2% a 3%, porém sua presença pode promover ou inibir o crescimento *in vitro*, dependendo da espécie e do tecido utilizado (VILLA et al. 2007). Neste trabalho a adição de 1 g L^{-1} de carvão ativado ao meio de cultura teve as melhores respostas no desenvolvimento da parte aérea e de raízes, quando comparado à ausência deste produto.

Estes benefícios no desenvolvimento *in vitro* vegetal podem ser explicados pela capacidade de adsorção do carvão ativado. Plântulas cultivadas em meio base, que é composto por sais minerais (macros e micronutrientes), sacarose, vitaminas e mio-inositol, possuem uma capacidade de absorção seletiva limitada, influenciada pela acidificação do meio, em virtude do metabolismo celular, podendo incorporar elementos não essenciais e/ou mesmo tóxicos como compostos fenólicos que podem levar até a morte da cultura (KERBAUY 2004, MULLER et al. 2007). Assim, a adição de carvão ativado pode impedir a acidificação do meio de cultivo, além de estabilizar o pH do meio e ainda pode servir como fonte alternativa de carbono para a cultura.

A adição de carvão ativado na etapa de enraizamento *in vitro* também foi testada por outros autores. O uso de concentrações crescentes (1 a 3 g L^{-1}) no cultivo *in vitro* de bananeira resultou em maior vigor radicular, além de maior número de raízes (COSTA et al. 2006). Resultado semelhante também foi encontrado para orquídea (*Cattleya pumila*) na qual a concentração de 1 g L^{-1} apresentou incremento na altura total das plântulas e no comprimento da raiz principal (GUSON et al. 2012). Já SARTOR et al. (2013) concluiu que o uso de carvão ativado, também na concentração de 1 g L^{-1}, não influenciou no cultivo *in vitro* de Jacarandá da Bahia (*Dalbergia nigra*).

Em contrapartida, VILLA et al. (2007) ao testar diferentes concentrações (0; 1,0; 2,0; 3,0 e 4,0 g L^{-1}) de carvão ativado em duas frutíferas de clima temperado, amoreira preta (*Rubus* spp.) e videira (*Vitis* sp.), concluiu que a presença do carvão ativado inibiu a multiplicação das brotações e crescimento de parte aérea e radicular. A adição de carvão ativado ao meio de cultura pode promover ou inibir o crescimento *in vitro*, conforme a espécie e o tipo de tecido utilizado, além da fonte do carvão, pureza e grau de ativação (PAN & STADEN 1998). Segundo os mesmos autores, a adsorção não-seletiva do carvão ativado, como por exemplo, em relação às vitaminas (tiamina, ácido nicotínico, piridoxina), além de quelatos de ferro e zinco, podem resultar em efeitos negativos para algumas espécies *in vitro*.

Para a variável porcentagem de calos houve interação dos fatores ($p<0,05$) carvão ativado e concentração de ágar (Tabela 2). A presença do carvão ativado e maiores concentrações (5 e 6 g L^{-1}) de ágar no meio de cultivo resultaram em maiores porcentagens (77 e 66%, respectivamente) de calos nos

explantes de sequoia.

Tabela 2. Calogênese (%) em explantes de sequoia *in vitro* cultivados em meio MS 50% com diferentes concentrações de ágar (4, 5 e 6 g L^{-1}) e da adição de carvão ativado (1 g L^{-1}).
Table 2. Calo (%) in in vitro sequoia explants cultured in 50% MS medium with different concentrations of agar (4, 5 and 6 g L^{-1}) and the addition of activated carbon (1 g L^{-1}).

Concentrações de ágar (g L^{-1})	Carvão ativado (1 g L^{-1})	
	Presença (%)	Ausência (%)
4	33,00 Aa*	0,01 Ba
5	77,00 Ab	1,00 Ba
6	66,00 Ab	11,00 Ba

*Médias seguidas por letras minúsculas diferentes na coluna ou maiúsculas na linha diferem entre si pelo Teste de Tukey ($p<0,05$).

Em plantas cultivadas *in vitro*, as raízes adventícias podem ser formadas, na fase de multiplicação, a partir de células de parênquima localizadas nas partes aéreas. Nessa condição a rizogênese pode ocorrer de modo direto ou indireto a partir de calos (GRATTAPAGLIA & MACHADO 1998). A calogênese na base dos explantes foi um processo recorrente durante todo o cultivo *in vitro* de sequoia (Figura 1).

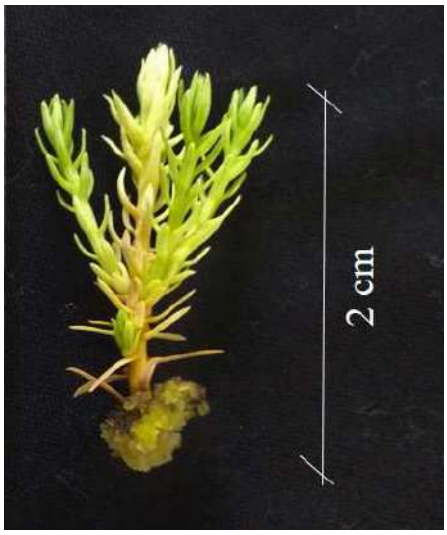


Figura 1. Formação de calos na base de explantes de sequoia cultivados em meio MS 50%.
Figure 1. Formation of callus in the base of sequoia explants grown in 50% MS medium.

A capacidade dos tecidos para formação de raízes depende de vários fatores endógenos e/ou exógenos e suas interações. Dentre estes fatores está a presença de substâncias como carvão ativado e o ágar, que podem interferir no desenvolvimento fisiológico e nas condições ambientais de crescimento do explante (SOUZA & PEREIRA 2007).

A luminosidade é um fator que normalmente influencia na indução de raízes. A presença do carvão no meio de cultivo pode simular fisicamente a condição de escuro, no qual as raízes podem se desenvolver melhor (GRATTAPAGLIA & MACHADO 1998) porém, concentrações elevadas de carvão ativado podem até mesmo impedir o processo de enraizamento. Segundo LEITZKE et al. (2009) com o aumento da concentração de carvão ativado (0, 10 e 20 g L^{-1}) houve uma redução na porcentagem de enraizamento e no comprimento médio das raízes de amoreira preta 'Xavante'. Já NEPOMUCENO et al. (2009), verificaram efeito positivo da adição do carvão sobre o número de raízes secundárias no cultivo *in vitro* de angico (*Anadenanthera colubrina*).

Sobre a influência do estado físico do meio de cultura, tem-se observado que algumas espécies podem estabelecer um sistema radicular mais vigoroso, quando cultivados em meio líquido com alguma fonte de aeração, como pontes de papel ou meio sob agitação. Embora o meio líquido interfira positivamente na disponibilidade de água, nutrientes, hormônios e níveis de oxigênio, a grande maioria dos protocolos de micropropagação é estabelecida em meio de cultura semi-sólido (SOUZA & PEREIRA 2007).

Altas concentrações de ágar podem ter efeito inibitório sobre a indução de raízes adventícias *in vitro*, sendo as causas exatas ainda não conhecidas. TIBOLA et al. (2004) atribuíram a possibilidade dos explantes não possuírem pêlos radiculares devidamente desenvolvidos, pela falta de oxigênio em meios solidificados com altas concentrações de ágar.

De acordo com HARTMANN et al. (2002), um meio de cultivo adequado para o enraizamento deve ser suficientemente poroso, a fim de permitir trocas gasosas eficientes. Possivelmente a menor concentração de ágar (4 g L^{-1}), testada neste trabalho, que tornou o meio semissólido, proporcionou uma melhor motilidade dos nutrientes e gases no cultivo *in vitro* resultando nos melhores resultados quando comparado a maiores concentrações de ágar e um meio mais sólido. Além disto, a utilização de menor concentração de ágar resulta em menores custos na produção final da muda.

CONCLUSÃO

Na etapa de enraizamento *in vitro* de sequoia recomenda-se a adição de carvão ativado (1 g L^{-1}) e baixa concentração de ágar (4 g L^{-1}) em meio de cultura MS 50% pois favorece o desenvolvimento da parte aérea e das raízes.

AGRADECIMENTOS

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo apoio financeiro e ao Centro de Ciências Agroveterinárias/Universidade do Estado de Santa Catarina (CAV/UDESC) pela estrutura e condições fornecidas para a execução do trabalho.

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SCIENTIA FORESTALIS
ISSN 2318-1222 (online)

ARTIGO ORIGINAL

Desempenho de espécies e procedências de *Eucalyptus* no Planalto Norte Catarinense, Brasil

Performance of Eucalyptus species and provenances in the Northern Plateau of Santa Catarina, Brazil

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Como citar: Frigotto, T., Navroski, M. C., Aguiar, N. S., Felippe, D., Borsoi, G. A., Pereira, M. O., & Lovatel, Q. C. (2020). Desempenho de espécies e procedências de *Eucalyptus* no Planalto Norte Catarinense, Brasil. *Scientia Forestalis*, *48*(127), e3273. https://doi.org/10.18671/scifor.v48n127.14

Resumo

O objetivo do estudo foi avaliar espécies e procedências de *Eucalyptus* spp., selecionando aquelas que apresentam elevada sobrevivência e capacidade produtiva nas condições da região do Planalto Norte Catarinense. O teste está localizado no município de Rio Negrinho, ao norte do Estado de Santa Catarina. O povoamento foi implantado em dezembro de 1999 com treze espécies do gênero *Eucalyptus*, oriundas de sementes de diferentes procedências. O experimento foi instalado em delineamento em blocos ao acaso, com três repetições e parcelas lineares. As variáveis avaliadas foram sobrevivência (%), altura (m), diâmetro a altura do peito (cm), volume (m^3) e bifurcação dos indivíduos. As espécies potenciais em relação às características quantitativas foram *E. smithii, E. dunnii, E. benthamii, E. badjensis* e *E. dorrigoensis*. Já para as variáveis qualitativas *E. badjensis*, *E. macarthurii*, *E. benthamii* e *E. dorrigoensis* apresentaram os melhores resultados. Foi possível verificar a ampla variabilidade das características avaliadas entre procedências, destacando a importância da escolha de materiais genéticos adaptados e com características quantitativas superiores. O estudo demonstra que outras espécies de *Eucalyptus*, além das usualmente plantadas na região, podem apresentar potencial para povoamentos comerciais, tolerando as baixas temperaturas, a ocorrência de geadas e apresentando boa produtividade.

Palavras-chave: Eucalipto; Sobrevivência; Crescimento; Geadas; Espécies potenciais.

Abstract

The objective of this study was to evaluate species and provenances of *Eucalyptus* spp., selecting those with high survival and productive potential under the conditions of the Santa Catarina Plateau. The test is located in the municipality of Rio Negrinho, northern Santa Catarina state. The stand was established in December 1999 with thirteen species of the genus *Eucalyptus*, originating from seeds of different origins. The experiment was carried out in a randomized block design with three replications and linear plots. The variables evaluated were survival (%), height (m), diameter at breast height (cm), volume (m^3) and bifurcation of the individuals. Potential species in relation to quantitative characteristics were *E. smithii, E. dunnii, E. benthamii, E. badjensis* and *E. dorrigoensis*. For the qualitative variables *E. badjensis, E. macarthurii, E. benthamii* and *E. dorrigoensis* presented the best results. It was possible to verify the wide variability of the characteristics evaluated between provenances, highlighting the importance of choosing adapted genetic materials with superior quantitative characteristics. The study shows that other *Eucalyptus* species, besides those usually planted in the region, have a potential for commercial stands, tolerating low temperatures, frost occurrence and good productivity.

Keywords: Eucalypts; Survival; Growth; Frost; Potential species.

Fonte de financiamento: FAPESC - grupo de pesquisa Gestão de Recursos Florestais (PAP2019031000055); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) código 001; Empresa Souza Cruz®.
Conflito de interesse: Nada a declarar.
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Recebido: 4 fevereiro 2019.
Aceito: 3 setembro 2019.
Editor: Paulo Henrique Müller Silva.

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INTRODUÇÃO

O gênero *Eucalyptus* pertence à família Myrtaceae e possui cerca de 600 espécies e subespécies, quase todas nativas da Austrália. O gênero se destaca pelas características de rápido crescimento, alta produtividade, ampla diversidade de espécies, grande capacidade de adaptação e aplicação para diversas finalidades, sendo amplamente utilizado para plantios florestais comerciais (Correia, 2011).

Os plantios de eucalipto ocupam no Brasil atualmente uma área superior a 5,7 milhões de hectares. O país se destaca pela produtividade florestal, sendo que em 2016 liderou o *ranking* global com uma média de 35,7 m^3 ha^{-1} ano^{-1} para os plantios de eucalipto (Indústria Brasileira de Árvores, 2017). Ao longo das últimas décadas se verificaram crescentes aumentos na produtividade das florestas de eucalipto, graças ao desenvolvimento de materiais genéticos de maior potencial produtivo, sobretudo pelo aprimoramento da hibridação e clonagem, bem como a evolução das técnicas de manejo florestal (Assis, 2014).

O gênero *Eucalyptus* apresenta ampla plasticidade e dispersão mundial, crescendo satisfatoriamente em diferentes situações edafoclimáticas, extrapolando aquelas das regiões de origem (Santos et al., 2001). O Sul do Brasil abrange áreas com climas subtropicais, com regularidade de chuvas ao longo do ano, enquadrados como *Cfa* (Clima subtropical, com verão quente) e *Cfb* (Clima temperado, com verão ameno), de acordo com a classificação de Köppen (Alvares et al., 2013). As áreas elevadas dos estados de Santa Catarina, Rio Grande do Sul e Paraná apresentam temperaturas relativamente baixas e número considerável de geadas, que afetam o cultivo de eucaliptos com baixa tolerância ao frio (Konzen et al., 2017).

Dessa forma, apesar do grande potencial florestal da região, a expansão de povoamentos do gênero *Eucalyptus* ainda tem ocorrido de forma limitada, principalmente devido à ocorrência de geadas e ao reduzido número de espécies que se adaptam a esta condição climática (Müller et al., 2017). As espécies deste gênero mais cultivadas atualmente nesses locais são *Eucalyptus benthamii* Maiden et Cambage e *Eucalyptus dunnii* Maiden. Contudo, outras espécies têm apresentado desenvolvimento silvicultural satisfatório em plantios experimentais, que podem vir a ampliar ou substituir esse número limitado de espécies (Brondani et al., 2010). A introdução de espécies com tolerância às baixas temperaturas ou mesmo híbridos com espécies alternativas é importante para aumentar a produção de eucaliptos em regiões mais frias (Konzen et al., 2017).

De acordo com Paludzyszyn Filho et al. (2006), o sucesso ou insucesso do plantio pode advir da escolha da espécie, da região de procedência geográfica e da região de procedência das sementes. Sabe-se que o comportamento das espécies frequentemente é inesperado quando introduzidas em novos locais (Ferreira & Couto, 1981). Assim, para a escolha das espécies que irão compor uma floresta com vistas a produção é imprescindível a realização de testes de espécies para avaliação da sua capacidade de aclimatação em cada local (Quiqui et al., 2001).

As variações genéticas existentes entre procedências dentro de uma mesma espécie surgem como resultado da adaptação das espécies às condições edafoclimáticas dos habitats (Kageyama et al., 1977). Assim, o comportamento de diferentes procedências de eucaliptos, quando submetidas a fatores ambientais diversos, também deve ser avaliado, certificando-se de que os plantios obterão êxito (Higa & Higa, 2000).

Diante do exposto, o objetivo do estudo foi avaliar espécies e procedências de *Eucalyptus* spp. na região do Planalto Norte Catarinense, selecionando aquelas que apresentam elevada sobrevivência e capacidade produtiva nestas condições.

MATERIAL E MÉTODOS

O povoamento de *Eucalyptus* spp. no qual foi realizado o estudo está localizado em Rio Negrinho, SC, Brasil. O município possui altitude próxima a 790 metros e solos predominantemente das classes Cambissolo Háplico Distrófico e Neossolo Regolítico Distrófico (Kobiyama et al., 2004).

A região do Planalto Norte Catarinense, onde se localiza o teste, é considerada de clima *Cfb* (clima temperado, subtropical úmido, com regularidade das chuvas ao longo de todo o ano) pela classificação de Köppen (Alvares et al., 2013). A temperatura média anual do município é de 16,8°C, com temperatura média mínima anual de 13°C e máxima de 22,8°C (Wrege et al., 2012). A precipitação pluviométrica total anual média é 1720 mm (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, 2009).

Na estação meteorológica de Rio Negrinho o número médio de geadas anuais foi de 10,21 entre os anos de 1990 e 2003; em outro município do Planalto Norte Catarinense, Matos Costa, o número médio de geadas anuais foi 18,5. Os episódios de geadas no Estado de Santa Catarina são mais frequentes entre os meses de maio e setembro, principalmente em junho e julho (Aguiar & Mendonça, 2004).

O talhão com as diferentes espécies/procedências foi plantado em dezembro de 1999, em espaçamento 3 x 2 metros. As treze espécies do gênero *Eucalyptus* sp. avaliadas são de origem seminal, provenientes de diversos locais, conforme demonstrado na Tabela 1.

Tabela 1 – Espécies e procedências de *Eucalyptus* implantadas em 1999 no município de Rio Negrinho, SC.

Espécie	Procedência das sementes	País
E. smithii	Papel e Celulose Catarinense (PCC-SC)	Brasil
E. smithii	Taboaganda	Austrália
E. smithii	Austrália	Austrália
E. smithii	Wilsons Promontory	Austrália
E. nitens	Taboaganda	Austrália
E. nitens	Austrália	Austrália
E. nitens	Macalista	Austrália
E. nitens	Glen Boc	Austrália
E. benthamii	Papel e Celulose Catarinense (PCC-SC)	Brasil
E. benthamii	Austrália	Austrália
E. dorrigoensis	Austrália	Austrália
E. macarthurii	Wilsons Promontory	Austrália
E. macarthurii	Austrália	Austrália
E. macarthurii	Pieter Maritz	Austrália
E. badgensis	Glen Boc	Austrália
E. badgensis x nitens	Glen Boc	Austrália
E. deanei	Papel e Celulose Catarinense (PCC-SC)	Brasil
E. deanei	Klabin/PR	Brasil
E. deanei	Manville Produtos Florestais/SC	Brasil
E. viminalis	Nacional Campo	Brasil
E. viminalis	Bonani	Brasil
E. viminalis	Manville Produtos Florestais/SC	Brasil
E. viminalis	BGNDO	Brasil
E. viminalis	Canela/RS	Brasil
E. obliqua	Riocell - Celulose Riograndense/RS	Brasil
E. dunnii	Acacia Creek N. Lgguns	Austrália
E. grandis	Lajeado/RS	Brasil

O experimento foi instalado com delineamento em blocos ao acaso, de três repetições e parcelas lineares. Cada linha de plantio foi composta por uma espécie e/ou procedência, com 45 árvores por linha. Nos locais em que houve a mortalidade de indivíduos foi realizado o replantio com mudas de *E. dunnii*, devido à dificuldade de encontrar mudas do mesmo material genético. As mudas replantadas não foram avaliadas.

A avaliação do experimento foi realizada em 2015, sendo consideradas as seguintes variáveis: sobrevivência - SOB (%), altura – H (m), diâmetro na altura do peito - DAP (cm), volume - VOL (m^3 ind^{-1}) e bifurcação - BIF (%), aos 16 anos. A avaliação da bifurcação foi realizada como critério binomial (0 - não bifurcado e 1 - bifurcado). A obtenção do volume individual foi realizada selecionando 20 árvores-amostra por espécie/procedência, contemplando todas as classes diamétricas. Por se tratar de uma área experimental com vistas para a seleção de material superior, foi considerada inviável a utilização de métodos destrutivos para obter o volume individual. Portanto, foi realizada a cubagem pelo método de *Smalian*, sendo utilizado o dendrômetro Criterion RD1000.

Para estimativa do volume individual foram testados doze modelos, conforme trabalho de Frigotto et al. (2015) realizado com estes dados. Com base nos resultados apresentados na análise gráfica de resíduos, erro padrão da estimativa (S_{yx}) e coeficiente de determinação ajustado ($R^2_{ajustado}$), o modelo que gerou estimativas com melhor ajuste foi o modelo de Stoate. A equação ajustada foi $v = 0,08357 - 0,0002.dap^2 + 0,0000404\left(dap^2.h\right) - 0,0028.h + \varepsilon_i$, em que: v= volume; dap= diâmetro na altura do peito; h= altura total; ε_i = erro absoluto. A equação apresentou $R^2_{ajustado}$ de 0,9696 e S_{yx} de 15,1%.

A normalidade do conjunto de dados foi confirmada por meio do teste de Kolmogorov-Smirnov e a homogeneidade pelo teste de Bartlett. A análise de variância (ANOVA) foi realizada e as médias foram comparadas pelo teste de Scott-Knott a 5% de probabilidade de erro.

Estimativa dos componentes genéticos

As estimativas dos parâmetros genéticos e fenotípicos foram efetuadas através do programa genético-estatístico Selegen-Reml/Blup (Resende, 2006). Utilizou-se o modelo 24: blocos ao acaso, teste de populações ou procedências, várias plantas por parcela, em que y = Xr + Zg + Wp + e, em que y é o vetor de dados, r é o vetor dos efeitos de repetição (assumidos como fixos) somados à média geral, g é o vetor dos efeitos genotípicos de populações (assumidos como aleatórios), p é o vetor dos efeitos de parcela, e é o vetor de erros ou resíduos (aleatórios).

A análise gerou estimativas dos seguintes parâmetros genéticos: V_g: variância genotípica entre populações ou procedências; V_{parc}: variância ambiental entre parcelas; V_e: variância residual dentro de parcela; V_f: variância fenotípica individual; h^2_g: herdabilidade individual no sentido amplo; C^2_{parc}: coeficiente de determinação dos efeitos da parcela; h^2_{mp}: herdabilidade da média da procedência, assumindo estande completo; Ac_{proc}: Acurácia da seleção de procedências, assumindo estande completo. A utilização do método REML para estimar os componentes da variância, deve-se ao desbalanceamento experimental em termos do número desigual de árvores sobreviventes por parcelas.

RESULTADOS E DISCUSSÃO

Seleção de espécies

A análise de variância revelou diferenças significativas (p<0,005) para todas as variáveis avaliadas, mostrando potencial para seleção de espécies e entre essas, das melhores procedências.

Para a variável quantitativa diâmetro na altura do peito (DAP) o conjunto de espécies composta por *Eucalyptus smithii* R.T. Baker, *Eucalyptus dunnii* Maiden, *Eucalyptus benthamii* Maiden & Cambage, *Eucalyptus badjensis* Beuzev. & Welch, *Eucalyptus dorrigoensis* (Blakely) L.A.S. Johnson & K.D. Hill, *Eucalyptus deanei* Maiden e o híbrido *Eucalyptus badjensis* Beuzev. & Welch x *Eucalyptus nitens* Maiden apresentaram os melhores resultados, não diferindo estatisticamente entre si. Estas mesmas espécies, com exceção de *E. deanei* e o híbrido *E. badjensis x E. nitens* também apresentaram os maiores valores de altura e volume individual (Tabela 2).

Tabela 2 - Média das variáveis diâmetro na altura do peito - DAP (cm), altura - H (m), volume - VOL (m^3 ind^{-1}), sobrevivência - SOB (%) e bifurcação do fuste - BIF (%) para as treze espécies de *Eucalyptus* aos 16 anos de idade.

Espécie	DAP (cm)	H (m)	VOL (m^3 ind^{-1})	SOB (%)	BIF (%)
E. smithii	30,7 a*	37,0 a	1,9195 a	32 c	11 b
E. dunnii	30,0 a	36,9 a	1,9094 a	83 a	16 a
E. benthamii	29,7 a	36,5 a	1,8831 a	55 b	1 b
E. badjensis	29,0 a	36,0 a	1,8544 a	42 b	5 b
E. dorrigoensis	25,4 a	35,6 a	1,8262 a	59 b	2 b
E. deanei	23,5 a	32,9 b	1,6588 b	55 b	18 a
E. badjensis x E. nitens	23,4 a	32,8 b	1,6476 b	32 c	23 a
E. viminalis	22,4 b	32,8 b	1,6462 b	34 c	10 b
E. macarthurii	21,1 b	31,6 b	1,5690 b	54 b	5 b
E. dalrympleana	21,0 b	31,4 b	1,5652 b	10 d	37 a
E. grandis	19,2 c	31,0 b	1,5360 b	47 b	13 a
E. nitens	18,1 c	29,6 c	1,4425 b	24 c	10 b
E. obliqua	13,6 c	27,7 c	1,3222 b	13 d	20 a

*Médias na mesma coluna seguidas por letras distintas diferem entre si pelo teste de Scott-Knott a $P < 0,05$.

E. smithii apresentou os melhores resultados para todas as variáveis dendrométricas (DAP= 30,7 cm; H= 37,0 m; VOL= 1,9195 m^3). Entretanto, em relação à sobrevivência (SOB) a espécie não obteve bons resultados, apresentando somente 32% aos 16 anos. Higa et al. (1997) em região fria do estado do Paraná encontrou valores semelhantes para a sobrevivência, sendo inferior a 25%. Para Yi et al. (2014) em uma região fria da China, encontraram 57% dos indivíduos de *E. smithii* com danos severos e extremamente severos devido ao congelamento, demonstrando a baixa resistência ao frio extremo.

Entretanto, pode-se dizer que apesar da alta mortalidade, os indivíduos remanescentes se recuperaram dos danos sofridos e apresentaram excelente desenvolvimento. A espécie é conhecida em algumas regiões da China pela alta taxa de crescimento, boa forma do caule, tolerância relativa ao frio e alto rendimento de óleos essenciais nas folhas (Chen, 2002; Zhang et al., 2003). Pesquisas na África do Sul também mostraram que a espécie tem rápido crescimento inicial, é moderadamente resistente ao congelamento e a seca, sendo plantada em grande escala em algumas áreas mais frias da África do Sul para a produção de óleos essenciais e madeira para celulose em rotação curta (Swain & Gardner, 2003). Em estudo realizado na China por Arnold et al. (2004), *E. smithii* se destacou no crescimento em diâmetro e altura, sendo recomendada para o plantio.

A espécie *E. dunnii* é uma das espécies de maior crescimento dentre os eucaliptos, sobretudo dentro da secção Maidenaria (Fonseca et al., 2010). Ao analisar a espécie, nota-se que a mesma obteve bons resultados para as variáveis dendrométricas. A espécie apresenta alto potencial de produção em área de plantio em clima temperado, com potencial de produção de até 50 m^3 ha^{-1} ano^{-1} de madeira para múltiplos usos. Vale ressaltar que *E. dunnii* pode apresentar crescimento inicial semelhante ao *E. grandis* em climas tendendo a tropical. Após esse período, há tendência de declínio do crescimento relativo se não houver compensações de retenção de umidade no solo (Paludzyszyn Filho et al., 2006). Higa et al. (1997) enquadrou *E. dunnii* no grupo que apresentou maior crescimento em experimento no Paraná.

E. benthamii também faz parte do grupo de espécies que se destacou nas caraterísticas quantitativas. No Sul do Brasil, *E. benthamii* tem mostrado bom crescimento e resistência a

geadas em Santa Catarina. A espécie também foi apontada como promissora em áreas montanhosas do Estado de Minas Gerais (Carpanezzi et al., 1988). Testes implantados em Colombo e Guarapuava (PR), indicam alta tolerância a geadas e crescimento médio superior ao *E. dunnii* (Paludzyszyn Filho et al., 2006). No estudo atual não houve diferença significativa no crescimento em diâmetro, altura e volume entre as duas espécies.

Em geral, *E. badjensis* surge como uma boa alternativa para o cultivo em área frias, com crescimento compatível a de outras espécies temperadas, tolerância a temperaturas abaixo de 0°C e apresenta mortalidade intermediária (Paludzyszyn Filho & Santos, 2011), fato que ocorreu nesse estudo. Em experimento instalado no Paraná, *E. badjensis* se apresentou como uma espécie de rápido crescimento, sendo considerada como uma espécie altamente potencial para plantios no Sul do Brasil em função da alta taxa de crescimento e resistência a geadas (Higa et al., 2002).

E. dorrigoensis faz parte do grupo de espécies que obteve melhores resultados para as variáveis dendrométricas e também se destacou para as variáveis qualitativas. Em um estudo comparando diferentes espécies de *Eucalyptus* em região fria da China foi observado que *E. dorrigoensis* juntamente com *E. benthamii* e *E. dunnii* apresentaram comportamento semelhantes para altura e diâmetro, aos 6 anos (Arnold et al., 2015), resultado semelhante ao encontrado no presente experimento, avaliado aos 16 anos de idade.

A espécie *E. deanei* e o híbrido *E. badjensis x E. nitens* apresentaram boas médias de DAP, porém, em relação à altura obtiveram médias menores, e consequentemente, menor volume médio individual. O híbrido *E. badjensis x E. nitens* ficou em posição intermediária entre as duas espécies nas variáveis quantitativas; assim, pode-se dizer que a hibridização com *E. nitens*, produziu plantas com menores dimensões.

Com relação à sobrevivência a média geral foi de 40%, variando de 10% a 83% para *E. dalrympleana* e *E. dunnii*, respectivamente. Como a área do estudo não apresentou avaliações nos primeiros anos após a implantação, não é possível determinar qual a causa exata da mortalidade das plantas. Contudo, por se tratar de um teste de espécies, no qual foi realizado o plantio de todos os indivíduos no mesmo momento, é possível supor que a principal causa de mortalidade seja o clima da região, especialmente a ocorrência de geadas. De acordo com Higa et al. (1997) um número reduzido de espécies do gênero *Eucalyptus* é indicado para plantios na região Sul do Brasil, onde as geadas são severas. Devido a essa condição determinante, a seleção de espécies e procedências de *Eucalyptus* resistentes ao frio é a primeira fase dos programas de melhoramento genéticos dirigidos ao aumento da resistência a geadas.

De acordo com Aguiar & Mendonça (2004), compilando dados de 33 estações meteorológicas do Estado de Santa Catarina, o ano de 1999 foi o que apresentou o maior número de episódios de geadas nos 24 anos de registros analisados. Já no ano de 2000 ocorreram as geadas mais intensas, principalmente no mês de julho. Coincidentemente, esses foram os anos dois primeiros anos após a instalação do teste, o que pode ter causado efeito direto na sobrevivência dos indivíduos.

A sobrevivência e a porcentagem de árvores bifurcadas podem ser utilizadas como indicadores da adaptabilidade às condições do local de plantio, especialmente em áreas com ocorrência de geadas (Higa et al., 1997). A amplitude de sobrevivência das espécies demonstra como o gênero *Eucalyptus* se adaptada a diferentes habitats e respondem às mesmas condições ambientais diferentemente.

As espécies que apresentaram as maiores taxas de sobrevivência foram: *E. dunnii* (superior às demais), *E. dorrigoensis*, *E. benthamii*, *E. deanei*, *E. badjensis*, *Eucalyptus macarthurii* H. Deane & Maiden, e *Eucalyptus grandis* W. Mill ex Maiden; todas com sobrevivência superior à média geral de 40%. Dentro deste grupo destaca-se as espécies que apresentaram menor taxa de bifurcação, inferior a 5% (*E. badjensis*, *E. macarthurii*, *E. benthamii* e *E. dorrigoensis*) demonstrando a adaptação às condições ambientais locais, com poucas árvores danificadas.

Entre as espécies citadas no grupo que se destacou nas variáveis qualitativas, *E. badjensis* obteve uma taxa relativamente alta de sobrevivência (42%) assim como baixo índice de bifurcação (5,6%). Em geral, *E. badjensis* aparece como uma boa alternativa para o cultivo em

área frias, com crescimento compatível a de outras espécies temperadas, tolerância a temperaturas abaixo de 0°C e apresenta mortalidade intermediária (Paludzyszyn Filho & Santos, 2011), assim como no presente estudo.

E. macarthurrii possui aptidão em resistir ao frio, confirmada pela satisfatória sobrevivência obtida (54%). Higa et al. (1997) já havia recomendado a espécie em estudo realizado em Campo do Tenente (PR), região fria com ocorrência de geadas, juntamente com *E. deanei*, que foram consideradas espécies promissoras para a região. Porém, o autor havia encontrado sobrevivência superior a 90%. A maior mortalidade nesse estudo pode ser decorrente de outros fatores, ou a ocorrência de geadas de maior intensidade.

A espécie *E. benthamii* vem sendo indicada para plantios na região Sul do Brasil sujeitas a geadas e temperaturas de até -6°C (Paludzyszyn Filho & Santos, 2011). Ainda sobre o desenvolvimento do *E. benthamii* no sul do Brasil, Benin (2014) cita o alto potencial da espécie no estabelecimento de reflorestamentos e suprimento de matéria-prima, devido a adaptação frente às condições climáticas encontradas nessa região. Estes fatos justificam os bons resultados encontrados para as características qualitativas, apresentando 55% de sobrevivência e apenas 1% dos indivíduos com bifurcação.

E. dorrigoensis apresentou 59% de sobrevivência e 2% de bifurcação. Em um estudo comparando diferentes espécies de *Eucalyptus* na China, foi observado que *E. dorrigoensis* juntamente com *E. benthamii* e *E. dunnii* apresentaram comportamento semelhantes para crescimento (altura e diâmetro), no entanto, a sobrevivência apresentou um percentual inferior em relação às outras duas espécies, devido à ocorrência de geadas severas e temperaturas mínimas de até -8 °C (Hesheng et al., 2003).

E. dunnii, *E. grandis* e *E. deanei* apesar de maiores taxas de sobrevivência (>47%), apresentaram maior taxa de bifurcação (entre 13 e 18%), diferindo estatisticamente das demais, efeito que pode ter sido causado por geadas e posterior recuperação das plantas. Entretanto, *E. dunnii* merece destaque em relação a sobrevivência, com 83%. Esse resultado pode ser justificado pela adaptação da espécie a regiões de clima frio e com presença de geadas, corroborando a indicação da espécie de Carpanezzi et al. (1988) para o plantio no estado de Santa Catarina, em altitudes de até 1000 m.

A espécie (*E. dunnii*) é indicada para plantios em regiões com temperaturas mínimas absolutas de até -5°C sob condições de aclimatação prévia, suportando até 22 geadas anuais (Food and Agriculture Organization, 1981). Estudos realizados em regiões frias no Brasil e em outros países demonstraram que com temperaturas de até -7,9°C a sobrevivência foi superior a 90%, indicando a alta resistência dessa espécie à baixas temperaturas, mesmo na fase juvenil (Lisbão Junior, 1980; Darrow, 1994; Higa et al., 1997; Mujiu et al., 2003). Porém deve ser considerada a taxa de bifurcação de 16%, dano que pode ter sido causado pela ocorrência de geadas severas. Em estudo realizado por Higa et al. (1997) *E. dunnii* foi uma das espécies mais danificadas pelas geadas, com 26% das plantas bifurcadas, mas apresentou a melhor capacidade de recuperação do crescimento.

E. grandis é a espécie de eucalipto mais utilizada em áreas tropicais e de transição para subtropicais no Brasil e em dezenas de outros países. Deve ser plantada em áreas livres de geadas e de períodos de seca muito prolongados; vale ressaltar a alta suscetibilidade ao frio dessa espécie (Paludzyszyn Filho et al., 2006). Apesar da recomendação, considerando o clima da região, a espécie merece destaque por conta das taxas de sobrevivência e de bifurcação satisfatórias, de 47% e 13%, respectivamente. Esse fato pode ter ocorrido por conta de adaptações das plantas matrizes ao clima da região, pois as sementes são provenientes do Sul do Brasil, gerando indivíduos mais tolerantes ao clima frio. Para comprovar essa hipótese os indivíduos merecem ser testados e propagados, verificando essa hipótese. A taxa de bifurcação sugere que as plantas sofreram danos por geadas, entretanto houve a posterior recuperação e crescimento destas.

E. deanei embora botanicamente semelhante de *E. grandis* e *E. saligna*, tem potencial de crescimento inferior a essas duas espécies, porém é mais resistente ao frio (Moura, 2001). Em sua área de distribuição natural suporta temperaturas mínimas entre 0°C e 5°C, geadas com frequência anual entre 0 e 50, ou mais. Na fase juvenil as plantas podem ser danificadas por

geadas severas, entretanto, apresentam boa capacidade de recuperação. No sul do Brasil, a espécie é considerada tolerante a geadas, após três anos de idade (Carpanezzi et al., 1988). No presente estudo apresentou 55% de sobrevivência e 18% de árvores bifurcadas, taxa relativamente alta, que pode ser explicada pelo dano causado por geadas e posterior recuperação.

Esperava-se que as espécies *Eucalyptus nitens* e *Eucalyptus viminalis* se destacassem nas características qualitativas, por serem características de regiões frias, apresentando resultados superiores aos encontrados para sobrevivência e bifurcação. Aparentemente as espécies não se adaptaram bem às condições locais ou algum outro fator biótico ou abiótico acarretou na mortalidade dos indivíduos.

Seleção de procedências

Em relação às variáveis quantitativas somente *E. smithii* e *E. viminalis* apresentaram diferenças entre as procedências (Tabela 3). Dentro da espécie *E. smithii*, que se destacou nas variáveis dendrométricas, a procedência Wilsons Promontory foi superior às demais (DAP= 36,7 cm; H= 39,9 m; VOL= 2,1021 m^3).

Tabela 3 - Média das variáveis diâmetro na altura do peito - DAP (cm), altura - H (m), volume - VOL (m^3 ind^{-1}), sobrevivência - SOB (%) e bifurcação do fuste - BIF (%) para diferentes procedências das espécies de *Eucalyptus* aos 16 anos de idade.

Espécie	Procedência	DAP (cm)	H (m)	VOL (m^3 ind^{-1})	SOB (%)	BIF (%)
E. smithii	Wilsons Promontory	36,7 a*	39,9 a	2,1021 a	28 b	17 a
	Taboaganda	28,7 b	36,3 b	1,8717 b	21 b	15 a
	PCC/SC	27,7 b	34,9 b	1,7793 b	40 a	11 a
	Austrália	26,4 b	31,7 b	1,5829 b	36 a	2 a
E. benthamii	PCC/SC	30,5 a	37,7 a	1,9608 a	60 a	11 a
	Austrália	26,6 a	34,2 a	1,7388 a	51 a	20 b
E. deanei	PCC/SC	24,1 a	33,4 a	1,6886 a	62 a	24 a
	Manville/SC	23,6 a	33,7 a	1,6831 a	76 a	16 a
	Klabin/PR	21,9 a	30,9 a	1,5296 a	28 b	8 a
E. macarthurii	Austrália	21,7 a	32,3 a	1,6169 a	61 a	10 a
	Pieter Maritz	20,9 a	31,6 a	1,5721 a	51 b	4 a
	Wilsons Promontory	20,5 a	30,6 a	1,5085 a	51 b	1 a
E. viminalis	Canela/RS	21,3 b	31,4 b	1,5585 b	26 b	6 a
	BGNDO	18,2 b	29,4 b	1,4328 b	31 b	8 a
	Nacional Campo	21,9 b	32,2 b	1,6120 b	37 a	19 a
E. nitens	Manville/SC	30,6 a	36,9 a	1,9136 a	41 a	12 a
	Austrália	19,7 a	30,0 a	1,4812 a	20,0 b	18,8 a
	Glen Boc	19,6 a	31,1 a	1,5406 a	40,0 a	3,1 a
	Macalista	15,9 a	28,8 a	1,3857 a	17,5 b	7,1 a
	Taboaganda	15,4 a	26,7 a	1,2625 a	20,0 b	18,8 a

*Médias na mesma coluna seguidas por letras distintas diferem entre si pelo teste de Scott-Knott a $P < 0,05$.

Para a estimativa dos parâmetros genéticos entre as procedências, as maiores herdabilidades (h^2_g) para as variáveis dendrométricas (DAP, H e VOL) foram obtidas para *E. viminalis*, obtendo valores entre 0,2132 e 0,2314 (Tabela 4). Valores dessa herdabilidade na faixa de 0,20 a 0,30 têm sido observadas para a maioria dos caracteres quantitativos em espécies florestais (Resende, 2002), representando potencial de seleção. A espécie (*E. viminalis*) também apresentou os maiores valores para V_g (variância genotípica entre procedências) e Ac_{proc} (Acurácia da seleção de procedências) reforçando que existe elevado potencial de seleção de procedências dentro da espécie.

De acordo com Fonseca et al. (2010), *E. viminalis* normalmente apresenta ampla variabilidade entre procedências, o que permite a seleção de genótipos superiores para o plantio. No presente estudo, dentre as procedências analisadas, pode-se destacar Manville/SC, a qual apresentou os melhores resultados para as variáveis quantitativas. Para as variáveis qualitativas não houve diferença significativa entre as procedências. De acordo com Konzen et al. (2017) a alta variabilidade genética dos indivíduos combinada com valores relativamente altos de herdabilidade pode implicar na previsão de ganhos genéticos consideráveis. Os autores destacam ainda que o ganho genético deve considerar a seleção de genótipos superiores e as melhores procedências.

Para *E. smithii* para os caracteres dendrométricos a h^2_g ficou próxima a 0,08 (8%), sendo a segunda espécie com maior valor de h^2_g. Para estes mesmos caracteres as outras espécies apresentaram valores de h^2_g próximos ou abaixo de 5%. Estes valores de herdabilidade representam baixo potencial de seleção das procedências. Em geral, herdabilidades individuais de baixa magnitude são comuns para caracteres quantitativos e, via de regra, conduzem a moderadas magnitudes das herdabilidades em nível de médias de progênies (Resende, 2002).

Estudo realizado por Konzen et al. (2017), revelou que procedências de *E. smithii* possuíam considerável variação genética para as variáveis diâmetro, altura, volume e retidão. Em geral, a maior parte da variação foi encontrada dentro das procedências. Em estudo na China realizado utilizando diversas procedências de *E. smithii*, em grande parte dos testes houve diferença entre procedências, demonstrando que a espécie apresenta grande variabilidade genética entre populações (Arnold et al., 2004).

Em relação à sobrevivência das plantas à campo, todas as espécies apresentaram diferenças entre procedências, com exceção de *E. benthamii*. A maior diferença entre procedências foi obtida para *E. deanei*, na qual as mudas originadas de sementes de Manville/SC apresentaram 76% de sobrevivência, enquanto as provenientes da Klabin/PR obtiveram 28% de sobrevivência. Esse resultado demonstra como o local de origem das sementes exerce influência sobre a sobrevivência e desenvolvimento da espécie em novos locais, sendo que a procedência pode ser determinante para o sucesso de um empreendimento florestal. Em relação aos parâmetros genéticos, *E. viminalis* apresentou as maiores possibilidades de seleção, com maiores estimativas de h^2_g e Ac_{proc}. No geral entre as espécies, a sobrevivência obteve parâmetros que expressam baixo potencial de seleção para a característica. Para bifurcação do fuste somente dentro de *E. benthamii* as procedências apresentaram diferença. Esta característica também não apresentou parâmetros genéticos que demostram potencial para seleção.

Para todos os caracteres a variância ambiental entre parcelas (V_{parc}) apresentou valores relativamente altos comparando-se com os valores de variância fenotípica individual, para a maior parte das variáveis, indicando forte influência do ambiente sobre os genótipos. Geralmente, para parâmetros importantes como V_g e h^2_g entre os caracteres avaliados mostraram que não ocorreram variações significativas entre as procedências testadas, contudo pode-se ressaltar que há diferenças no desempenho entre as características e que a variabilidade existente pode permitir avanços expressivos em futuros programas de melhoramento genético de algumas espécies, especialmente para *E. smithii* e *E. viminalis*

Tabela 4 - Resultado dos parâmetros genéticos para os caracteres diâmetro a altura do peito (DAP), altura (H), volume (VOL), sobrevivência (SOB) e bifurcação de procedências de *Eucalyptus* spp. em um plantio de 16 anos de idade em Rio Negrinho, SC. Brasil.

Espécies	Parâmetros genéticos	DAP	H	VOL	SOB	BIF
E. smithii	V_g*	17,1445	3,7954	0,0114	0,0407	0,0017
	V_{parc}	19,4040	5,2448	0,0211	0,0287	0,0175
	V_e	135,8401	36,7028	0,1515	0,1441	0,1053
	V_f	172,4082	45,7447	0,1885	0,2285	0,1246
	h^2_g	0,0992	0,0835	0,0842	0,1045	0,0145
	c^2_{parc}	0,1126	0,11464	0,1137	0,1256	0,1408
	h^2_{mp}	0,6962	0,6528	0,6414	0,3215	0,2080
	Ac_{proc}	0,8345	0,8077	0,8084	0,5936	0,4561
E. benthamii	V_g	1,0914	1,6315	0,0068	0,0201	0,0011
	V_{parc}	23,8014	9,7052	0,0398	0,0270	0,0278
	V_e	166,6442	67,9621	0,2785	0,2258	0,2047
	V_f	191,5425	79,3015	0,3250	0,2017	0,1048
	h^2_g	0,0065	0,02151	0,0208	0,0245	0,0189
	c^2_{parc}	0,1242	0,12214	0,1224	0,1047	0,1494
	h^2_{mp}	0,1076	0,30545	0,3081	0,3078	0,2510
	Ac_{proc}	0,3271	0,5535	0,5550	0,4632	0,4923
E. deanei	V_g	0,0860	0,0825	0,0003	0,0148	0,0007
	V_{parc}	19,9940	6,6114	0,0276	0,0389	0,0247
	V_e	119,9642	39,6684	0,1624	0,1547	0,1487
	V_f	140,0445	46,3614	0,1891	0,2014	0,1743
	h^2_g	0,0006	0,00185	0,0017	0,0412	0,0045
	c^2_{parc}	0,1428	0,14266	0,1426	0,1289	0,1422
	h^2_{mp}	1,1595	3,2678	3,1791	0,2396	0,0783
	Ac_{proc}	0,1077	0,1808	0,1783	0,3974	0,2798
E. macarthurii	V_g	0,0524	0,0422	0,0027	0,0104	0,0013
	V_{parc}	13,3051	5,2497	0,0215	0,0175	0,0085
	V_e	79,8036	31,4981	0,1284	0,1783	0,0493
	V_f	93,1554	36,7918	0,1501	0,1984	0,0589
	h^2_g	0,0061	0,0011	0,0012	0,0176	0,0229
	c^2_{parc}	0,1228	0,1421	0,1438	0,0987	0,1391
	h^2_{mp}	1,0487	2,135	2,1384	0,2178	0,3052
	Ac_{proc}	0,1025	0,1467	0,1474	0,3874	0,5525
E. viminalis	V_g	26,7745	8,2885	0,0346	0,0107	0,0002
	V_{parc}	10,5635	2,2985	0,0134	0,0178	0,0003
	V_e	88,1224	25,3185	0,1055	0,1078	0,095

Tabela 4 – Continuação...

	V_f	125,4678	35,9052	0,1542	0,3698	0,096
	h^2_g	0,2132	0,2314	0,2247	0,1784	0,002
	c^2_{parc}	0,0840	0,0640	0,0908	0,0201	0,003
	h^2_{mp}	0,9121	0,9335	0,9128	0,2178	0,302
	Ac_{proc}	0,9555	0,9661	0,9551	0,7851	0,549
	V_g	1,3665	2,1241	0,0090	0,0407	0,0001
	V_{parc}	12,48774	5,1282	0,0213	0,0287	0,015
	V_e	74,9264	30,7695	0,1278	0,1441	0,091
E. nitens	V_f	88,7807	38,0219	0,1582	0,2285	0,1068
	h^2_g	0,0153	0,0558	0,0573	0,1045	0,0017
	c^2_{parc}	0,1406	0,1348	0,1346	0,1256	0,1426
	h^2_{mp}	0,2165	0,51134	0,5182	0,3215	0,0295
	Ac_{proc}	0,4653	0,7150	0,7199	0,5936	0,1721

* Parâmetros genéticos: V_g: variância genotípica entre procedências; V_{parc}: variância ambiental entre parcelas; V_e: variância residual dentro de parcela; V_f: variância fenotípica individual; h^2_g: herdabilidade individual no sentido amplo; C^2_{parc}: coeficiente de determinação dos efeitos da parcela; h^2_{mp}: herdabilidade da média da procedência; Ac_{proc}: Acurácia da seleção de procedências.

De forma geral, pode-se dizer que pode haver outras espécies potenciais para o plantio no Sul do Brasil, como visto nos resultados do presente estudo, apresentando características qualitativas e quantitativas com potencial à seleção e multiplicação. Deve-se priorizar o uso de material genético adequado, considerando espécies e procedências, que entre outras características desejáveis, sejam tolerantes ao frio ou que apresentem boa capacidade de rebrota, possibilitando a regeneração das plantas em uma eventual ocorrência de geadas severas.

CONCLUSÃO

As espécies potenciais em relação às variáveis quantitativas foram *E. smithii, E. dunnii, E. benthamii, E. badjensis* e *E. dorrigoensis*. Já para as variáveis qualitativas pode-se destacar *E. badjensis*, *E. macarthurii*, *E. benthamii* e *E. dorrigoensis*, que apresentaram baixas taxas de mortalidade e de bifurcação, demonstrando boa adaptação nas condições ambientais locais.

Espécies como *E. dunnii* e *E. benthamii* já são plantadas comercialmente na região, porém, esse estudo mostra que outras espécies possuem potencial para serem exploradas, se adaptando ao frio, ocorrência de geadas, e apresentando elevada produtividade. Assim, são recomendados novos estudos principalmente com as espécies *E. badjensis e E. dorrigoensis*, que se destacaram nas variáveis qualitativas e quantitativas.

E. viminalis e *E. smithii* apresentaram os maiores valores para as estimativas dos parâmetros genéticos, demostrando que existo possibilidade de seleção dentro de suas procedências, principalmente para diâmetro, altura e volume.

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Contribuição dos autores: TF: investigação, metodologia, supervisão, software, escrita – primeira redação; MCN: conceituação, curadoria de dados, obtenção de financiamento, metodologia, supervisão, escrita – revisão e edição; NSA: validação, escrita – revisão e edição; DF: investigação, visualização, software; GAB: conceituação, obtenção de financiamento, administração do projeto; MOP: validação, visualização, escrita – primeira redação; QCL: visualização, escrita – primeira redação.

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Efeito das "falhas de pinha" da *Araucaria angustifolia* no enraizamento de miniestacas de *Sequoia Sempervirens*

Effect of *Araucaria angustifolia* "pinecone failures" on rooting of *Sequoia Sempervirens* minicuttings

DOI:10.34117/ bjdv6n7-341

Recebimento dos originais: 15/06/2020
Aceitação para publicação: 15/07/2020

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RESUMO
A propagação vegetativa por miniestaquia possibilita a obtenção de várias plantas idênticas a partir de uma única planta matriz. Entretanto, vários fatores podem influenciar a capacidade e a rapidez no enraizamento das estacas, como: os ambientais, o estado fisiológico, a maturação, o tipo de propágulo, o uso de fitormônios e substratos, e a época de coleta da estaca. Desse modo, este trabalho teve como objetivo testar o efeito das concentrações de "falhas de pinha" misturado ao substrato, no enraizamento de miniestacas de *Sequoia sempervirens*. O delineamento experimental foi inteiramente casualizado com cinco repetições de cinco estacas cada, sendo conduzido pelo esquema bifatorial (4x4), sendo quatro clones de sequoia e quatro concentrações de "falhas de pinha", o que se totalizou em 16 tratamentos com cinco repetições. As variáveis analisadas foram: porcentagem de enraizamento, número de raízes por miniestaca, e a formação de calos na base das miniestacas. Comparando-se todos os clones, A100 teve melhores médias para enraizamento e número de raízes, porém teve uma resposta negativa a adição das "falhas de pinha", ao contrário do clone A130, que aumentou as médias com a adição do material. Desta maneira, recomenda-se as "falhas de pinha" como insumo para produção de mudas originadas por miniestacas, já que pode favorecer a produção de acordo com o clone utilizado.

Palavras-chave: Propagação vegetativa, Silvicultura, Miniestaquia, Produção de mudas

ABSTRACT
Vegetative propagation by minicutting makes it possible to obtain several identical plants from a single matrix plant. However, several factors can influence the capacity and speed in the rooting of the cuttings, such as: the environmental ones, the physiological state, the maturation, the type of propagule, the use of phytohormones and substrates, and the time of collection of the cutting. Thus, this work aimed to test the effect of concentrations of "pinecone failures" mixed with the substrate, in the rooting of Sequoia sempervirens minicuttings. The experimental design was completely randomized with five replications of five cuttings each, being conducted by the bifactorial scheme (4x4), with four sequoia clones and four concentrations of "pinecone failures", which totaled 16 treatments with five repetitions. The variables analyzed were: rooting percentage, number of roots per mini-cuttings, and callus formation at the base of the mini-cuttings. Comparing all the clones, A100 had better averages for rooting and number of roots, but it had a negative response to the

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addition of “pinecone failures”, unlike clone A130, which increased the averages with the addition of the material. In this way, "pinecone failures" is recommended as an input for the production of seedlings originated by mini-cuttings, since it can favor production according to the clone used.

Keywords: Vegetative propagation, Forestry, Mini cuttings, Seedling production

1 INTRODUÇÃO

A *Sequoia sempervirens* (D. Don) Endl. é uma espécie nativa da costa da Califórnia dos Estados Unidos, que possui suas particularidades de proteção por ser a única espécie viva do gênero *Sequoia*, tendo muitos indivíduos com uma idade extremamente longa, chegando a mais de 4.000 anos (HAN e LI, 2015). É uma árvore de crescimento rápido, atingindo de 1 a 5 m de crescimento em altura por ano, sendo assim, uma das espécies mais altas do mundo, alcançando 120 m. Seu desenvolvimento ocorre em regiões úmidas e em altitudes que variam de 30 a 750 m, assim, sua dispersão estende-se até 900 m acima do nível do mar (HAN e LI, 2015; PEREIRA et al., 2019).

Dentre as características que garantem os múltiplos usos da *Sequoia sempervirens*, destacam-se: o armazenamento de grandes quantidades de biomassa, representadas pelo valor de incremento anual de 33,2 m³, considerado alto se comparado a de outras espécies encontradas no ecossistema onde está inserido; a casca e a madeira possuem tanino, que é bastante utilizado na produção industrial; apresenta extrativos, o que torna a madeira mais resistente a fungos e ataque de insetos; possui densidade baixa, mas é amplamente utilizada para a construção de utensílios na construção civil (decks, cercas, janelas, portas, persianas, acabamentos interiores), destino o qual a aparência e a estabilidade são requisitos importantes (JONES e O’HARA, 2012; SCOTT MC et al., 2016; COWN, 2008).

Pela ampla diversidade em seu uso, houve aumento do seu cultivo, consequentemente, incentivo aos plantios, em alguns países. Como exemplo tem-se o Brasil, que possui características ambientais propícias para o desenvolvimento da espécie, destacando-se como a principal a presença de altas altitudes, na região sul, localizada na Serra Gaúcha e no Planalto-Serra-Catarinense (NAVROSKI et al., 2015). Todavia, para o estabelecimento de bons povoamentos florestais é necessário obter sucesso na produção de mudas, tanto na qualidade como na quantidade. Com esse intuito, vários estudos científicos e avanços técnicos têm sido realizados com o objetivo de melhorar a qualidade das mudas florestais, assegurando boa adaptação e crescimento após o plantio (HOPPE, 2004).

Na produção de mudas, para reduzir os problemas com a baixa germinação das sementes e viabilidade das plântulas, pode ser utilizada a propagação vegetativa por estaquia. Esse tipo de

propagação possibilita a obtenção de várias plantas idênticas a partir de uma única planta matriz (FACHINELLO et al., 2005; LIU et al., 2006). Entretanto, vários fatores podem influenciar a capacidade e a rapidez no enraizamento das estacas, destacando-se: os ambientais, o estado fisiológico, a maturação, o tipo de propágulo, o uso de fitormônios e substratos, e a época de coleta da estaca (PAIVA e GOMES, 2001). Além destes, o potencial genético de cada cultivar também exerce influência no enraizamento (TREVISAN; SCHWARTZ; KERSTEN, 2000).

Após a escolha do tipo de propagação, é preciso considerar os fatores que influenciam na qualidade das mudas, e entre os existentes, a escolha do substrato ou de um componente adicional ao substrato, são os de maiores graus de importância, devido principalmente às suas características físico-químicas que podem favorecer ou prejudicar o desenvolvimento da muda (CALDEIRA et al., 2011). De acordo com Rodrigues et al. (2016) insumos não convencionais, de fácil aquisição, tornam-se atraentes pelo baixo custo e por melhorar as propriedades do solo e aumentar a produção vegetal, o que facilita o transporte das mudas para o campo.

Como alternativa para o emprego, enquanto componente adicional em substrato tem-se a "falha de pinha" ou "escamas de preenchimento", que pode considerar-se um insumo não convencional encontrada no estróbilo feminino da *Araucaria angustifolia*, e são caracterizadas como escamas estéries das pinhas (MATTOS, 1994; MANTOVANI; MORELLATO; REIS, 2004). Essa parte vegetativa não possui nenhuma destinação exclusiva, e por esse motivo torna-se importante a produção de trabalhos que auxiliem a determinar um destino para o seu uso, comprovando que esse material é viável economicamente.

Neste contexto, este trabalho teve como objetivo testar o efeito das concentrações de "falhas de pinha" misturado ao substrato, no enraizamento de miniestacas de *Sequoia sempervirens*.

2 MATERIAL E MÉTODOS

O experimento foi conduzido na casa de vegetação do Centro de Ciências Agroveterinárias (CAV), no campus de Lages, da Universidade do Estado de Santa Catarina (27°47'33.62"S; 50°18'4.60"O), durante o período de abril de 2017 e avaliado 100 dias após a instalação.

No município de Lages, de acordo com a classificação de Köppen, o clima é do tipo Cfb, ou seja, é quente e temperado, com uma pluviosidade significativa ao longo do ano, e mesmo que se tenha um mês seco a pluviosidade continua a existir. A precipitação média anual é de 1300 a 1500 mm, e a temperatura média anual é de 15º C (ALVARES et al., 2014).

As miniestacas de *Sequoia sempervirens* foram obtidas a partir de podas em indivíduos instalados em jardim clonal, de aproximadamente 45 anos de idade, localizadas na Floresta Nacional

de São Francisco de Paula, em São Francisco de Paula – RS. O tamanho das miniestacas variou de 7 a 9 cm.

As "falhas de pinha", de *Araucaria angustifolia*, utilizadas no experimento foram oriundas de florestas naturais localizadas na cidade de São Joaquim – SC. Após a coleta, o material foi encaminhado para o CAV-UDESC, onde foi espalhado e seco em ambiente natural, e ao obter seu estado apropriado, foi triturado em moinho de martelo industrial, para assim obter boa granulometria.

O experimento foi representado por um esquema bifatorial, sendo quatro plantas matrizes denominadas A100, A130, A135, A138, e quatro formulações, com diferentes proporções, de substrato comercial Maxfértil (composto por casca de pinus, fosfato natural, casca de arroz carbonizada, vermiculita e fertilizante NPK) mais a "falha de pinha" (100% substrato comercial; 20% falha + 80% substrato comercial; 40% falha + 60% substrato comercial e 60% falha + 40% substrato comercial).

O delineamento experimental foi inteiramente casualizado com cinco repetições de cinco estacas cada, sendo conduzido pelo esquema bifatorial (4x4), o que se totalizou em 16 tratamentos com cinco repetições, gerando 80 unidades experimentais.

Ao longo do experimento as variáveis analisadas foram: porcentagem de enraizamento, número de raízes por miniestaca, e a formação de calos na base das miniestacas.

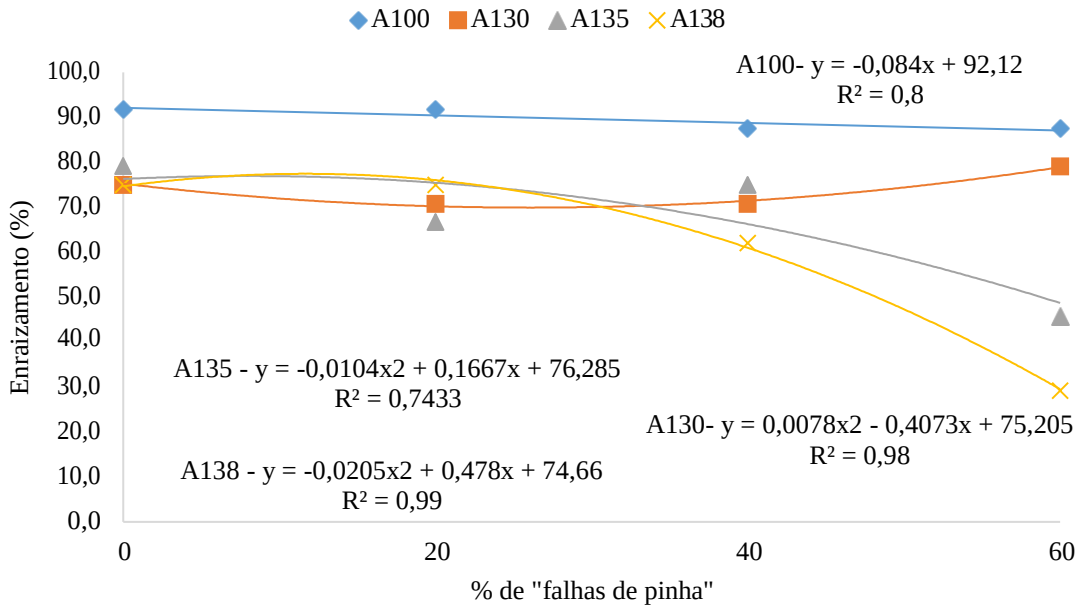
Os dados foram submetidos à análise de variância a 5% de probabilidade, utilizando-se o *software* Sisvar® (FERREIRA, 2011), e a comparação estatística entre os tratamentos foi realizada pelo teste de médias de Scott-Knott.

3 RESULTADOS E DISCUSSÃO

Dentre as variáveis analisadas a porcentagem de enraizamento obteve como média geral o valor 72,6%, com variação entre 60,3% (A138) a 89,6% (A100) (Figura 1).

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Figura 1. Regressão quadrática para porcentagem de enraizamento de cada clone de acordo com a porcentagem de falha adicionada ao substrato.



Em seu trabalho, Pereira et al. (2017), ao analisar o enraizamento de estacas individuais de plantas matrizes de *Sequoia sempervirens*, obtiveram valor médio de aproximadamente 80% na porcentagem de enraizamento, tendo valor máximo de 100% e valores menores que 40%. Alto valor também foi encontrado por Moraes et al. (2014), ao avaliarem a porcentagem de enraizamento de miniestacas caulinares e foliares de *Toona ciliata* M. Roemer em substrato comercial Plantmax, sendo mais de 96% de miniestacas enraizadas. Já Navroski (2015), ao verificar o resgate vegetativo, o enraizamento de estacas e a adaptação das mudas enraizadas em sistema de minijardim clonal de *Sequoia sempervirens*, utilizando diferentes doses de ácido indolacético (AIA), encontrou a maior taxa de enraizamento no valor de 80,9%.

De acordo com Wendling e Xavier (2003) vários são os fatores que irão determinar o sucesso no enraizamento, consequentemente, na sobrevivência das miniestacas, ou seja, para que isso ocorra é necessário que haja boas condições de temperatura, umidade e manejo, aliado ao vigor fisiológico. Ligado a esses fatores tem-se também o uso de substâncias ou materiais que auxiliam a regular o crescimento vegetal e a manter a sustentação das estacas, mantendo uma boa aeração e umidade, como é o caso dos hormônios vegetais e os substratos. O próprio silvicultor precisa realizar todas essas análises para assim obter mudas de boa qualidade.

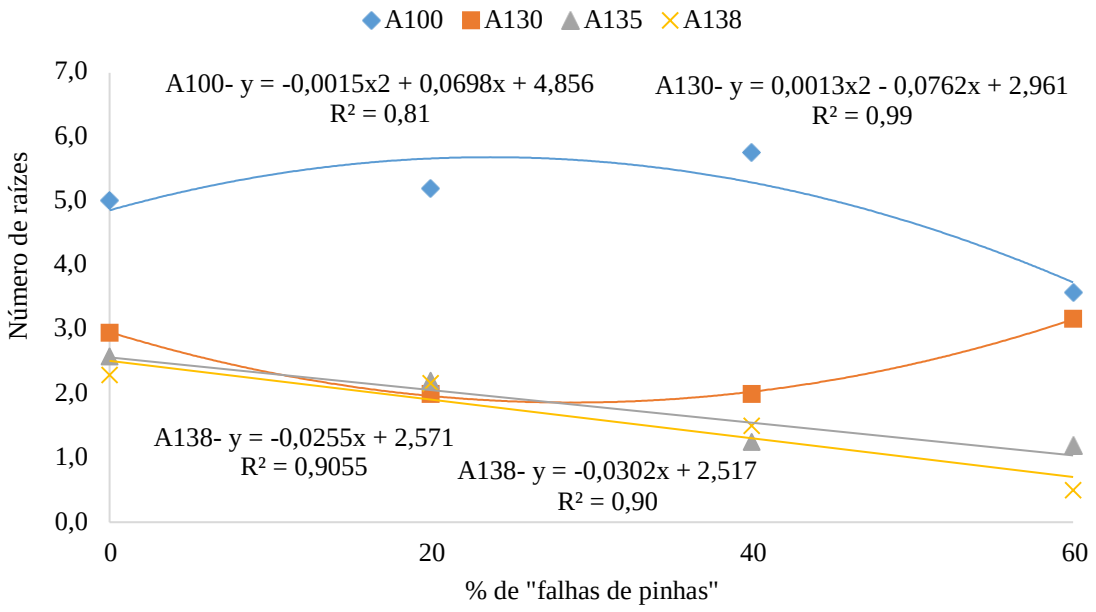
Neste trabalho, o aumento das médias se deu devido ao acréscimo de “falhas de pinhas” de araucária como componente adicional ao substrato, demonstrando que o uso do material foi eficaz para o desenvolvimento das estacas. Em seu trabalho, Dias et al. (2012) afirma que as propriedades

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físicas, químicas e biológicas de um substrato influenciam no enraizamento e no desenvolvimento da muda. Desta maneira, pelo resultado obtido na variável porcentagem de enraizamento, pode-se confirmar que o uso deste material é recomendável.

O número de raízes formadas por miniestaca, de *Sequoia sempervirens*, obteve o valor de 2,7 para a média geral, tendo o clone A100 maior média, no valor de 4,91, e o clone A138 menor média, com valor de 1,61 (Figura 2).

Figura 2. Regressão quadrática para número de raízes de cada clone de acordo com a porcentagem de falha adicionada ao substrato.



De acordo com Lima e Ohashi (2016) é importante não apenas a percentual de raiz formada nas estacas, mas também, o número de raízes formadas no sistema radicular. Por exemplo, Pereira et al. (2017) encontrou uma alta variação entre clones para o número de raízes em miniestacas de *Sequoia sempervirens* submetidas à hormônios vegetais, onde o valor da média variou de 1,4 a 16,8. Entretanto, Lima e Ohashi (2016) ao avaliar a influência de substratos e do tempo no número de raízes de mudas de *Schizolobium parahyba* var. *amazonicum*, encontrou nas combinações realizadas com os substratos vermiculita e fibra de coco como complementos de areia, valor média que variou entre 5,13 e 12,92.

O número de raízes é completamente dependente do tempo a qual as miniestacas estão submetidas. Pereira et al. (2019) relatam que o enraizamento de *Sequoia sempervirens*, quando submetido ao ácido indolbutírico (AIB), apresenta formação de novas raízes entre 70 e 80 dias. Ou seja, pode-se supor que ao usar o AIB mais a “falha de pinha” como componente, o número de

raízes ao completar 100 dias pode ser maior. Desta maneira, pode-se recomendar que futuros trabalhos sejam elaborados para determinar resultados com o devido tema.

Além disto, quando as variáveis: número e comprimento de raízes são estabelecidos, por exemplo, é possível indicar se as mudas possuirão maiores chances de sobrevivência quando encaminhadas ao campo. Em sua pesquisa, Luna (2008) destaca que estas variáveis são importantes no processo de enraizamento das miniestacas de sequoia, pois muitas vezes, quando é apresentado enraizamento, a taxa de mortalidade pode ser alta, devendo-se, principalmente às condições ambientais em que as miniestacas estão inseridas.

Os resultados apresentados na Tabela 1 mostram que a origem das raízes pode variar de acordo com o material genético. O clone A130 teve maior porcentagem de raízes originadas diretamente do tecido, e o clone A135 a partir dos calos.

Tabela 1. Origem das raízes em diferentes clones de *Sequoia sempervirens*.

	Origem das raízes (%)		
Clone	Tecido	Calo	Tecido + Calo
A100	18,6 b*	24,4 b	59,3 a
A130	45,0 a	25,3 b	29,5 b
A135	14,0 c	54,6 a	31,2 b
A138	27,5 b	44,8 a	32,7 b

*Médias na mesma coluna seguidas por letras distintas diferem entre si pelo teste de Scott-Knott, ao nível de 5% de significância.

Frequentemente as raízes emergem dos calos, levando a acreditar que sua formação é essencial para o enraizamento. Em espécies de fácil enraizamento, a formação de raízes é independente da formação do calo, embora ambos envolvam a divisão celular. A simultânea ocorrência é devido à independência das mesmas condições internas e ambientais. Para espécies de difícil enraizamento, a origem das raízes adventícias tem sido associada com a formação de calos (HARTMANN et al., 2002). Portanto, segundo essa definição, neste trabalho, o clone A130 teria um enraizamento mais fácil, porém como observado na porcentagem de enraizamento, este clone apresentou valor de 75% inferior ao clone A100.

O período em que é realizada a coleta das estacas, como citado, pode influenciar a formação de calos e raízes em algumas espécies florestais. Por este motivo é interessante que seja avaliado o período em que é realizada a coleta da sequoia, seja ela no inverno ou no verão, já que ambos os períodos podem inibir o crescimento. Em altas temperaturas, com baixo grau de lignificação, pode ocorrer a desidratação, e se no inverno, maior grau de lignificação, pode acarretar em mais

dificuldade na modificação dos tecidos que auxiliam no crescimento (FACHINELLO; HOFFMANN; NACHTGAL, 2005).

Portanto, a partir das análises realizadas, pode-se afirmar que o desenvolvimento da miniestaquia pode ser útil para avaliações preliminares de algumas características de interesse em programas de melhoramento da *Sequoia sempervirens*. Isso ocorre com a seleção de genótipos mais promissores a campo e com melhor desenvolvimento. Afinal, qualquer técnica de propagação vegetativa multiplica o genoma completo da planta, formando clones da mesma (WENDLING; FERRARI; DUTRA, 2005).

Ademais, a "falha de pinha" se comportou como um bom complemento caso deseje-se agregar volume ao substrato, pois não influenciou negativamente no desenvolvimento das mudas de sequoia. Concordando com Lemos et al. (2016) que utilizaram o material para o desenvolvimento de mudas de *Eucalyptus benthamii*. Assim, o que era utilizado apenas para a fabricação de pellets; como adubo, no acúmulo de matéria seca para composteira; como cobertura de solo, para diminuição de capina e para a não utilização de herbicidas; e para tingimento natural de lãs; agora pode ser indicado, como componente de substrato na silvicultura, na área de produção de mudas (JACINTO et al., 2017).

4 CONCLUSÃO

Para a produção de mudas de *Sequoia sempervirens* por meio da miniestaquia, as "falhas de pinha" pode ser um recurso utilizável como componente ao substrato, desde que misturada com proporções adequadas.

O material genético das estacas pode determinar uma resposta positiva ou negativa com adição das "falhas de pinha", visto que cada clone possui uma resposta distinta à medida que é adicionado o material.

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ENFLO
Ecologia e Nutrição Florestal/*Ecology and Forest Nutrition*
www.enflo.com.br

ISSN: 2316-980X http://dx.doi.org/10.5902/2316980X47063 Scientific Article/Artigo Científico

Gas exchange of *Eucalyptus camaldulensis* Dehnh under saline water influence[1]

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Abstract: In view of the increasing expansion of the use of wood and non-timber products from the *Eucalyptus* genus, the identification and impact caused by the salinity of Brazilian soils in the development and growth of the species are of fundamental importance for a better utilization of salinized areas in the country. In order to contribute to the understanding of this problem, the present work evaluated the effect of different levels of salinity (brackish water) on gas exchange in *Eucalyptus camaldulensis* Dehnh plants. The levels analyzed were: 0, 50, 100, 200, 400 µM NaCl. A completely randomized design with 10 replicates (plants) was used, in which the gaseous exchanges were evaluated with the aid of a portable photosynthesis meter (IRGA), determined the values of photosynthesis (A), stomatal conductance (gs), rate transpiration (E), the relation between the intercellular and atmospheric concentration of CO^2 (Ci / Ca) and water use efficiency (WUE). Initially the seedlings did not undergo physiological changes, showing a certain tolerance of the species to high levels of salinity. Reduction in gas exchanges was only more noticeable after reapplication of the salt (after 7 days). Due to the results obtained, high tolerance of the species to salinity is observed.

Keywords: Salinity; Photosynthesis; Tolerance

Trocas gasosas de *Eucalyptus camaldulensis* Dehnh sob influência de água salobra

Resumo: Perante a crescente expansão do uso de produtos madeiráveis e não madeiráveis provindos do gênero *Eucalyptus*, a identificação e o impacto ocasionado pela salinidade dos solos brasileiros, no desenvolvimento e crescimento da espécie são de fundamental importância para o melhor aproveitamento das áreas salinizadas no país. Visando contribuir para o entendimento desta problemática, o presente trabalho avaliou o efeito de diferentes níveis de salinidade (água salobra) nas trocas gasosas em plantas de *Eucalyptus camaldulensis* Dehnh. Os níveis analisados foram: 0, 50, 100, 200, 400 µM de NaCl. Utilizou-se o delineamento inteiramente casualizado com 10 repetições (plantas), onde as trocas gasosas foram avaliadas com auxílio de medidor portátil de fotossíntese (IRGA), determinando-se os valores de fotossíntese (A), condutância estomática (gs), taxa transpiratória (E), relação entre a concentração intercelular e atmosférica de CO^2 (Ci/Ca) e eficiência no uso da água (WUE). Inicialmente as mudas não sofreram alterações fisiológicas, mostrando certa tolerância da espécie aos altos níveis de salinidade. Reduções nas trocas gasosas só foram mais perceptíveis após sete dias, com reaplicação do sal. Pelos resultados obtidos, percebe-se elevada tolerância da espécie à salinidade.

Palavras – chave: Salinidade; Fotossíntese; Tolerância

[1] Received on 08 August 2020 and accepted for **scientific article** publication on 21 September 2020.
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Introduction

The growing expansion of the forestry industry in Brazil, promoted by the introduction of exotic species, has been the object of study and research, mainly due to the adaptation of these species to the climatic conditions in the country. The introduction of species in sites other than its place of origin is always a process that requires studies aimed at its survival, considering the productivity of the species in the region to be inserted.

In order to have a successful implantation of productive forest stands, a combination of several factors is necessary, such as genetic materials adapted to the location and edaphoclimatic conditions that favor the development of the species (BRUNINI and CARDOSO, 1998). However, with the expansion of forest plantations in less favorable areas, there is a need to know the behavior of different species. Among the exotic species introduced in Brazil, the genus *Eucalyptus* has been growing in prominence in reforestation programs, due to its rapid growth, easy handling, great rate of increment, biomass and mainly for its varied purposes in the industry.

Within the *Eucalyptus* genus, the *E. camaldulensis* species has been gaining space in the Brazilian market. The species is native to Australia, in the subtropical and temperate climate and occurs naturally at altitudes ranging from 30 m to 600 m above sea level. The annual rainfall index of the region of origin is between 250 mm to 600 mm with a dry period of 4 to 8 months, maximum temperature is 35°C and the minimum is 11°C. *Eucalyptus camaldulensis* is of great economic interest due to its ability to grow in poor soils, resistance to long periods of drought, tolerance to excessive rainfall, as well as hard and heavy wood (LIMA et al., 2010). Due to its physical-mechanical characteristics, *E. camaldulensis* wood has been used for civil construction, crossties, floors, framework, fences, turned wood, firewood, coal and in the manufacture of plywood (BOLAND et al., 2006).

The success of *E. camaldulensis* plantations as an exotic species is attributed to its superiority in wood production in relation to other species cultivated in adverse environmental conditions. This is because the species thrives in dry and sterile places, has tolerance to drought and high temperature, rapid growth and good regrowth capacity, tolerance to periodic flooding or waterlogging lasting 4 to 5 months, tolerance to soil salinity, some frost tolerance, in addition to the usefulness of its wood (ELDRIDGE et al., 1993).

Considering the adaptation of the species in forest areas in critical zones, it has been introduced in Brazil with high frequency, mainly in the Cerrado of Minas Gerais state and in São Paulo. Aiming to expand the region of the country in which the species is introduced, it is necessary study and researches the natural adversities that may or may not be tolerated by *E. camaldulensis*, and the physiological responses of the species. Scientific knowledge may subsidize some silvicultural treatments for the cultivation of the species.

One aspect that can influence directly in the development of the species is the salinity of the soil, usually caused by natural processes and, mainly, by the misuse of the land. Approximately 30 million hectares of soil on the planet are affected by salt, and 0.25 to 0.5 million hectares of productive area are lost each year, due to soil salinization (FAO, 2002).

The choice of tolerant species to critical environments, such as places with high levels of salinity, is of great importance for the success of the forestry activity. Thus, the present study aims to assess whether there is an effect of increased sodium chloride (NaCl) concentration on physiological parameters of *Eucalyptus camaldulensis* seedlings.

Material and methods

The experiment was carried out between October and December 2018, in a greenhouse in the forest nursery of the Center of

Agricultural Sciences - CAV (UDESC), located in the municipality of Lages, state of Santa Catarina, in the coordinates 27 ° 47'33 "S and 50 ° 18'4 "W. The site has an altitude of approximately 900 meters, whose climate, according to the Koppen classification, is temperate (humid mesothermal and mild summer) - Cfb, with average annual precipitation between 1300 and 1500 mm and average annual temperature of 15 ° C (ALVARES et al ., 2013). The region belongs to the hydrographic basin of the Uruguay River, a hydrographic sub-basin of the Pelotas River.

Seedlings of *Eucalyptus camaldulensis*, produced via cuttings, were put in 55 cm³ tubes filled with commercial substrate based on peat and pine bark. After 120 days, the seedlings were transferred to pots with five liters of capacity, filled with soil from the region. The pots remained in the shade house of the forest nursery for approximately five months. During the experiment, the plants were placed in a greenhouse, without any type of irrigation. The experimental design was completely randomized, with 10 repetitions (plants) per treatment, with the treatments being composed of different concentrations of sodium chloride - NaCl (0, 50, 100, 200 and 400 µM) diluted in distilled water.

The first application of the saline solution occurred on October 24, 2018, where 500 ml of the solution were applied per plant, in concentrations that varied according to the treatments. Subsequently, on October 30, a second application was performed, as the seedlings did not show any symptomatic variation initially. The only irrigation carried out during the experiment was with the saline solution, applied on the 7th day.

The gas exchange evaluations were performed with the aid of a portable photosynthesis meter, brand Liquor, model Li-6400xt, in five repetitions, where the following variables were measured, photosynthesis - A (µmol CO2 m^{-2} s^{-1}), stomatal conductance - gs (mol m^{-2} s^{-1}), transpiratory rate - E (mmol H2O m^{-2} s^{-1}), relation between intercellular and atmospheric concentration of CO^2 - Ci / Ca (µmol CO^2) and water use efficiency - WUE ([µmol m^{-2} s^{-1}] $^{-1}$). The photosynthetically active radiation used during the measurements was 900 µmol of photons m^{-2} s^{-1}. This value was determined by the luminous saturation curve measured for eucalyptus, between 800 and 1,000 µmol of photons m^{-2} s^{-1} (SILVA and KLAR, 2004). Photosynthetically active radiation (RFA) was provided by an artificial light source (LI-6400-40), with a blue light percentage of 10% of the total RFA.

The gas exchange evaluations were performed at 24 h (1 day) and 48 h (2 days), 7, 12 and 14 days after the first application of saline solution. For the evaluations, the same leaf from each plant was used throughout the experimental period. The readings were stable after 2-3 minutes. Water use efficiency (WUE) was calculated by dividing A by E.

The normality and homogeneity of the data variance were tested by the Kolmogorov-Smirnov and Bartlett tests, respectively. For the statistical analysis of the experiment, ANOVA analysis of variance (p <0.05) was used. When significant, the data were adjusted to the response surface graphs in the Statistica 6.0 software.

Results

The treatments differed for all physiological variables, with variations in adaptation and responses depending on the NaCl concentrations. When measuring the photosynthetic rate, a drop in photosynthesis is seen, mainly from the 7th day onwards when the second application of saline occurred (Figure 1). It is observed that, with the passage of days, under saline conditions, the photosynthetic rates were lower and lower, but even in high concentrations of NaCl, the plants have a capacity for tolerance to salinity.

The stomatal conductance (gs) of the plants was significantly reduced (Figure 2) by the salinity of the applied solution. There were linear decreases in gas due to the increase in the concentration of saline solution.

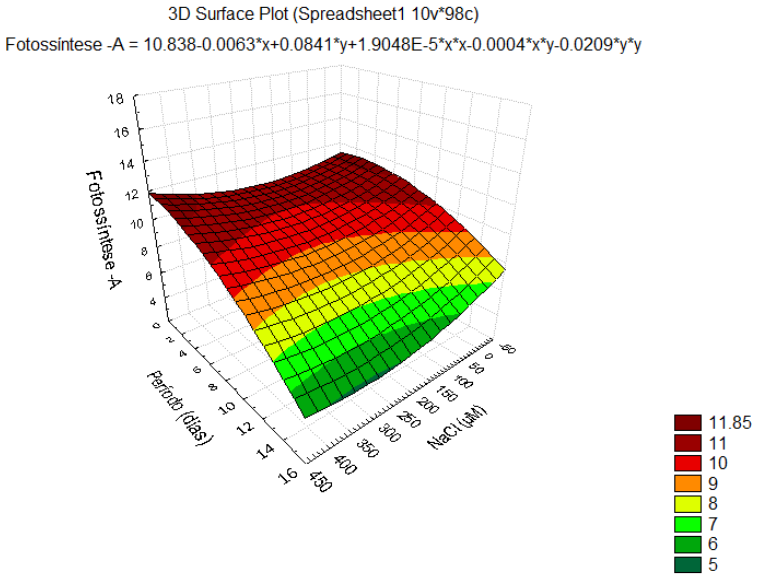


Figure 1 - Photosynthetic rate of *E. camaldulenis* seedlings as a function of application of different concentrations of saline solution (NaCl).
Figura 1 - Taxa fotossintética de mudas de *E. camaldulensis* em função da aplicação de diferentes concentrações de solução salina (NaCl).

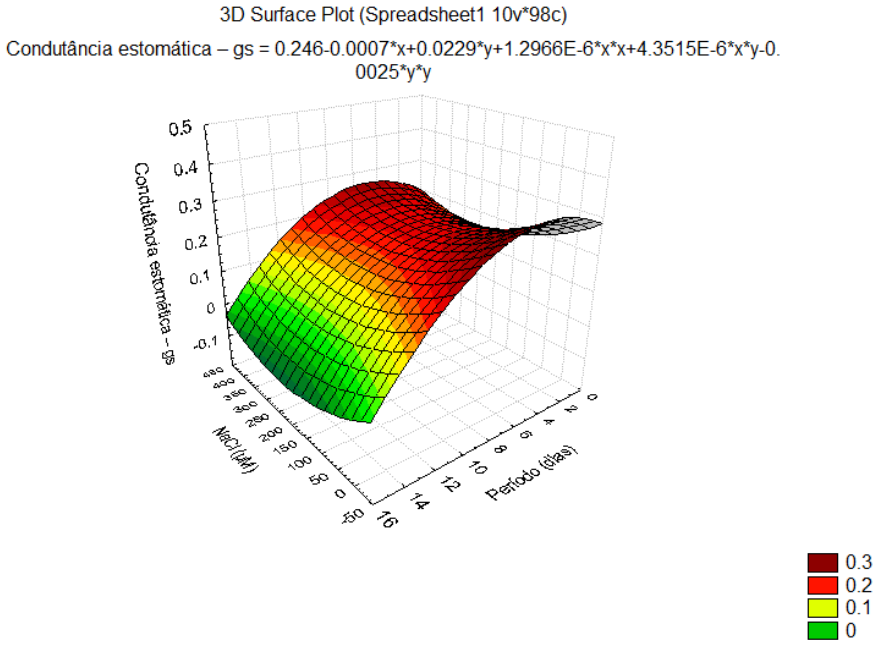


Figure 2 - Stomatic conductance (gs) of *E. camaldulensis* seedlings as a function of NaCl concentration variation.
Figura 2 - Condutância estomática (gs) de mudas de *E. camaldulensis* em função da variação da concentração de NaCl.

Transpiration (E) (Figure 3) had the same trend observed in stomatal conductance, with lower values after the first application, tending to decrease in the subsequent application.

Under saline conditions in the soil, the plants showed very efficient stomatal control since the lowest transpiratory rate was observed from the

second application of the saline solution onwards.

During the evaluation period, there was a drop in the ci/ca ratio (Figure 4), due to stomatal closure, which in general ended up reducing the exchange of gases between the environment and the interior of the leaf.

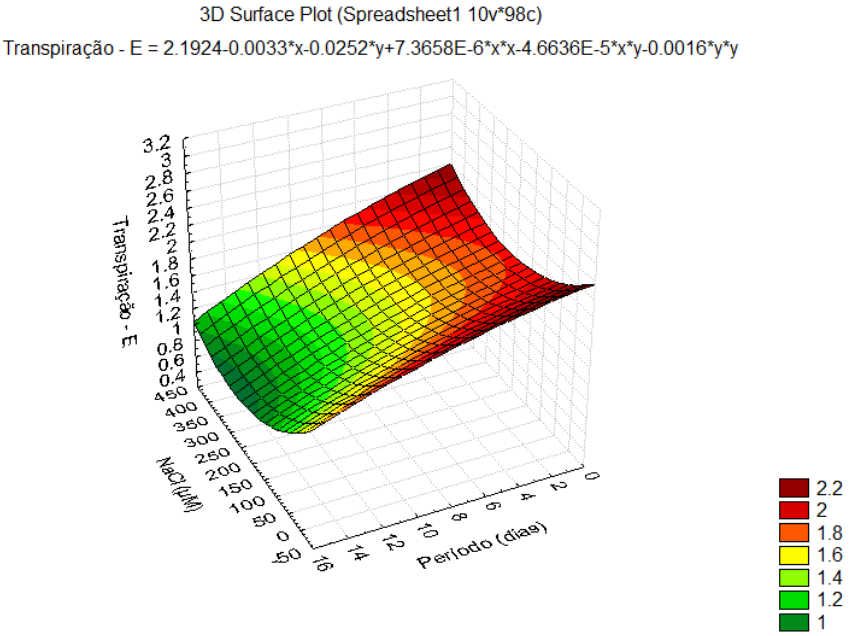


Figure 3 - Transpiration (E) of *E. camaldulensis* as a function of the different NaCl concentrations.
Figura 3 - Transpiração (E) de mudas de *E. camaldulensis* sob diferentes concentrações de NaCl.

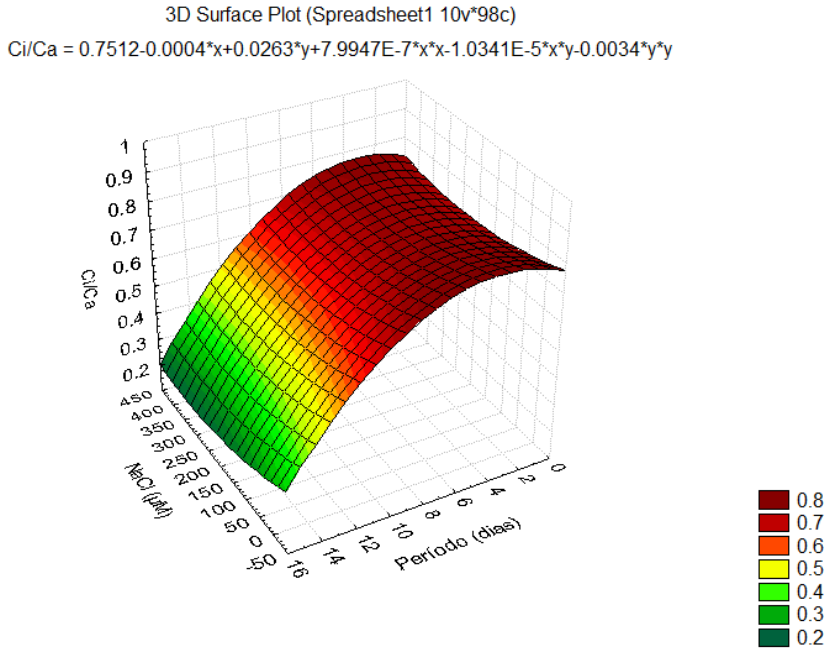


Figure 4 - Relationship between intercellular and atmospheric CO^2 (ci / ca) concentration in *Eucalyptus camaldulensis* as a function of days under salt stress and NaCl concentrations.
Figura 4 - Relação entre a concentração intercelular e atmosférica de CO^2 (ci/ca) em *Eucalyptus camaldulensis* em função dos dias sob estresse salino e concentrações de NaCl.

When analyzing the efficiency in the use of water (WUE) during the experimental period, it is noted that the plants subjected to the highest concentrations of salt (which reflects in water deficiency), had greater efficiency in the use of water (Figure 5). It was observed that the effects of different concentrations of saline solution on stomatal conductance were quite expressive, that is, when the seedlings received higher saline levels, there was better use of water.

In general, gas exchange was significantly affected after the second application of sodium chloride saline (NaCl). The water deficit caused by salinity was a limiting factor for stomatal opening, which contributed to the decrease in the assimilation of carbon by photosynthesis, because the higher the salinity, the lower the water availability, consequently, the values of A, gs and E will be smaller; water efficiency (WUE) increased when the saline concentrations were higher.

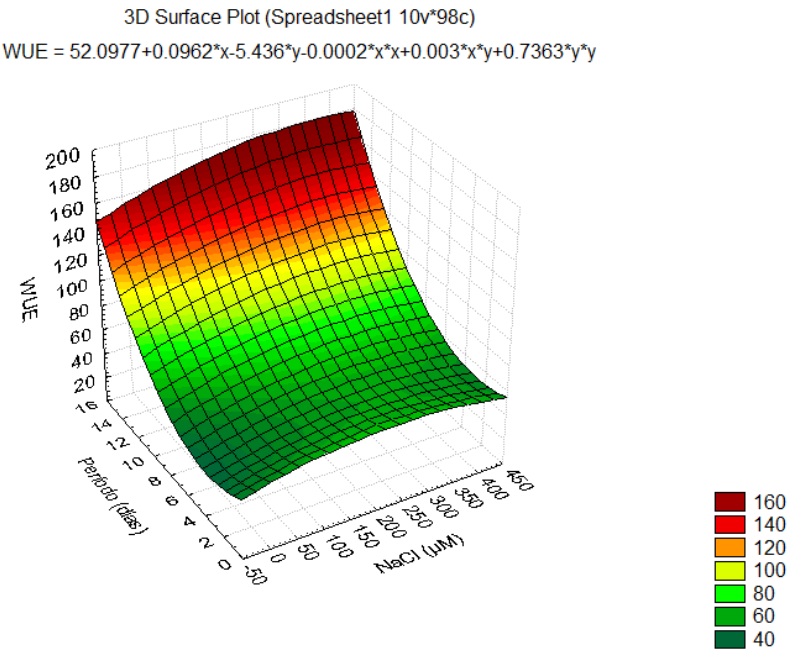


Figure 5 - Water use efficiency (WUE) in *Eucalyptus camaldulensis* under saline stress.
Figura 5- Eficiência no uso da água (WUE) em *Eucalyptus camaldulensis* sob estresse salino.

Discussion

The results of the present study demonstrate the potential for adaptation of the species *Eucalyptus camaldulensis* in places with greater salinity. The responses of plants to hydric stress, caused by salinity, included physiological changes such as stomatal closure, reduction in photosynthetic rates, changes in hormone levels and changes in the performance of genes. The photosynthetic rate (A) may or may not be related to stomata movement (SILVA and KLAR, 2004). The application of the saline solution shows that the increase in salinity levels reduced the stomatal conductance, which resulted in a decrease in the partial pressure of intercellular CO^2, negatively interfering in the assimilation of CO^2 by the photosynthetic apparatus (ANGELOCCI, 2002).

The plant response to water stress caused by salinity included physiological changes, such as stomate closure and reduction in photosynthetic rates. The decrease in stomatal conductance is directly related to the drop in the water potential of the leaves. Salinity reduces the photosynthetic rate per unit leaf area of most cultivated plants, due to the decrease in stomatal conductance (O'LARYARY, 1995). However, the reduction of stomatal opening can limit the rate of CO^2 diffusion into the leaf, with direct effects on photosynthesis and growth (EL‐SHARKAWY, 2007).

The similarity between gs and E in the evaluations is expected, due to the closing of stomata, thus showing that with the passing of the day the perspiration ends up suffering a drop in its levels. This effect can cause osmotic stress, reducing the availability of water for individuals, consequently affecting gas exchange and plant growth.

The concentration of CO^2 is generally attributed to stomatal behavior, in which with the closure of stomata there is a greater resistance to the influx of CO^2 and, therefore, less concentration in the sub-stomatal chamber (PRAZERES et al., 2015). It is likely that efficiency in this case is linked to a lower rate of transpiration by plants.

The use of water comprises the processes of transpiration and evaporation of water in the soil, however, the WUE is sensitive to environmental conditions and changes (LACLAU et al., 2016). When evaluating *E. camaldulensis* plants under conditions of salt stress, it is important to differentiate the changes that occur during the individual's life and those that are the result of several cycles of natural or artificial selection of genes and, therefore, are part of the genetic structure of the species. In the first case, the changes occur as a result of temporary physiological changes, involving a series of metabolic processes in response to the presence of stress, giving rise to a process known as acclimatization. In the second, there are a series of definitive morphophysiological changes that lead to the evolution of the species, in a process called adaptation. Although the processes of acclimatization and adaptation involve changes in gene expression, only the adaptation mechanisms are transmitted from one generation to another (BOSCO, 2010).

Conclusions

Eucalyptus camaldulensis plants showed physiological resistance after application of sodium chloride saline (NaCl) to the substrate, especially in the first seven days, with low reduction of gas exchange.

Due to the physiological responses presented by the plants, such as the ability to tolerate salinity and the efficiency in the use of water, since with the higher the level of salinity, the better results were obtained, showing that there was better use of water, indicating satisfactory responses from *Eucalyptus camaldulensis* to high salinity conditions, indicating good adaptation of the species to soils with higher salinity.

Acknowledgements

The author thanks FAPESC for funding the research group Management of planted and natural forests (PAP2019031000055).

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Hindawi
International Journal of Forestry Research
Volume 2020, Article ID 8850641, 8 pages
https://doi.org/10.1155/2020/8850641

Research Article

Liming and Fertilization on the Growth of *Eucalyptus benthamii* and *Eucalyptus dunnii* in Brazil

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Received 25 August 2020; Revised 9 October 2020; Accepted 20 October 2020; Published 30 October 2020

Academic Editor: Qing Lai Dang

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The use of mineral fertilizers has shown substantial productivity gains for the vast majority of *Eucalyptus* forests. The objective of the present work is to evaluate the response of *Eucalyptus benthamii* and *Eucalyptus dunnii* at 48 months of age, to different doses of limestone, soluble NPK, and natural phosphates. The experiment was conducted in a plantation located in South Brazil. The experimental design was a randomized block with three replications, and the treatments consisted of combinations of doses of natural phosphate (NP) (0, 400, 600, and 800 kg ha^{-1} of Gafsa reactive NP P_2O_5), limestone (0, 3, 5, 6, and 10 Mg ha^{-1} of dolomitic limestone), and mineral fertilizer–NPK (0, 100, 133, and 167 kg ha^{-1} of mixed mineral fertilizer 6-30-6). Height and diameter measurements were taken after 48 months. There was a positive response with the increase of NPK fertilizer dose, and the dose where the highest averages were obtained was 167 kg ha^{-1}. The doses of NP had significant effects on the increase of the variables up to 600 kg ha^{-1}, also the averages decreased with the increase of the dosage. For liming, positive results were observed from its absence to the maximum dose, justifying its use in minimum dose in order to supply the necessary amounts of Ca and Mg in the soil. The use of NPK, natural phosphate, and limestone is recommended, causing an increase in the growth of both species of *Eucalyptus* studied.

1. Introduction

The introduction of species from other regions is a process that needs studies aimed at assessing their adaptability and survival, as well as their reflexes on economic productivity. A combination of several factors is required for the establishment of productive forests, such as site-adapted genetic materials, favorable soil and climate conditions, proper management, and high-quality seedlings [1].

The need for fertilization in forest species comes from the fact that the soil is not always able to provide all the nutrients in quantity that the plants need for proper growth, and the amount depends on the nutritional needs, soil fertility, reaction of fertilizers with soil, and fertilizer efficiency. Proper nutrition in *Eucalyptus* plantations is essential for the establishment of high-yielding forests [2].

Eucalyptus species are considered nutrient-demanding, mainly phosphorus in the early phase and potassium in the juvenile and adult phase [3]. Given this feature, along with the fact that they are under subtropical conditions, it is relevant to promote rapid early development with gains in height and diameter, so that plants reach a size where frost damage is minimized. However, regarding nitrogen fertilization, Sette et al. [4] did not obtain increment for *E. grandis*, as well as no changes in wood structure.

Most Brazilian soils have inadequate chemical characteristics for intensive production, among them a high natural acidity, making it necessary to use limestone for its suitability, increasing the pH and calcium and magnesium levels in the soil, and allowing the full development of the culture [5]. According to Barros et al. [6], liming calcium is the second nutrient most absorbed by most forest species. The

most accumulated nutrients in components exported from the *Eucalyptus* production area are Ca and Mg, placed in order, with Ca occupying the first place, with approximately 75% of this element exported from the site with the harvest. Over several years of cultivation, nutritional deficiency can be promoted for plants and limits the productivity of future cycles due to Ca depletion [7]. In addition, nutritional imbalances may be produced by providing inadequate Ca^{2+} and Mg^{2+} ratios, which may induce plant deficiency and compromise growth.

Fertilization can increase the growth of *E. benthamii* and *E. dunnii* forests, resulting in an increased final yield and may even reduce the rotational cycle. There is a direct relationship between the different doses of liming, phosphating, and NPK with the growth of *Eucalyptus* species. In view of this, it is expected that different growth behaviors will be expressed as a function of fertilizer doses, and the higher the dose, the higher the growth of dendrometric variables.

Thus, the objective of the study was to quantify the growth of *E. benthamii* and *E. dunnii*, in response to fertilization, for the different doses of dolomitic limestone, reactive natural phosphate, and NPK, aiming to define the specific fertilization management for each studied species.

2. Materials and Methods

2.1. Study Area. The study was carried out in Rio Negrinho (latitude 26°06′41″ south and longitude 49°48′19″ west), located in the microregion of the northern plateau of Santa Catarina State (Brazil). With an average altitude of 800 m above the sea level, the municipality presents regular surface and plateau relief, with hills of small altimetric amplitude.

Regarding the climate, in the Köeppen classification, the northern Santa Catarina plateau is Cfb (constantly humid temperate climate, no dry season, with cool summer). The average annual temperature ranges from 15.0°C to 17.0°C, with average maximum temperatures ranging from 24.0°C to 26.6°C and minimum from 10.8°C to 11.8°C. The average annual precipitation is 1720 mm, with maximum and minimum of 2500 mm and 1082 mm, respectively. The relative humidity can vary from 80.0% to 86.2% [8]. Considering the hydraulic demand of *Eucalyptus*, which is 800–1,200 mm [9], the study area has the amount of rain necessary to meet the average requirements of the genus.

Prior to the implementation of the experiment, no natural corrective or phosphates had been used in the field, only base fertilization in this area, which was cultivated with *E. viminalis* in the first planting cycle, planting in 1983 and clear-cutting in 2011.

Soil composite samples were collected in the 0–20 cm layer to characterize it in the experimental area (Table 1). Chemical analyses were performed according to the methodology described by Tedesco et al. [10] by the Soil Routine Analysis Laboratory of the Department of Soils and Natural Resources of Santa Catarina State University, Lages, SC (Brazil). The soil of the experiment site was classified as Humic Cambisol (Cambisols).

Table 1: Chemical and physical characteristics of the soil in the 0–20 cm depth layer of the study area.

Attributes	Values	Interpretation*
pH $H_2O^{(1)}$ (1 : 1)	4.28	No pH for reference**
SMP buffer	4.20	—
$Ca^{(2)}$ ($cmol_c dm^{-3}$)	0.88	≥4.0 $cmol_c dm^{-3}$
$Mg^{(2)}$ ($cmol_c dm^{-3}$)	0.76	≥1.0 $cmol_c dm^{-3}$
$Al^{(2)}$ ($cmol_c dm^{-3}$)	4.64	—
P Mehlich$^{(2)}$ (mg dm^{-3})	0.72	≥15.1 mg dm^{-3}
$K^{(2)}$ (mg dm^{-3})	73.80	≥106 mg dm^{-3}
OM (%)	3.45	≥5.00%
Effective CEC ($cmol_c$ dm^{-3})	6.46	—
CEC pH 7.0 ($cmol_c$ dm^{-3})	36.32	15.1 a 30.0
Basis saturation (%)	5.00	—
Al saturation (%)	71.84	—
Clay (%)	18.50	Class 4

[1]Methodology used by Tedesco et al. [10]; OM, organic matter. *Values are subjected to interpretation. **Culture tolerant to soil acidity and has no pH for reference.

2.2. Experimental Design and Treatments. The experiment was set up in a randomized block design with three replications of 15 trees each. The treatments consisted of combinations of doses of natural phosphate (0, 400, 600, and 800 kg ha^{-1}), limestone (0, 3, 5, 6, and 10 Mg ha^{-1}), and NPK mineral fertilizer (0, 100, 133, and 167 kg ha^{-1}), at planting fertilization, for *E. benthamii* and *E. dunnii*. *E. benthamii* seedlings of seminal origin produced in the region of São Bento do Sul, SC (Brazil), and clonal plants of *E. dunnii* produced in the region of Canoinhas, SC (Brazil), were used. For both species, the seedlings planted were approximately 30 cm height and had shoot diameter greater than 2 mm.

The amounts of nutrients used in the experiment were determined from soil analysis performed before planting. The 400 kg dosage of natural phosphate is the standard dosage of the company where the study was conducted, with 800 kg being double the dosage and 600 the average of the two doses. The NP used was the Tunisia-branched Gafsa reactive (NPGR) (28% total P_2O_5).

For the limestone factor, the company uses 3.5 Mg ha^{-1} as the standard farm dosage. The dosage of 10 Mg ha^{-1} is almost half the dosage of 19 Mg ha^{-1}, recommended by the Rede Oficial de Laboratórios de Análise de Solos (ROLAS) (Official Network of Soil Analysis Laboratories) [11], and as intermediate dose was used, 6 Mg ha^{-1}. For NPK, the dose of 133 kg ha^{-1} is recommended by the interpretation of soil analysis, with 167 kg ha^{-1}, the maximum dose, and 100 kg ha^{-1}, the minimum dose. The source of NPK was mixed mineral fertilizer with formulation 6-30-6 (NPK), providing the amount of 6 kg ha^{-1} of N and K_2O and 30 kg ha^{-1} of P_2O_5 for 100 kg ha^{-1} fertilizer dose, 8 kg ha^{-1} of N and K_2O and 40 kg ha^{-1} of P_2O_5 for 133 kg ha^{-1} of fertilizer, and 10 kg ha^{-1} of N and K_2O and 50 kg ha^{-1} of P_2O_5 for 167 kg ha^{-1} of fertilizer.

First, cultural treatments were made in the area in accordance with the company's operational plan. Operations began in November 2012 with the cleaning of the area by mechanized mowing and chemical weeding in the total area. The tillage was mechanized, performed in minimum

cultivation with subsoiling at 45–50 cm deep in the planting line, with spacing of 3.0 m between the rows. After subsoiling, dolomitic limestone (80% PRNT) was applied according to the treatments.

The planting, the application of NP, and the fertilization of planting were carried out in January 2013. The planting was performed manually in spacing of 3×3 m, and the NP was filleted over the subsoiling line. There was an application of 500 mL of hydrogel per planting pit. Planting fertilization was carried out in two lateral "pits" per plant, at a distance of 10–15 cm from them, using manual fertilizers adjusted at the dosages fixed in the treatments.

Coverage fertilization was carried out two months after planting, along with the surface replanting, in the crown projection, at a dose of 133 kg ha^{-1} of mineral fertilizer with formulation 20-00-20 (N-P-K), providing the amount of 27 kg ha^{-1} of N and K_2O. Coverage fertilization was performed at the same dose for all plots.

The fight against leaf-cutting ants was carried out by applying 4 kg ha^{-1} of formicide bait at the time of planting and 20 days after planting. Ten months after planting, a mechanized mowing was performed between the rows to control weed competition.

2.3. Data Collection and Analysis. The dendrometric data of total tree height (m) and BHD diameter at breast height (cm) were collected with the help of the Haglöf and Suta clinometer, respectively, in February 2017. All diameters were collected within each portion and five heights. The other heights were estimated using a hypsometric equation, with the Kopezky–Gehrhadt model chosen for *E. benthamii*: $ht = \beta 0 + \beta 1 \cdot BHD^2$, with a coefficient of determination (adjusted R^2) of 0.3983; estimate standard error (Syx%) of 11.25%; and coefficients $\beta 0$ 12.4166 and $\beta 1$ 0.0178. For *E. dunnii*, the model chosen was Schneider's $ht - 1.3 = \beta 0 + \beta 1 \cdot BHD$, with an adjusted R^2 of 0.5482; Syx% of 7.13, and $\beta 0$ 5.3158 and $\beta 1$ 0.6080. The volume was calculated by the expression, $V = g * h * f$, where V is the volume (m^3); g is the basal area (m^2); h is the height (m); and f is the artificial form factor considering 0.4624 [12]. After estimating the variables, the data were submitted to Tukey's means test at 5% error probability. The analyses were performed using the variations between the treatments (NPK, natural phosphate, and limestone) and the two species (*E. benthamii* and *E. dunnii*).

3. Results

3.1. Species and NPK. Regarding the diameter, for *E. benthamii*, the highest average was observed for 167 kg ha^{-1}, not differing from 133 kg ha^{-1} and being 13% higher in relation to the worst observed average, which was the control (Figure 1(a)). For *E. dunnii*, the highest average was presented by 167 kg ha^{-1}, which does not differ from 133 kg ha^{-1}. The lowest average was presented by the control, being 21% smaller, not differing from 100 kg ha^{-1}.

There was a statistical difference for treatments in *E. benthamii* in relation to height, where the lowest result was observed for the control, with an average of 37% lower compared to the average of the other treatments (Figure 1(b)). For dosages of 100, 133, and 167 kg ha^{-1}, there were no differences. For *E. dunnii*, the lowest mean height was also observed for the control, being 19% lower, not differing from 100 kg ha^{-1}. The highest averages were observed with 133 kg ha^{-1} and 167 kg ha^{-1}.

For the volume in *E. benthamii*, the lowest average was presented by the control, differing from all other treatments (Figure 1(c)). The highest average was presented by 167 kg ha^{-1}, not differing from 133 kg ha^{-1}, and 63% higher than the lowest average observed. *E. dunnii* obtained the highest average volume for the treatment 167 kg ha^{-1}, while the lowest average obtained was for the control treatment, 53% lower than the highest average, not differing from 100 kg ha^{-1}.

3.2. Species and Natural Phosphate (NP). Regarding the means of treatments with NP for *E. benthamii* in relation to BHD, the lowest mean observed was in the control treatment that did not differ from 400 kg ha^{-1} (Figure 2(a)). The other treatments had the highest averages, 13% higher than the lowest average. For height (Figure 2(b)), the control also obtained the lowest average, being 37% lower than the others, which did not differ from each other. For *E. dunnii*, the lowest mean was observed for the control, both in relation to BHD and in relation to height, being 18% and 23%, respectively, lower than the other averages.

Regarding the volume, the highest average for *E. benthamii* was presented by 600 kg ha^{-1}, not differing from 0 kg ha^{-1} and 800 kg ha^{-1} (Figure 2(c)). The lowest average obtained was for the control treatment, which differed from the other averages, and was 62% lower than the best average. For *E. dunnii*, the treatment with 600 kg ha^{-1} of NP also had the highest average; however, it differed from the other treatments. The lowest average was observed for the control treatment, which was 48% lower than the treatment with the highest average.

3.3. Species and Liming. For *E. benthamii* BHD, the control treatment presented the lowest average, 18% lower than the average of the other treatments (Figure 3(a)). For height, the same behavior was observed, with the control treatment obtaining the lowest average (Figure 3(c)). The other treatments showed no difference between them, being 38% superior in relation to the control. Regarding volume, the lowest mean was also for the control, differing from the other averages. The highest average obtained was for 6 Mg ha^{-1}, not differing from 0 Mg ha^{-1}, 61% higher than the lowest average obtained.

The mean BHD for *E. dunnii* did not differ for liming doses, being 19% higher when compared to the control with the lowest average (Figure 3(a)). Regarding height and volume (Figures 3(b) and 3(c)), the lowest average was observed for the control, 24% and 41%, respectively, lower than the other fertilizers, which did not differ from each other.

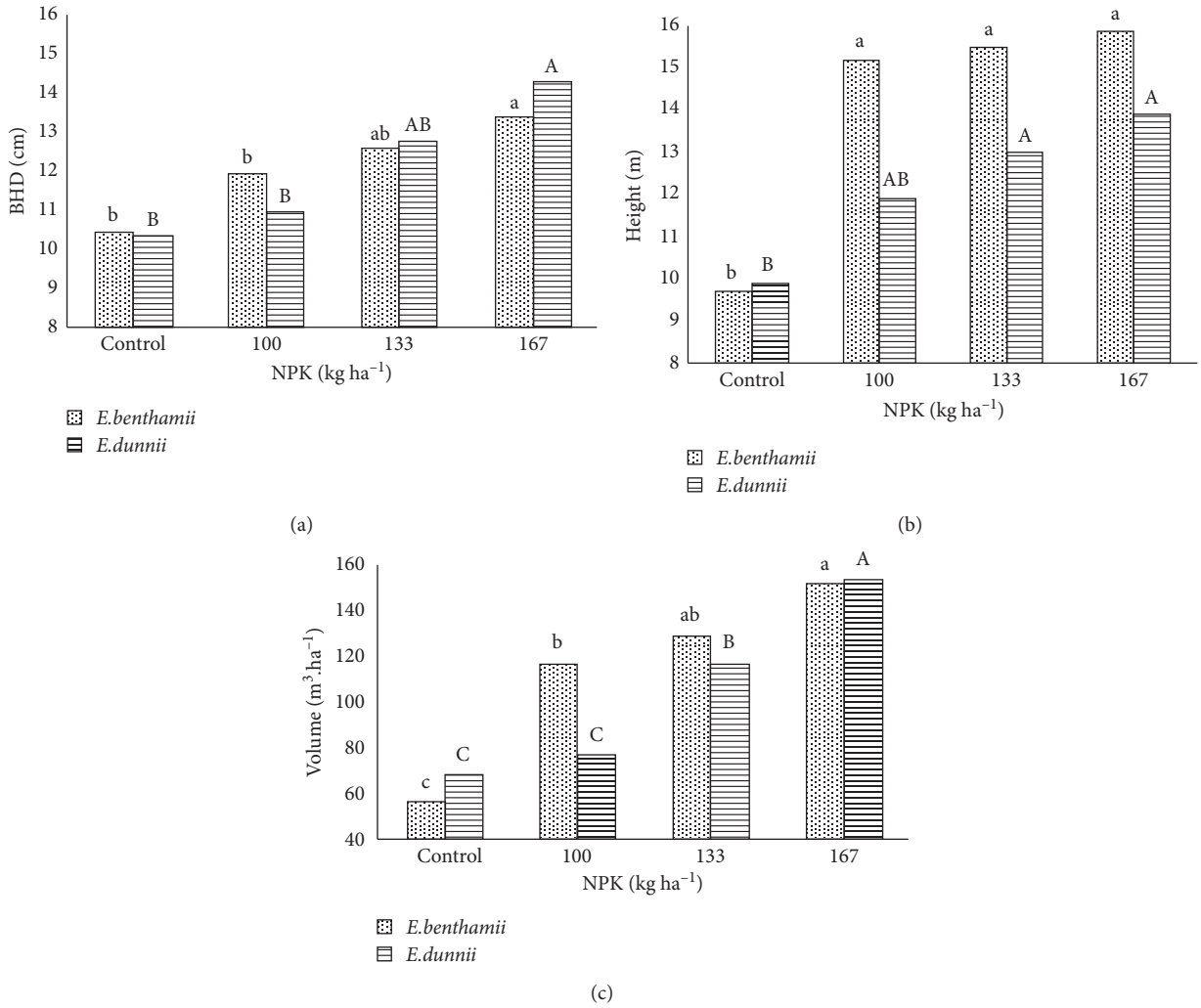


Figure 1: (a) Breast height diameter (BHD, cm), (b) height (m), and (c) volume ($m^3\ ha^{-1}$) in *Eucalyptus benthamii* and *Eucalyptus dunnii* in relation to NPK fertilizing. Averages followed by the same letter, lower and uppercase, do not differ between them by the Tukey test at 5% probability. Lowercase letters compare fertilization in relation to *E. benthamii*. Uppercase letters compare fertilization in relation to *E. dunnii*.

3.4. Averages of Treatments and Species. Regarding BHD, there was no statistical difference between species averages for all treatments (Figure 4). For height, there was a statistical difference between the species, and the highest averages were obtained for *E. benthamii*, around 16% higher when compared to the *E. dunnii* averages. The averages obtained for volume also present a statistical difference between species and behaved on an average 13% higher for *E. benthamii* when compared to *E. dunnii.*

4. Discussion

Positive results were observed in all NPK dosages applied to the treatments for both *E. benthamii* and *E. dunnii*, highlighting the 167 kg ha^{-1} treatment, in diameter, height, and volume. It can be noted that with the omission of NPK application, the variables also suffer a reduction in response. Cicek et al. [13] working with the effect of NPK fertilization on initial field performance with *Fraxinus angustifolia* (ash tree), with fertilization of 67 g $plant^{-1}$ NPK (10-10-10) and 133 g $plant^{-1}$ NPK (20-20-20), observed positive effects on increases in BHD and H during the first 36 months of growth, and when compared to the control (without fertilization), the averages were 24% higher. Bavoso and Bassaco [14], in their work with *E. dunnii* growth with increasing doses of NPK, stated that the absence of NPK limited the development of plants, and in contrast, growth had a positive relationship with increasing dosage. This positive growth response is due to the greater amount of nutrients essential for the development of the plant that are available to be absorbed by it. The decrease in plant growth without initial fertilization, as observed in our study with both species of *Eucalyptus*, is a function of the participation of elements in the structure of numerous molecules, being the main limitation of growth, being part of proteins, nucleic acids, and many other cellular constituents, such as membranes and hormones [15].

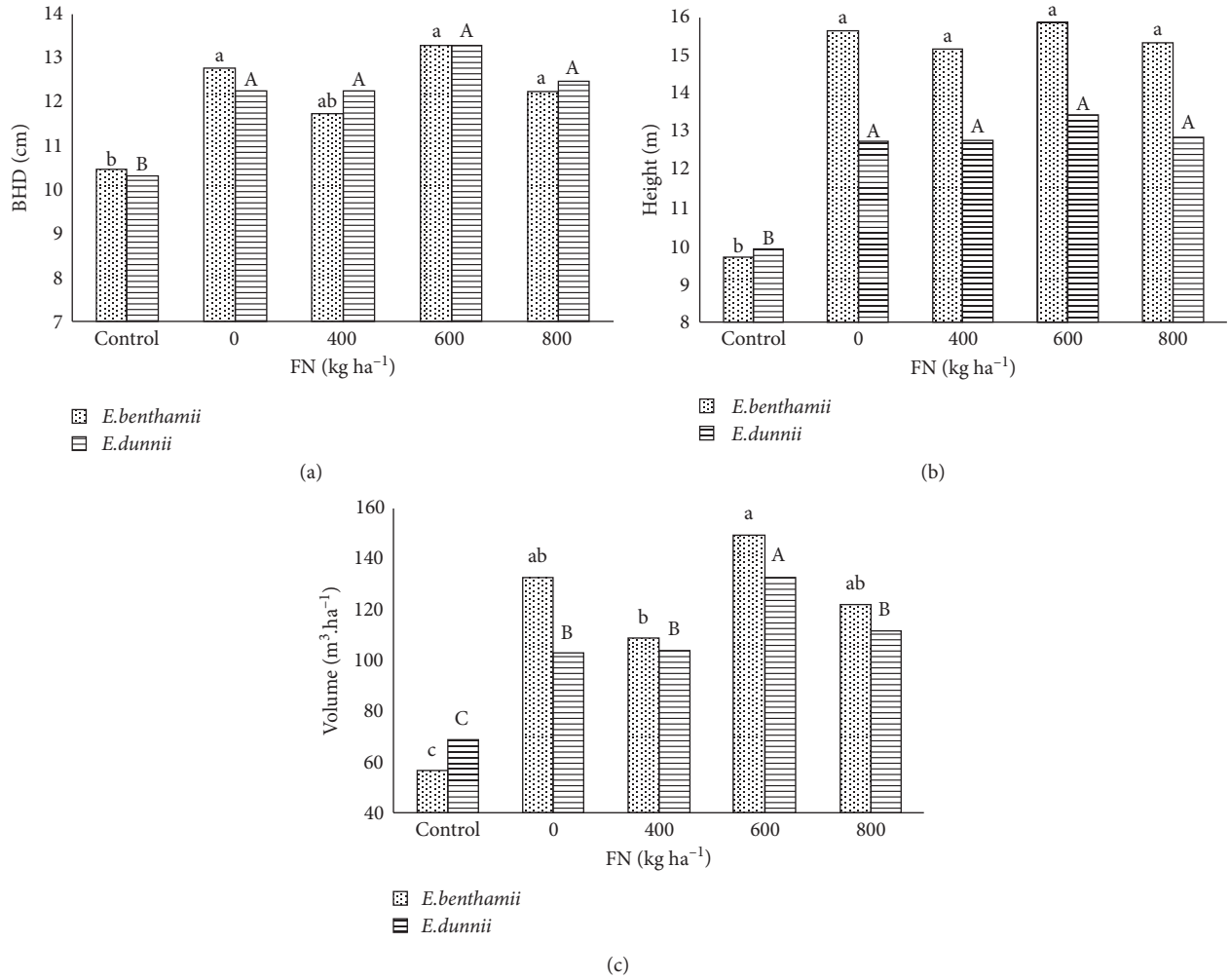


Figure 2: (a) Breast height diameter (BHD, cm), (b) height (m), and (c) volume (m³ ha^{-1}) in *Eucalyptus benthamii* and *Eucalyptus dunnii* in relation to natural phosphate (FN) fertilizing. Averages followed by the same letter, lower and uppercase, do not differ between them by the Tukey test at 5% probability. Lowercase letters compare fertilization in relation to *E. benthamii*. Uppercase letters compare fertilization in relation to *E. dunnii*.

Comparing the species, there is a difference in growth in relation to the variables height and volume. *E. benthamii* stands out with higher averages, and one of the possible explanations for the volume average to be higher is to be directly related to height, since the two species do not present differences in BHD. Moro et al. [16], in the study on response of *Pinus taeda* with different ages and NPK fertilization in the southern Santa Catarina plateau (Brazil), observed that the wood volume increased in all ages in response to NPK fertilization. According to the authors, adequate fertilization of the three chemical elements is important. Treatments that omitted some nutrients had lower volume averages.

Regarding the conditions of the present study, the positive response of mineral fertilization with NPK from the lowest doses can be explained by the low presence of *P* and *K* and O.M. content and clay (Table 1) in the soil, with data found by Muniz et al. [17] who consider that the amount of NPK fertilizer directly influences the development of *Eucalyptus* change. According to Epstein and Bloom [18], the application of *P* is necessary to guarantee the production and the ideal quality of the plants, when still young and when the root system is not yet developed to explore larger soil volume. There is a response of K in the growth for *Eucalyptus* species [19] stressing that its application to the study soil was extremely important. Since one of the main sources of *N* in the soil comes from the O.M. contents, the application of this element may have been fundamental for the development of the planting in the study soil.

In treatments with natural phosphate (NP), a positive response of the averages above the control in BHD, height, and volume for both *E. benthamii* and *E. dunnii* can be observed at all applied doses. This may be related to the phosphorus (*P*) requirement that *Eucalyptus* has in the stand implementation phase, being the highest critical *P* level in the early development phase [3]. This response to *P* is likely to decrease for both species over time, decreasing the influence of nutrient on growth.

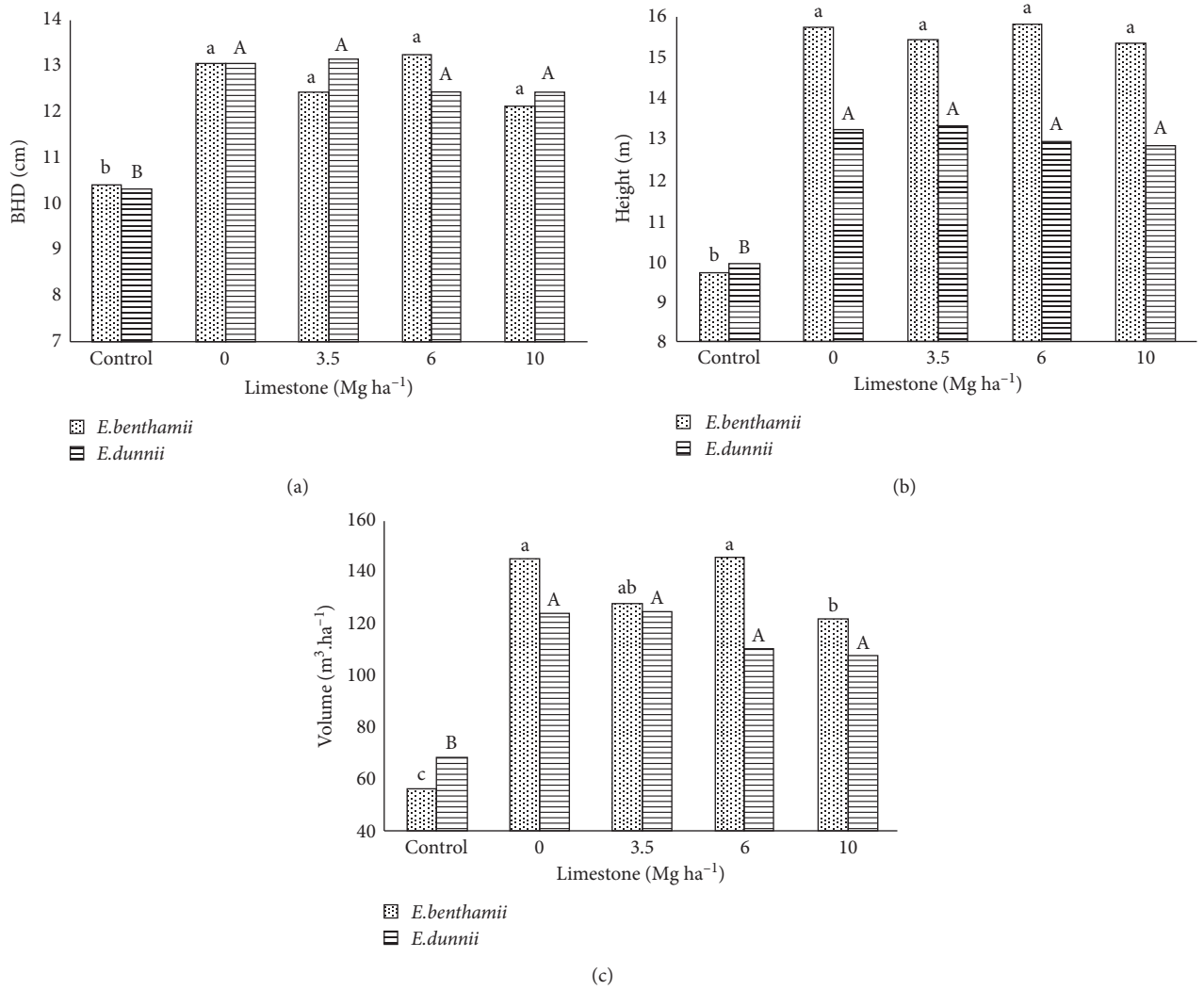


Figure 3: (a) Breast height diameter (BHD, cm), (b) height (m), and (c) volume (m³ ha^{-1}) in *Eucalyptus benthamii* and *Eucalyptus dunnii* in relation to liming. Averages followed by the same letter, lower and uppercase, do not differ between them by the Tukey test at 5% probability. Lowercase letters compare fertilization in relation to *E. benthamii*. Uppercase letters compare fertilization in relation to *E. dunnii*.

When comparing the species, *E. benthamii* stands out in height and volume, with no difference between the response for variable diameter. This behavior may be explained by the better response of *E. benthamii* to fertilization, while *E. dunnii* does not have such a strong but significant response.

Regarding liming, positive results were obtained at all dosages for *E. benthamii* and *E. dunnii* in relation to BHD and height, showing higher growth when compared to control, although the species is tolerant to soil acidity, as a function of low pH. However, the fact that the treatments presented higher averages than the control may be related to the Ca and Mg supply through liming, favoring the species development. Tucci et al. [20] observed a significant effect on the growth of forest species with liming from the application of 0.75 Mg ha^{-1}, also increasing Ca and Mg and decreasing Al content.

In the present study, there was also a positive relationship between the volume variable and liming. For both species, the treatments with limestone application were superior to the control, showing that liming positively affected the growth. In a similar study, Maeda and Bognola [21] obtained a liming effect on *E. dunnii* growth, increasing shoot dry matter production, seedling growth, accumulation, and *P* use efficiency in *Eucalyptus*.

E. benthamii presented the highest averages of height and volume in the comparative between species, reinforcing the assumption that the response to fertilization of the species is higher when compared to *E. dunnii*. It can be said that under the conditions of the present study, fertilization was of supreme importance in plant growth, demonstrating that with the application of nutrients in the soil, the species responded to growth. This response may be directly related to Ca and Mg supply through liming and increase of *N*, *P*, and *K* levels with NPK application and phosphate fertilization, factors that mainly contributed to the initial development and establishment of the plantation.

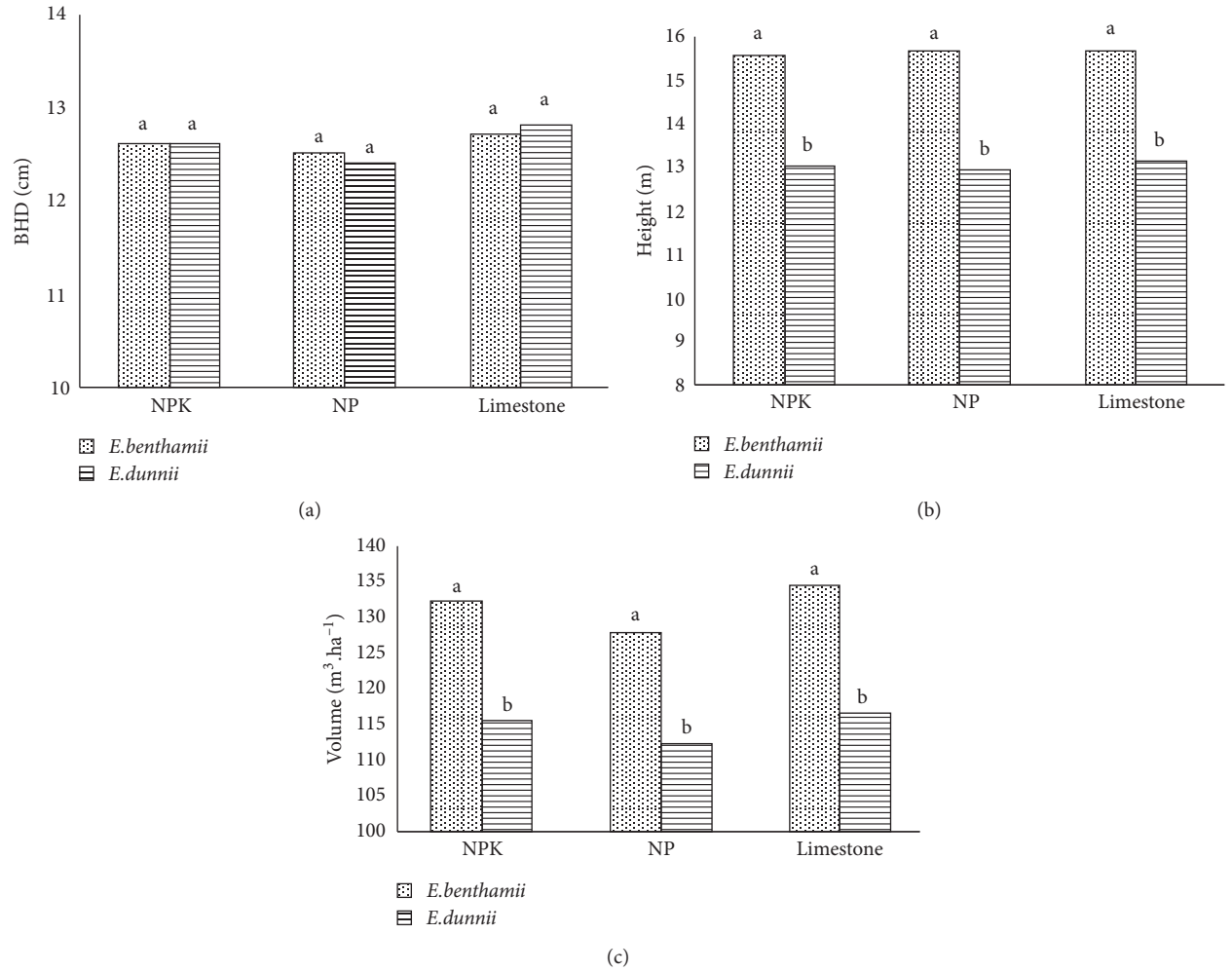


Figure 4: (a) Breast height diameter (BHD, cm), (b) height (m), and (c) volume ($m^3\ ha^{-1}$) in *Eucalyptus benthamii* and *Eucalyptus dunnii*. Averages followed by the same letter, lower and uppercase, do not differ between them by the Tukey test at 5% probability. Lowercase letters compare fertilization in relation to *E. benthamii*. Uppercase letters compare fertilization in relation to *E. dunnii*.

Maximum growth limiting doses were not found for the studied species, but unnecessary liming may affect the availability of other nutrients and cause restrictions on plant growth, also increasing production costs.

5. Conclusions

For NPK, the recommendation is 167 kg ha^{-1}, which presented the highest growth averages for all variables.

For NP, the recommendation is 600 kg ha^{-1}, which is the dosage that presented the growth peak of the study variables.

For limestone, the dose application recommendation is 3.5 Mg ha^{-1}, aiming to supply the Ca and Mg amounts in the soil.

E. benthamii has the higher demand for NP, NPK fertilization, and liming than *E. dunnii*.

Data Availability

The data used to support the findings of this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors acknowledge the University of the State of Santa Catarina (UDESC) for the institutional support for conducting the master's research in Forest Engineering by Diego Pereira da Rosa (first author of the article), with a

dissertation entitled "Calagem e adubação no crescimento de *Eucalyptus benthamii* Maiden et. Cambage e *Eucalyptus dunnii* Maiden." They would also like to acknowledge FAPESC for funding the research group Management of Planted and Natural Forests (PAP2019031000055) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for partial finance support, Brazil (Finance code 001). They also thank the company Souza Cruz®.

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MINI-CUTTINGS ROOTING OF *Sequoia sempervirens* AT DIFFERENT IBA CONCENTRATIONS AND CLONES

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Received for publication: 29/10/2018 - Accepted for publication: 21/10/2019

Resumo

Enraizamento de miniestacas de Sequoia sempervirens em diferentes clones e concentrações de AIB. Sequoia é uma espécie que proporciona grandes volumes de madeira, é resistente à decomposição e tem a produção de mudas por miniestaquia como uma alternativa viável. O objetivo do trabalho foi avaliar o enraizamento de miniestacas de diferentes clones de *Sequoia sempervirens* e posterior emprego de distintas concentrações de AIB em clones com baixo enraizamento adventício. Foram utilizadas miniestacas de diferentes clones, oriundas de minijardim clonal. No experimento com regulador de crescimento utilizaram-se diferentes concentrações de AIB (de 0 mg L^{-1} a 3000 mg L^{-1}). O material foi colocado para enraizar em estufim e avaliado aos 90 dias. Todos os experimentos foram instalados em delineamento inteiramente casualizado, com posterior avaliação da sobrevivência (%), miniestacas com calos (%), miniestacas enraizadas (%) e número de raízes (somente no experimento com diferentes concentrações de AIB). No primeiro experimento a sobrevivência das miniestacas foi alta (próximo a 95%) e o enraizamento apresentou grande variação entre os clones (22,8 a 100%). No segundo experimento, utilizando-se clones com enraizamento, anteriormente avaliado abaixo de 50%, submetidos à aplicação de AIB, observou-se alta sobrevivência (88,1 a 99,4%). Os clones apresentaram respostas variadas entre si, mas com melhora no enraizamento utilizando-se regulador de crescimento.

Palavras-chave: propagação vegetativa, regulador de crescimento, AIB, raízes.

Abstract

Sequoia is a species that provides large volumes of wood, is resistant to decomposition and has seedling production by mini-cutting as a viable alternative. The aim of this work was to evaluate the rooting of mini-cuttings of different clones of *Sequoia sempervirens* and subsequent use of different concentrations of IBA in clones with low adventitious rooting. Mini-cuttings of different clones were used, from clonal mini-garden. In the experiment with the growth regulator, different concentrations of IBA (from 0 mg L^{-1} to 3000 mg L^{-1}) were used. The material was placed to be rooted in a mini-tunnels and evaluate at 90 days. All experiments were installed in a completely randomized design, with a subsequent evaluation of survival (%), mini-cuttings with calluses (%), mini-cuttings rooted (%) and number of roots (only in the experiment with different concentrations of IBA). In the first experiment, the survival of mini-cuttings was high (close to 95%) and rooting showed great variation among the clones (22.8 to 100%). In the second experiment, using clones that previously presented rooting less than 50%, submitted to IBA application, high survival (88.1 to 99.4%) was observed. The clones presented different responses among themselves, but with improve in the rooting using growth regulator.

Keywords: vegetative propagation, growth regulator, IBA, root.

INTRODUCTION

Sequoia sempervirens (D. Don) Endl.), is a conifer native to the west coast of the state of California and the southwest coast of the state of Oregon in the United States, corresponds to a well-known species, mainly because it is one of the highest, reaching more than 100 meters in ideal conditions of growth. It is a species used when there is a need for wood resistant to decomposition (OLSON *et al*., 1990). The tree is highly valuable, not only for ornamental purposes but also for industrial purposes, due to its rather vigorous growth. The species is rarely attacked by disease or insects and is resistant to strong winds and other adverse weather conditions. Seed germination is highly variable, presenting many empty seeds and with malformed or infected embryos with several

parasites. Like most forest species, seed propagation is predominant for the species, although vegetative propagation through root shoots or lateral sprouts also occurs (KORBAN; SUL, 2007).

Vegetative propagation was used to produce sequoia seedlings (LUNA, 2008) by cuttings, but few large-scale that work in this sense have been reported in the literature (OLSON *et al*., 1990). For species that present seasonality in the production and storage difficulty of the seeds, mini-cutting is an alternative. By means of this technique, it is possible to form a clonal bank in a greenhouse, with a supply of diversified material for the production of seedlings aiming at the conservation of the species, besides allowing the selection of matrices with superior phenotypic characteristics for commercial production, as wood quality for the production of wood (OLIVEIRA *et al*., 2016).

The mini-cutting provides increases in rooting, in addition to favoring the development of a better quality root system for vigor, uniformity and volume, which positively reflects the survival and performance of the clone in the field (OLIVEIRA *et al*., 2012). However, it is important to know the factors that affect root formation, directly related to the success of cuttings production.

Some of the main factors involved in the rooting of mini-cutting are the hormonal balance, genetic constitution of the plant (potential and genetic variability within the species), endogenous level of inhibitors, nutritional conditions, propagation/youthfulness effect of propagule, season is collected, abiotic factors (temperature, light, humidity), substrate quality and the use of growth regulators. Regarding growth regulators, auxins were usually, considered as the main inducers of adventitious rooting, especially in difficult rooting species (DIAS *et al*., 2012). Among the most used, indole butyric acid (IBA) is used because of its efficiency, stability and lower toxicity over a wide concentration range. The sequoia cuttings respond favorably to the hormonal treatment (NAVROSKI *et al*., 2015). As the maturation effect of the donor plant of the species increases, the auxin concentrations increase (up to a certain limit) so that there is a maximization in the success in rooting (LUNA, 2008).

Considering that the clonal effect and the application of exogenous auxin present high importance in the clonal propagation, it was expected to find substantial differences between the tested clones, mainly due to the high number of genetic materials. For low-rooting clones without the use of growth regulator, it was expected that their use may increase root formation. In this context, we aim to evaluate the rooting of mini-cuttings of different clones *Sequoia sempervirens* and the subsequent use of different concentrations of IBA in clones with low adventitious rooting.

MATERIALS AND METHODS

As propagule sources for mini-cuttings were select from sequoia matrices (located in Sao Francisco de Paula, Rio Grande do Sul state, Brazil) of approximately 40 years of age, propagated by the conventional cutting process, 120 days after partial or complete annealing of the matrix tree. The material was collected from the parent trees and transported to the Forest Nursery located in Lages, Santa Catarina state, Brazil. After that, cuttings with a size of 10.0±2 cm of length and 0.7±2 cm of diameter containing a pair of acicular leaves reduced to 50% of the leaf area and treated with 6.000 mg L^{-1} of indole-3-butyric acid (IBA). Then, they were inserted in tubes (180 cm^{-3}) containing vermiculite of medium granulometry and commercial substrate (1:1 v/v) and arranged in a mini-tunnel for rooting. The mini-tunnel is a structure located under the shadehouse and with the following dimensions: 8.0 x 1.20 x 0.9 m (length x width x height).

After cutting, five-month-old rooted cuttings were acclimatizing in a shade house for 30 days, then transferred to greenhouse to complete the acclimation for another 30 days until it the seedlings reaches 20 cm in height. Subsequently, were transferred to pots with 5L vases filled with peat and pine bark-based commercial substrate (50%) and medium-sized vermiculite (50%). Fifteen days after the installation, the time necessary to adapt the seedlings to the system, the apex of the cuttings the main bud was cut at a height of 10 cm (± 2 cm), forming the mini-stumps for the establishment of the clonal mini-garden. Monthly, for four months, pruning of the mini-stumps was carried out.

The nutrient solution used in the clonal mini-garden was the base of the commercial fertilizer composed of 10% N (water soluble); 42% P_2O_5 (water soluble); 10% K_2O (water soluble); 0.6% Mg; 0.1% Fe and 0.02 Br. Fertigation was done twice a week, with each mini-stump receiving 50 ml of solution composed by diluting 1.5 grams of the fertilizer per liter of water. After the period of formation of the mini-stumps, the sprouts were collect to perform the experiments.

Mini-cutting of different clones and application of IBA

The first experiment consisted of the rooting of mini-cuttings of different clones kept in a clonal mini-garden system. Sixteen treatments (clones) were used: A100, A113, A115, A116, A117, A126, A127, A130, A131,

A133, A135, A136, A138, A140, A227 e A228). A completely randomized design was used, with 10 replicates of 10 stakes each. No growth regulator was used for this experiment.

With the clones that low rooting (<50%), a second experiment was realized, using different concentrations of IBA. The first factor was composed of 4 clones (A100, A117, A126 and A138) and the second factor was IBA concentrations (0, 1000, 2000 and 3000 mg L^{-1}). Eight replications of 10 mini-cuttings each used; the design was completely randomized in a two-factorial scheme.

Mini-cutting and growing conditions

From each clone, mini-cuttings were prepared whit 8 to 10 centimeters in length, the basal portion being bevel cut and the upper portion being incised transversely, a pair of a pair of acicular sheets being cut in half. In the first experiment, there was no use of growth regulator. In the second experiment, the cuttings were immersed in hydroalcoholic solution, with the base of the cuttings remaining in contact with the solution for a period of 15 seconds. After preparation, the mini-cuttings were arrange to be rooted in 180 cm^3 polypropylene tubes containing vermiculite of medium granulometry and commercial substrate (1: 1 v/v) with the addition of 6 g L^{-1} of Osmocote®, where it was made the insertion of approximately 2 cm of the base of the mini-cutting in the substrate.

The commercial substrate for plants, according to the manufacturer, is composed of peat, expanded vermiculite, pine bark and charcoal. The chemical and physical characteristics, after analysis of the substrate, are as follows: pH = 6.6; electrical conductivity = 0.13 $mScm^{-1}$; wet density = 450.8 kg m^{-3}; dry density = 302.7 kg m^{-3}; current humidity = 32.8 (%); total porosity = 72.2 (%); aeration space = 17.0 (%); readily available water = 12.2 (%); buffer water = 2.4 (%); remaining water = 40.5 (%); CRA10 - water holding capacity at 10 cm = 55.1; CRA50 = 42.9 and CRA100 = 40.5. The physical and chemical characterization of the substrate was Laboratory of Substrates of the Department of Horticulture and Forestry of the Federal University of Rio Grande do Sul.

The mini-cuttings were a condition in mini-tunnel (structure presenting 8.0 m long x 1.20 m wide x 0.9 m high). The temperature inside the mini-tunnel generally ranges between 20-32 °C and relative humidity of the air remains above 80%, and irrigation was performed by micro-sprinkler for 5 minutes, 5 times a day. The mini-cuttings remained in this condition for 90 days, at which time the evaluation of the experiment was performed.

Evaluated variables and statistical analysis

In both experiments, the percentage of survival, percentage of mini-cuttings that had calluses and percentage of rooted mini-cuttings were evaluated. In the experiment with different concentrations of growth regulator in low rooting clones, the number of roots was also evaluate, a parameter that was not analyzed in the previous experiment, since the seedlings from this experiment were destined to the field planting. The count of the number of roots implies in the disintegration of the substrate, causing serious damages to the roots of the cuttings. To evaluate the number of roots, the complete removal of the substrate, subsequent root washing and counting of all roots were performed visually.

Surviving mini-cuttings were those that had live wood, old leaves or young shoots, rooted or not. For calluses, the mini-cuttings were considered alive, with undifferentiated cell mass at the base, rooted or not. The percentage of rooting was considered on the total, not only on the surviving. It was considered as rooted mini-cuttings with induction of root origin of at least 2 mm in length.

After checking the normality of the data by means of the Kolmogorov-Smirnov and homogeneity by the Bartlett test, the analysis of variance was performed. When necessary, the data were transform by the function $(x + 0.5)^{0.5}$ and the means were compared by the Scott-Knott test, at 5% probability.

RESULTS

In the first experiment, evaluating the rooting of mini-cuttings of different clones, there was a significant difference between the three variables analyzed (Table 1). Mean survival between clones was high, almost 95%. Only four clones showed mini-cutting survival less than 90%. Regarding callus formation, with the exception of clones A116 and A140, all others presented more than 80%.

The rooting, the main variable observed when working with the mini-cutting technique, showed great variation among the clones. Some clones (A113, A115, A127 and A136) showed 100% or very close rooting. Half of the evaluated clones (eight) showed rooting indices between 66.7% and 87.8%, and only four of them (A100, A117, A126 and A138) presented rooting less than 50%.

For the four clones that obtained rooting less than 50% in the first experiment, after being submitted to IBA application, there was a significant effect of the clone factor for survival (%) and callus formation (Table 2). For clone root number and IBA concentrations, there was no significant effect on the three variables analyzed.

The survival of mini-cuttings varied between clones, ranging from 88.1% (A138) to 99.4% (A126). Callus formation was also high for most clones, remaining close to 90%, except for A117, which presented lower calogenic formation. The number of roots presented an average of 3.5, showing no significant difference between clones and concentrations of IBA used.

In relation to the rooting percentage, there was an interaction between the clones and the IBA concentrations. For all clones, the quadratic function presented a better adjustment (Figure 1). For clones A126, A138 and A100, rooting without growth regulator was less than 25%, with significant elevation when 1,000 mg L-1 of IBA was used. Clone A117 was the only one to present rooting near 40% without the use of IBA and to maintain rooting near this value with the use of growth regulator.

Tabela 1. Sobrevivência (%), formação de calos (%) e enraizamento (%) de miniestacas de *Sequoia sempervirens* obtidas de diferentes clones.

Table 1. Survival (%), callus formation (%) and rooting in the mini-cuttings of *Sequoia sempervirens* obtained from different clones.

Clone	Variable		
	Survival (%)	Callus (%)	Rooting (%)
A136	100.0 a*	95.2 a	98.1 a
A133	100.0 a	90.3 a	84.1 b
A131	100.0 a	100.0 a	66.7 b
A138	100.0 a	88.3 a	22.8 d
A100	100.0 a	87.9 a	36.9 c
A127	98.7 a	82.0 b	98.7 a
A115	98.4 a	100.0 a	95.4 a
A113	98.1 a	95.0 a	100.0 a
A227	95.6 a	91.0 a	87.8 b
A130	97.2 a	86.7 a	77.5 b
A116	96.4 a	75.0 b	84.2 b
A135	95.2 a	80.4 b	79.5 b
A117	88.7 b	98.0 a	48.7 c
A140	86.5 b	78.7 b	86.0 b
A228	86.4 b	85.1 b	86.4 b
A126	82.1 b	83.5 b	39.8 c
P-valor	<0.001	0.035	<0.001
CV%	19.2	24.4	22.1
Overall average	94.4	88.6	73.3

* Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test, 5% error probability.

Tabela 2. Sobrevivência (%), formação de calos (%) e número de raízes em miniestacas de S*equoia sempervirens* em função dos diferentes clones e concentrações de AIB.

Table 2. Survival (%), callus formation (%) and number of roots in *Sequoia sempervirens* mini-cuttings according to the different clones and IBA concentrations.

		Survival (%)	Callus (%)	Number of roots
CLONE	A100	92.1 b*	89.4 a*	3.8^{ns}
	A117	92.4 b	76.5 b	3.7
	A126	99.4 a	92.3 a	3.2
	A138	88.1 b	92.5 a	3.4
IBA (mg L^{-1})	0	94.2 ^{ns}	92.5 ^{ns}	2.9 ^{ns}
	1,000	92.7	84.0	3.3
	2,000	94.3	85.0	3.7
	3,000	91.7	85.0	3.8
	Média	93.0	87.2	3.5
	CV (%)	14.0	15.3	37.2

* Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test, 5% error probability. ^{ns} - Not significant at 5% probability of error.

For clones A126, A117 and A100, the concentration indicated by the fit of the equations is close to 1,500 mg L^{-1} of IBA. Only for clone A138 that the maximum response can be obtain with concentration, close to 2,000 mg L^{-1}. For all clones, the use of the highest concentration of IBA (2000 mg L^{-1}) presented similar results.

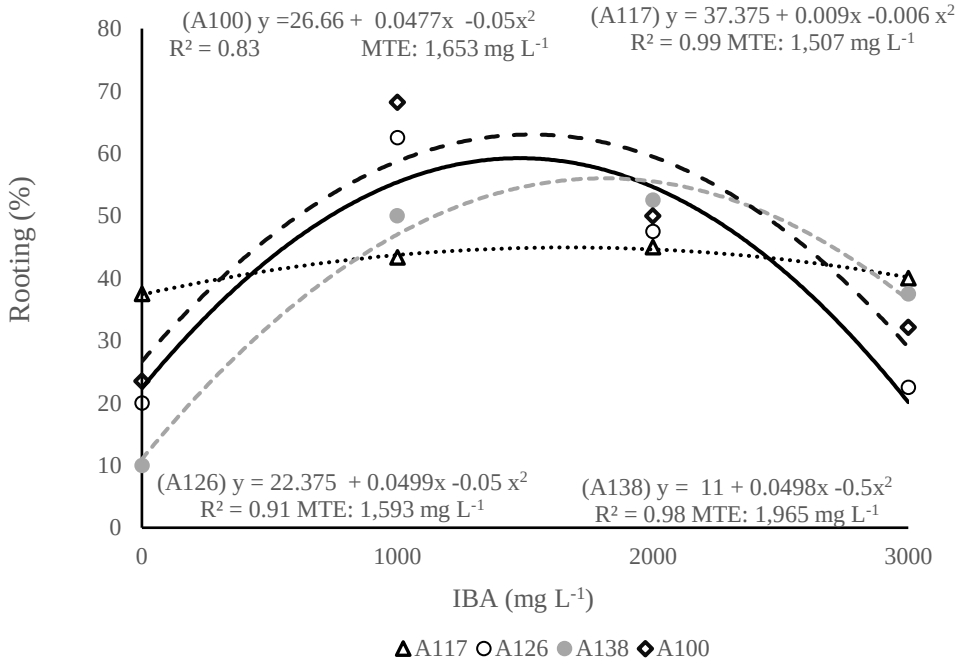


Figura 1. Enraizamento de miniestaquia de *Sequoia sempervirens* (Clones: A100, A117, A126 e A138) em diferentes concentrações de AIB.

Figure 1. Mini-cutting rooting of *Sequoia sempervirens* (Clones: A100, A117, A126 and A138) at different IBA concentrations.

DISCUSSION

The good survival results of the mini-cuttings for the 16 clones demonstrate an adequate control of temperature and, especially, humidity provided in the rooting environment (mini-tunnel) during the conduction of the experiment, and it is indispensable that it presents adequate conditions for the maintenance of mini-cuttings (STUEPP *et al*., 2013). In addition, the very ability of the mini-cuttings of the species to survive, resembling other vegetative propagation species such as *Eucalyptus* (BRONDANI *et al*., 2010).

Callus formation in sequoia mini-cuttings is common (NAVROSKI *et al*., 2015). Some clones present all of these with the presence of calogenic structures, some of which are less well formerd, such as clone A116 (75%). The roots of the sequoia mini-cuttings, as well as other genus of conifers (*Cryptomeria, Larix, Podocarpus, Picea* e *Taxodium*) usually have roots arising from external calogenic tissues (HARTMANN *et al*., 2011). According to the same authors, in some species the formation of callus is a precursor of the formation of adventitious roots. However, calogenesis has been reported as an obstacle to the rooting of propagules of various forest species (SINGH; ANSARI, 2014).

In relation to rooting, a high variation was find in the results, with good indexes for most clones, with only four of them with averages below 50%. The low rate of rooted mini-cuttings may be relate to the recalcitrance of these four clones. Thus, indirect selection may be unfavorable for growth or adaptive characteristics due to the propagation of specific genotypes (more likely to be mini-cutting), resulting in loss of clones in the selection process (OLIVEIRA *et al*., 2015).

For the great majority of the clones, the rooting indexes were superior to 80% and can be consider as very promising for the species, mainly because it is a material only reinvigorated by the technique of girdling in the field trees and one pruning in the clonal mini-garden. Work developed with *Sequoia sempervirens* by Luna (2008) showed inferior rooting to the present study, with values between 30 to 35%. Similar rooting (80%) was achieved only in the work of Navroski *et al*. (2015). All the works referenced made use of cuttings for the rooting, and no reports of works were done using clonal mini-garden, with later use of mini-cutting in the production of cuttings of the species.

This great difference in rooting between clones reveals possible genotypic effects for this trait. Dantas *et al.* (2016) comments that the genetic component is one of the factors that most influence the rooting, having a greater or lesser natural ability to form roots between the different clones.

In a study with *Eucalyptus cloeziana*, propagated by mini-cutting, there was the indication of an expressive genetic control in rooting (OLIVEIRA *et al.*, 2015). The same authors inferred that there were experimental rigor and good reliability in the prediction of genotypic values, with the possibility of identifying superior clones for adventitious rooting. Still according to these authors, the presence of considerable variation in rooting between clones indicates the possibility of practicing selection among sequoia clones propagated through mini-cutting, and it is possible to obtain significant genetic gains.

The lack of work related to the selection of superior genotypes for rooting in a forest improvement program is probably a consequence of the elimination of productive genotypes to other characteristics of interest. The selection of rooting of cuttings/mini-cuttings occurs naturally in nurseries, since recalcitrant genotypes/clones for rooting was discarded (OLIVEIRA *et al*., 2015), especially in species already cultivated. However, for species still in initial breeding trials, such as sequoia, the disposal of these less rooting materials should not be done, since clones with difficulty in being propagated by vegetative means may have good silviculture characteristics in the field, making them interesting long-term.

Concerning the application of IBA to clones with roots of less than 50%, observed in the first experiment, a difference was again observed between the clones for survival and callus formation in the mini-cuttings. In relation to rooting, the effect of the exogenous application of auxin on the four clones was observed. Without the use of IBA, three of the four clones showed low root formation (<25%). Already with the use of 1,000 mg L^{-1} of IBA there was increased in rooting, reaching almost 70% for clone A100.

Adventitious root formation is a demanding process of high energy, involving cell division, in which the predetermined cells move from their morphogenetic pathway to act as mother cells for the beginnings of the root; therefore, need hormonal stimuli for root initiation (GEHLOT *et al*., 2014). This efficiency for root induction depends on the presence of endogenous auxin levels, which may vary according to age, propagule position and genotype. When the responsible factor is the genotype or clone, one can make use of exogenous growth regulators, especially in cases of superior clones (WENDLING *et al*., 2014).

The rooting of cuttings/mini-cuttings was especially controlled by endogenous levels of plant regulators, mainly auxins (LI *et al*., 2009). As noted in some clones in the present experiment, exogenous auxin applications may increase rooting percentage due to its ability to act on plant tissues located near the excision region (ZHU *et al*., 2010), and may be related to concentrations and time of immersion (WENDLING *et al*., 2010). Therefore, the application of IBA acts as a metabolizing agent and signal for the induction of rooting. During external contact with the cell, IBA induces changes in the metabolism of enzymes, carbohydrates, RNA, DNA and proteins. These changes in the rooting region may inhibit or promote adventitious root regeneration, especially during cell division and differentiation (KOMATSU *et al*., 2011).

Generally, the concentrations considered optimal for root formation are very particular to each situation (BRONDANI *et al.*, 2010). This fact was observed in the present study, where the clones presented different behaviors regarding the need to use IBA (Figure 1). Almeida *et al.* (2007), testing concentrations of 0 to 6000 mg L^{-1} of IBA observed that some clones of *Eucalyptus cloeziana*, a species considered difficult to vegetative propagation, presented higher rhizogenic potential of mini-cuttings only when treated with the highest concentrations of the plant regulator. The application of plant regulators is subject to the physiological and genetic conditions of the matrix plant and may not result in a significant increase in rooting, as was observed for clone A117 in this study.

The application of auxin induces the rooting in species of conifers as *Pinus elliottii* var. *elliottii x P. caribaea* var. *hondurensis* (HUNT *et al*., 2011), *Pseudotsuga menziesii* (COPES; MANDEL, 2000) and *Pinus radiata* (RICCI *et al*., 2008), among others. In general, auxin may be more beneficial in species or clones difficult to root or with less predisposition to rooting (HUNT *et al*., 2011), as observed in the present study. However, in many cases, auxin does not provide an improvement in rooting (DIAS *et al.*, 2012), showing that some species have a natural ability to rooting mini-cuttings, not justifying the application of indolebutyric acid for the induction of adventitious roots.

Above 2,000 mg L^{-1}, the application of IBA caused a reduction in the rooting of the mini-cuttings. This is because the exogenous auxin has exceeded its maximum utilization point, from which point it becomes inhibitory (POP *et al.*, 2011). In this sense, Hartmann *et al*. (2011) states that when applying plant regulators, there is a stimulating effect on the induction of roots, but this stimulus reached a maximum point, from which it becomes inhibitory. A similar result, with a reduction in rooting from an ideal concentration, has also been report in *Sequoia sempervirens* (NAVROSKI *et al.*, 2015) and other species such as *Taxus wallichiana* (KAUL, 2008) and *Handroanthus heptaphyllus* (OLIVEIRA *et al*., 2015).

CONCLUSIONS

- There is wide variation in mini-cutting rooting among the sequoia clones evaluated.
- The survival and callus formation exhibit less variation, presenting an average of close to 90%.
- Clones that presented rooting less than 50% in the first experiment and were submitted to application with IBA reported varied responses, but with improved in the rooting.

ACKNOWLEDGMENTS

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship awarded.

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Ciência Rural, Santa Maria, v.50:6, e20190581, 2020
http://dx.doi.org/10.1590/0103-8478cr20190581
ISSNe 1678-4596
BIOLOGY

Morphological and physiological variation in *Toona ciliata* under water and salinity stress

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ABSTRACT: *The objective of this paper was to test the effects of water deficit, saturation and salinity in individuals of Toona ciliata. Seven treatments were tested: the control group, the complete suspension of irrigation, the permanent saturation of the pot soil and four doses of NaCl (50, 100, 200 and 400 µmol). The experimental design was completely randomized, with 8 repetitions in each treatment. Physiologic evaluations were carried out during the experiment, and morphologic evaluations at the end. The plants were responsive to the applied stresses comparatively to the control group. Reductions were observed in morphological and physiological variables in all treatments under stress. All individuals under stress recovered slightly in the end of the experiment, and began to sprout again, representing a probable adaptation to the conditions.*
Key words: *Toona ciliata, water saturation, water deficit, salinity.*

Variação morfológica e fisiológica em *Toona ciliata* sob efeito de estresse hídrico e salino

RESUMO: *O trabalho teve como objetivo mensurar os efeitos de déficit hídrico, saturação e efeito da salinidade em mudas de Toona ciliata. Foram testados sete tratamentos: controle, suspensão completa de irrigação, saturação permanente do solo no vaso e quatro diferentes doses de NaCl (50, 100, 200 e 400 µmol). O delineamento experimental utilizado foi inteiramente casualizado, com oito repetições. Foram realizadas avaliações fisiológicas ao longo do experimento e ao final, avaliações morfológicas. As plantas foram responsivas aos estresses aplicados comparativamente ao controle. Havendo redução em variáveis morfológicas e fisiológicas em todos os tratamentos sobre estresse. Todos os indivíduos sob estresse tiveram uma pequena recuperação ao final do experimento, iniciando a rebrotar, representando uma possível adaptação ao estresse.*
Palavras-chave: *Toona ciliata, saturação hídrica, déficit hídrico, salinidade.*

INTRODUCTION

Amongst the tree species used for commercial production of wood that were introduced in Brazil, *Toona ciliata* M. Roemer var. *australis*, commonly known as Australian red cedar has stood out recently, having some characteristics of economic importance, such as a short growth cycle, good wood productivity and high value in both domestic and foreign markets. Originally from the tropical regions of Australia, according to SOUZA et al. (2010) it has adapted well in Brazil, having reported favorable edaphoclimatic conditions for developing itself, its main advantage over the native cedar from Brazil being the absence of attacks by *Hypsipyla grandella*, a plague that attacks the apical bud of Meliaceae, causing the forking of the tree trunk. Its wood, of noble use, is indicated for fine furniture manufacturing and construction work (ALBINO et al., 2011).

According to MELOTTO et al. (2019), the environment conditions in the majority of states in Brazil are more advantageous for forest plantations than most wood producing countries, but even then the choice of which species to introduce depends on a variety of factors, such as adaptability to the local conditions, its susceptibility to local diseases and pests, the invasive potential of the species, use of quality seedlings and adequate planning of the products that will come from the plantation. However, choosing species that have tolerance of stresses such as droughts, flooding or salinity is also of high importance.

Hydric deficit causes morphologic, physiologic and nutritional alterations, which have influence in the seedling's capacity to resist adverse

Received 08.06.19 Approved 03.11.20 Returned by the author 04.24.20
CR-2019-0581.R2

conditions of its surroundings, and consequently, in its quality (LISAR et al., 2012). Water stress can be classified as water deficit, the lack of water in the soil, or as water saturation (overflow), the excess of water. The water deficit stress induces physiologic and biochemical alterations in plants; blocking cellular growth and photosynthesis, whilst altering respiration, those being the first effects to appear (VELÁZQUEZ & HERNÁNDEZ, 2013).

Overflow is a stress factor normally caused by flooding in plantations, it consisting in the total saturation of the soil pores by water. This layer can be superficial or deep, which can cause the partial or total submersion of the plants. Hydric saturation reduces the availability of nutrients, the diffusion of O_2 and cellular respiration, influencing the plant's hydric relations and impairing the biomass gain (NAJEEB et al., 2015). In plants, the excess of salt induces a stress, constituting a limiting factor to the distribution and productivity of the species (YILDIRIM et al., 2009).

Saline stress can cause low osmotic potentials and high cation concentrations, among other alterations that include compromising the opening and closing of stomate, slowing the growth of leaves and biomass production and lowering the relative water content, turgor pressure and hydric cellular potential (TOPPA & BRAMBILLA, 2011).

Considering the Australian red cedar's potential in the forestry industry, and the importance of knowing the effects of different stresses in the development of plants, this present research aimed to evaluate the effects of hydric deficit, saturation and salinity in seedlings of *Toona ciliata.* The expected response from the individuals under stress was a reduction in the photosynthetic rate and lighter mass at the end of the experiment.

MATERIALS AND METHODS

The experiment was developed during October 2018 and February 2019, in the plant nursery of the University of the state of Santa Catarina, located in Lages, Santa Catarina, Brazil, whose coordinates are 27°47'33"S e 50°18'4"W, it has an approximate altitude of 900 meters. According to the Köppen classification, the climate of the city of Lages/SC is subtropical highland with uniform rainfall (Cfb-temperate). The annual average temperature is 15° C, and the annual average precipitation fluctuates between 1300 and 1500 mm. The temperature and relative air humidity during the experiment were 26 °C and 76,9%, respectively. These values were collected inside the greenhouse, with a datalogger, gathering information every 10 minutes.

Toona ciliata seedlings, at around two months of age, were manufactured in the university's plant nursery. The sowing was made inside tubes of 180 cm^3, containing a commercial substrate based in *Pinus* bark, with addition of 6 g L^{-1} of Osmocote® fertilizer, in 19-6-10 (NPK) formulation, characterized by its slow release of 4 to 5 months. The seedlings were kept in the nursery for approximately 120 days and then moved to a greenhouse, and kept there for 30 more days. As soon as the seedlings reached an average height of 25 cm (October 2018), they were transferred to vases with 10 L capacity. The vases were filled with soil from the region, classified as Cambissolo Húmico alumínico argiloso (Inceptisol). The seedlings received the conventional irrigation from the greenhouse (approximately 8 daily mm) for 40 days. After this period the induction of the stress treatments began.

The experimental design was randomized; seven treatments were tested, each having eight repetitions, constituted by the plant in the vase. The treatments were as follows: a control group; suspension of irrigation (hydric deficit); saturation of the soil and application of the saline stress, in four different concentrations of NaCl (50, 100, 200 and 400 µmol L^{-1}).

The plants under hydric deficit were allocated inside the greenhouse, without any irrigation, their vases were covered with plastic bags, to avoid accidental irrigation. For the saturation of the soil, the holes in the bottom of the vases were covered, and then they were filled with water all the way to the border, being monitored daily and irrigated in case of the water level lowering. The saline stress was inducted with the NaCl concentration (50, 100, 200 and 400 µmol L^{-1}) diluted in water. The solution was applied in the day before each photosynthesis measurement. These treatments were also removed from irrigation, being the saline solution, the only irrigation used. The control group was kept in the area, under conventional irrigation of 8 mm per day.

The biometric evaluations of height and stem diameter were realized in two different times, the first in the day of the stresses implementation and the second in the end of the experimental period, 36 days after. The data from those evaluations were utilized to obtain the height increment (IH), stem diameter increment (IDAC) and height/stem diameter ratio (h/d). Thirty days after the beginning of the experiment, a visual analysis of symptoms due to stress was made, grades ranging from 1 to 5

were given to each plant, the grades were as follows: 1) normal; 2) spots in the leaves; 3) winding of the leaves 4) both previous symptoms in one plant and 5) leaf shedding.

Five plants from each treatment were selected for photosynthesis measuring, realized with a portable photosynthesis system IRGA (Infra-Red Gas Analyzer), model LI-6400 (LI-COR). Five analysis were made, 3, 9, 16, 23 e 33 days after the induction of the stresses. Measurements were made between 8 and 10 AM in each day, keeping the randomness of treatments.

The following variables were measured with the IRGA: liquid photosynthetic rate (A - µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (Gs - mol m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}), intercellular concentration of CO_2 in the mesophyll (Ci - µmol CO_2) and external CO_2 concentration (Ca - µmol CO_2). With those variables, the ratio of intercellular/external concentration of CO_2 (Ci/Ca- µmol CO_2) and the water use efficiency of the plant (WUE – [µmol m^{-2} s^{-1}]$^{-1}$) were estimated.

At the end of the experimental period, the dry mass of the plants was manually collected, by the destructive method. Samples were divided in root and shoot, which were then put inside paper bags, and kept inside a drying oven with forced air circulation (60 ± 3 °C) until reaching constant mass (grams) after 96 hours. After, they were weighed in a precision scale (0,01 g) in order to find out the ratio of dry mass in each plant. The Dickson quality index (IQD) was also estimated.

The normality of the data was ascertained with the Shapiro-Wilk test as was the homogeneity of variances with Bartlett's test. They were then submitted to an analysis of variance (ANOVA). And with significant F values ($p<0,05$), Tukey's range test ($p < 0,05$) was used to find the differences of means.

RESULTS

In relation to the morphologic variables (IH, IDAC, h/d ratio, visual analysis, dry mass and IQD) measured during the experiment, all of them had significant differences (p-value<0.05) in the different treatments applied to the Australian red cedar plants, with the exception of h/d ratio (Table 1).

Amongst the treatments, the tallest average of height increment was from the control group while the worst was the saline stress of 50 µmol of NaCl. The control group was the only treatment that was significantly different from the others. The control group was also the one with highest value in stem diameter increment, and the water deficit treatment had the worst average, in this variable, the control group was also the only to be significantly different than the rest. In the height/stem diameter ratio, there was no difference among the treatments.

In the visual analysis the control group had the best results, with an average grade of 1.0 (the smallest average being better). Both water saturation and deficit had the worst results, their averages both being 4.6. The control group was not statistically different from the 50 and 100 µmol NaCl doses, which themselves didn't differ from the 200 and 400 µmol NaCl doses, the water saturation and deficit.

In relation to each treatment's dry mass, the highest average of shoot dry mass (MSA) belonged to

Table 1 - Morphologic variables obtained in the evaluation of *Toona ciliata* seedlings, according to the different stresses.

Treatment	----------Variables----------							
	IH (cm)	IDAC (mm)	h/d	AV	MSA (g)	MSR (g)	MST (g)	IQD
Control Group	14.7a*	3.64a	4.98ns	1a	8.62a	8.61a	17.2a	2.93a
Saturation	3.12b	1.38b	4.81	4.6b	2.83b	1.3b	4.13b	0.6b
Hydric deficit	3.04b	0.84b	5.49	4.6b	3.14b	1.88b	5.02b	0.69b
50 µmol NaCl	2.94b	1.61b	4.75	3.2ab	4.04b	2.62b	6.65b	1.06b
100 µmol NaCl	4.8b	1.58b	4.78	3ab	3.46b	2.78b	6.25b	1.05b
200 µmol NaCl	5.22b	1.04b	5.17	3.4b	3.4b	2.71b	6.11b	0.94b
400 µmol NaCl	3.34b	1.35b	4.75	2.6ab	2.88b	2.17b	5.04b	0.84b

*Equal letters don't differ between treatments according to Tukey test at 5% probability. ns - no significant effect. Where: IH: Height increment; IDAC: Stem diameter increment; h/d: Height/diameter ratio; AV: Visual analysis of seedlings; MSA: Shoot dry mass; MSR: Root dry mass; MST: Total dry mass; IQD: Dickson quality index.

the control group, it being different to all the other treatments. The same result happened in root dry mass (MSR), total dry mass (MST) and Dickson quality index (IQD).

In the physiological variables (photosynthetic rate, stomatal conductance, Ci/Ca ratio, transpiration and water use efficiency), there was difference (p-value <0.05) between all stress treatments from the second evaluation onwards, 9 days after the first. The great effect of the stress in the saturation treatment can be observed in the behavior of the photosynthetic rate (Figure 1a). In the first evaluation (day 3 of the experiment) it was already possible to notice a reduction in its photosynthetic rate. In the ninth day a big difference among the treatments can be seen, with the control group maintaining similar values to the first analysis, while the majority of other treatments began to show stress signals, highlighting the hydric saturation and deficit treatments.

The third and fourth evaluations (16 and 23 days after the start respectively) presented a similar behavior, in which the control group kept its photosynthesis values close to the first two, while the others continued to decrease. In the last evaluation (day 33 of the experiment), the control group was once again the one with highest values. The saline stress treatments with 50, 100 and 200 µmol NaCl, recovered their photosynthetic rate from the previous analysis (23 days).

In the first evaluation, all treatments were similar for stomatal conductance (Figure 1b). In the ninth day, the stress signals began to appear in some treatments, the control group had the best conductance, not differing from the 50 and 100 µmol NaCl treatments. The water saturation treatment had the worst conductance values. The last three evaluations, in days 16, 23 and 33, had similar behavior, the control group staying on top and now differing from all others. In the last evaluation all treatments except the water saturation recovered the conductance slightly.

In the first evaluation for Ci/Ca ratio, all treatments were statistically similar (Figure 1c). In the second, the hydric deficit treatment began to drop, showing the worst ratio and being the only one to differ significantly from the best (control group). In the evaluation from day 16, the control group kept the best Ci/Ca ratio, maintaining that in all later analysis. All treatments recovered the ratio in the last analysis.

In day 3 of the experiment, all treatments were close in transpiration. With 9 days, it was possible to notice significant differences, with the saturation treatment having the worst value (Figure 1d). The last three evaluations had similar behavior, the control group in all three had the best transpiration, both saturation and saline dose of 100 µmol NaCl had significant recovery in the last analysis.

The first evaluation had similar values for all treatments in water use efficiency (Figure 1e). The control group had stable behavior in *WUE* during all analysis. Both water and saline stress treatments elevated the most in the intermediate evaluations (days 16 and 23) and fell back to values close to the ones from the beginning after a month passed from the application of the stresses.

DISCUSSION

The application of the stresses resulted in contrasting effects in all cases, hydric deficit being the one who yielded the biggest symptoms, exemplified by the small increment in height, smaller dry mass and IQD value, and worst grade in visual analysis. In the physiological variables, the effect in this treatment was also fast and easily seen, its photosynthetic rate among the smallest right from the beginning, decreasing even more during the experiment.

This effect in the seedlings is a consequence of the limitation in the carbon assimilation from the photosynthesis. A detail that can be highlighted from the treatment was the precocious loss of leaves, which is an eventual response to the hydric deficit (MAFAKHERI et al., 2010). Hydric stress usually affects the leaf area and leaf mass (VON ARX et al., 2012). The water deficit promoted smaller biomass production in all parts of the plant; however, it also had bigger water use efficiency, because with the reduction in leaf area there was a lesser loss of water, therefore the carbon/transpiration ratio was positive (CERNUSAK et al., 2011).

In the same way, the effects of the stress were fast in the soil saturation treatment, it, alongside with the deficit treatment being the treatments with most symptoms, and in general the more intense effects. Its photosynthetic rate was the smaller among the treatments from the start, the stress effects noticeable from the beginning, maintaining the smallest value until the end of the experiment. Its stomatal conductance, Ci/Ca ratio, and transpiration had similar behaviors, starting the experiment among the smaller values, significantly reducing until the middle and recovering in the end of it.

Saturated soils inhibit root growth, leaf area expansion, and photosynthesis because of the lack of oxygen and cooler soil temperatures. Yellow

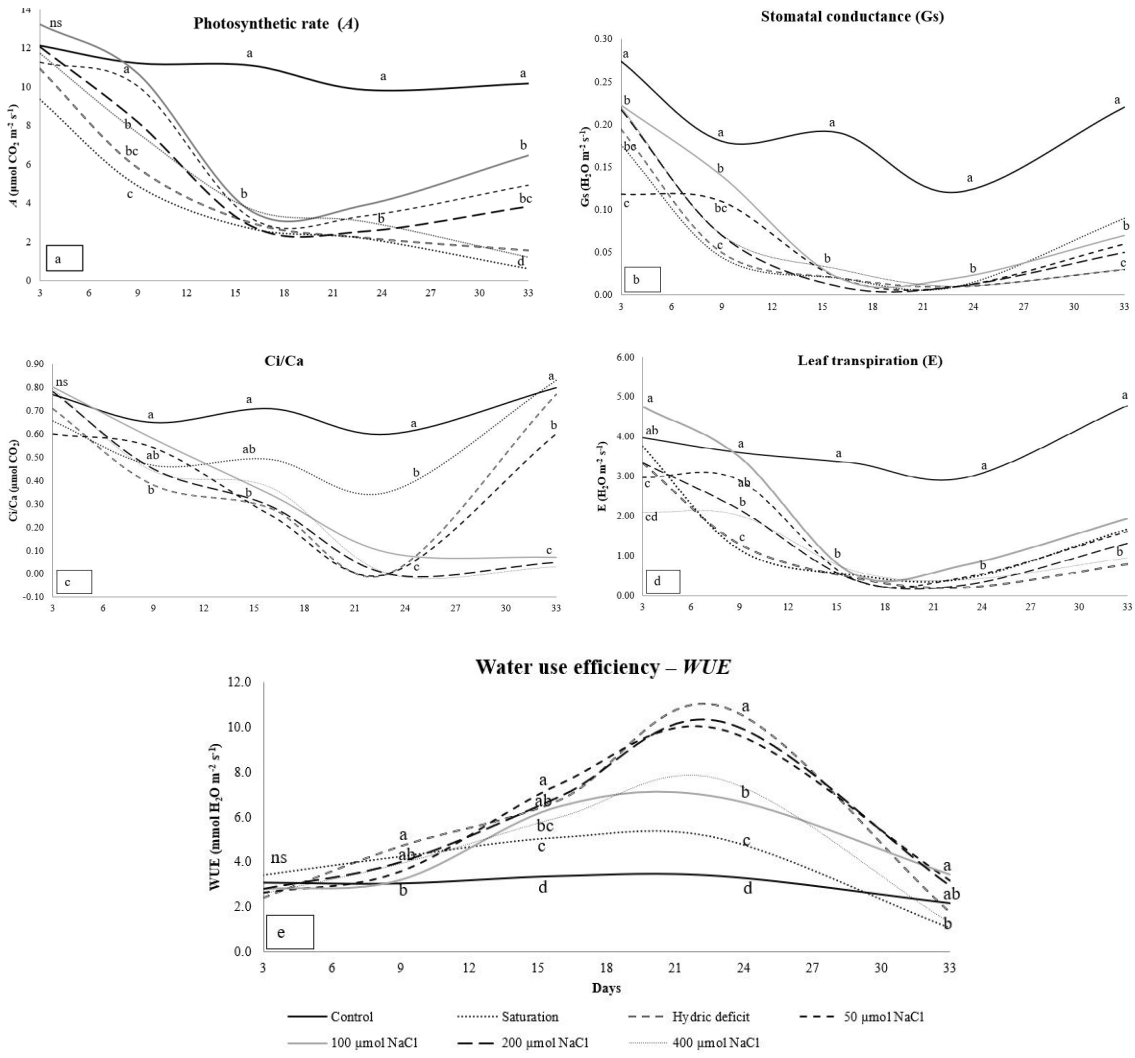


Figure 1 - a) Photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), b) Stomatal conductance (H_2O m^{-2} s^{-1}), c) Ci/Ca ratio - Ci/Ca (Intercellular CO_2 concentration/external CO_2 concentration ratio in μmol CO_2), d) Leaf transpiration (H_2O m^{-2} s^{-1}), e) Water use efficiency – *WUE* (mmol H_2O m^{-2} s^{-1}) in *Toona ciliata* seedlings according to the applied stresses during the experiment (between days 3 and 33). Letters correspond to differences between treatment groups by Tukey test.

leaves indicated a slowing of photosynthesis and plant growth. Leaves and sheaths may turn purple from accumulation of sugars if photosynthesis continues but growth is slowed (BASU et al., 2016).

Although, root growth can compensate to some extent later in the season, a saturated profile early in the season can confine the root system to the top several inches of soil, setting up problems later in the season if the root system remains shallow. Plants with shallow root systems also become more susceptible to stand ability problems during periods of high winds (BASU et al., 2016).

In these cases, abscisic acid, ethylene and its precursors can form in large quantities, which would cause the closing of stomata in leaves, a hyponastic response and frequently leaf abscission. Insuccession, the membrane cellular system degenerates, mitochondria are disintegrated and their enzymes are

partially inhibited (LARCHER et al., 2016), decreasing the growth and development of plants.

The effects of stress were also notable in the saline stress treatments, in less quantity than both hydric ones however, indicating the species bigger resistance to this type of stress. According to FATEMI et al. (2019), responses in gene expression of related genes may lead to mechanisms of ionic and osmotic homeostasis, growth control and repair of cell damage. Taken together, these responses may result in the plant's tolerance or sensitivity to stress.

The treatments under stress had similar behavior during the evaluation of the physiological variables, bottoming (peaking in the case of water use efficiency) around the 21st day, and recovering afterward, in some cases ending the experiment close to the control group again, showings a possible adaptation to the conditions they were under. Contributing to this, is the fact that during the recover, some individuals started to sprout again.

CONCLUSION

Observing the analysis results, it can be noticed that the *Toona ciliata* seedlings are susceptible to all three stress types that were applied. Plants under the effect of hydric deficit and saturation were affected earlier and in a more expressive way, indicating that the species might suffer more in flooded soils, or in cases with rain deficit. In the case of hydric saturation, the symptoms were faster to appear. As for the saline stress, the symptoms were milder and slower to appear, which might indicate the species has a certain capacity to adapt to saline soils.

ACKNOWLEDGEMENTS

The authors thank the Universidade do Estado de Santa Catarina (UDESC), for the institutional support for this research. To FAPESC for funding the research group Management of Planted and Natural Forests (PAP2019031000055) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for partial finance support, Brasil - Finance code 001.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript. All authors critically revised the manuscript and approved of the final version.

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SCIENTIA FORESTALIS

ISSN 2318-1222 (online)

ORIGINAL ARTICLE

Rooting cuttings of Ilex paraguariensis native to southern Brazil according to mother tree genotype, rooting environment and IBA use

Estaquia de Ilex paraguariensis nativa do Sul do Brasil de acordo com o genótipo da planta matriz, ambiente de enraizamento e uso de AIB

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How to cite: Nascimento, B., Sá, A. C. S., Moraes, C., Santos, J. C. P., Pereira, M. O., & Navroski, M. C. (2020). Rooting cuttings of *Ilex paraguariensis* native to southern Brazil according to mother tree genotype, rooting environment and IBA use. *Scientia Forestalis*, *48*(128), e3087. https://doi.org/10.18671/scifor.v48n128.24

Abstract

Vegetative propagation is an effective tool for the establishment of plants with superior characteristics. Therefore, it is necessary to test the rooting of each genotype in relation to the environment and observe the response of the species to the use of growth regulators. The objective of this search was to evaluate the rooting of *Ilex paraguariensis* cuttings according to genotypes in two environments, and test doses of IBA. The cuttings were obtained in Urupema, Santa Catarina. Mother trees 15 years old on average, were selected randomly, and canopy shoots were collected and transported to UDESC, in Lages. The shoots were sectioned into cuttings and put to root according to the experiments: I) Six genotypes were conditioned in two environments: a mini tunnel system and a greenhouse with intermittent nebulization (GWIN) with temperature control, II) In a mini tunnel with IBA concentrations: (mg L^{-1}): 0, 2,500, 5,000, 7,500 and 10,000. Survival (%), callus (%), rooting (%), number and mean root length (cm) were evaluated. Survival was superior in mini tunnels (>20.0%), but its rooting percentages were lower (35.0%) than the GWIN (>50.0%). Genotype G6 was higher in all variables, with survival (>70.0%) and rooting (>30.0%). The highest IBA dose reached twice as much rooting as the control, but it was still low (15.0%). The correct selection of the genotype and environment are crucial for the successful rooting of *I. paraguariensis* cuttings. In addition, rooting seems to be more associated to the time factor than to the use of IBA itself.

Keywords: Cuttings; Growth regulator; Mate tree; Micro sprinkler; Nebulization.

Resumo

A propagação vegetativa é uma ferramenta efetiva no estabelecimento de plantas com características superiores. Para tanto, é necessário testar o enraizamento de genótipos em relação ao ambiente e observar a resposta da espécie ao uso de reguladores de crescimento. O objetivo deste trabalho foi avaliar o enraizamento de genótipos de *Ilex paraguariensis* em dois ambientes e testar doses de AIB. Os propágulos foram obtidos em Urupema, Santa Catarina. Com média de 15 anos de idade, as árvores matrizes foram selecionadas aleatoriamente, tendo as brotações de suas copas coletadas e transportadas para a UDESC, em Lages. As brotações foram seccionadas em estacas e postas para enraizar de acordo com os experimentos: I) Seis genótipos acondicionados em dois ambientes, sendo um estufim e outro uma casa de vegetação com nebulização intermitente e controle de temperatura, II) Em estufim sob as doses de AIB: (mg L^{-1}): 0, 2.500, 5.000, 7.500 e 10.000. Foram avaliados: sobrevivência (%), calos (%), enraizamento (%), número e comprimento médio (cm) de raízes. Sobrevivência foi maior

Financial support: FAPESC - grupo de pesquisa Gestão de Recursos Florestais (PAP2019031000055), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) código 001.
Conflict of interest: Nothing to declare.
Corresponding author: b.nascimento_92@hotmail.com
Received: 20 September 2018.
Accepted: 26 November 2019.
Editor: Paulo Henrique Müller Silva.

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em estufim (>20,0%), mas o enraizamento foi menor (35,0%) que na casa de vegetação (>50,0%). O genótipo G6 foi superior em todas as variáveis, principalmente quanto sobrevivência (>70,0%) e enraizamento (>30,0%). A maior dose de AIB atingiu o dobro de enraizamento da não aplicação, mas ainda considerado baixo (15,0%). A seleção correta do genótipo e ambiente são cruciais no sucesso do enraizamento de estacas de *I. paraguariensis*. Ainda, o enraizamento aparenta estar mais associado ao fator tempo do que o uso de AIB.

Palavras-chave: Estaquia; Regulador de crescimento; Erva-mate; Microaspersão; Nebulização.

INTRODUCTION

The use of the sexual route in breeding is important because it ensures greater genetic diversity and provides that certain desirable characteristics of the mother tree are inherited (Xavier et al., 2013). However, in addition to not having a standardization of the seedlings, there may be a low number of seeds produced and associated with the possibility of dormancy, long periods of germination and high genetic heterogeneity are caused by sexual reproduction (Xavier et al., 2013; Wendling & Brondani, 2015).

Vegetative propagation can solve some of these problems by using genetically superior mother trees, with cuttings being one of the most used techniques for this purpose (Wendling & Brondani, 2015). Certain advantages of this technique are: direct transmission of desirable characteristics, homogenization of the plants produced and possibility of continuous production throughout the year (Dias et al., 2012). The main factor that guarantees the success of this technique lies in the adventitious rooting of the cuttings produced (Hartmann et al., 2011). Therefore, some characteristics should be taken into account: the genotype used (Wendling & Dutra, 2010), the rooting environment (Pires et al., 2013) and the use of growth regulators (Hartmann et al., 2011).

One of the most important factors for success in rooting is genetics when different genotypes of the same species respond differently to cutting rooting (Franzon et al., 2010). Environments with nebulization irrigation favour the survival of cuttings and their rooting, mainly by keeping the leaves functional for longer (Wendling & Brondani, 2015). An environment with high and constant humidity is essential for maintaining the survival of cuttings (Xavier et al., 2013). The use of growth regulators in cuttings, such as IBA, increase auxin levels in the basal cuttings, helping in the root formation by cell differentiation (Hartmann et al., 2011).

Ilex paraguariensis Saint Hilaire, Aquifoliaceae family, also known as mate, is a characteristic species with a strong cultural link in Southern Brazil, and has a wide potential of use, from consumption in the less industrialized form, such as teas, to the pharmaceutical and cosmetic industry (Dartora et al., 2013). Like other crops, *I. paraguariensis* plantations should be constituted by superior plants, selecting seedlings of productive characteristics of interest. Although it is the best technique for selection in genetic improvement, the vegetative propagation of this species does not yet have efficient protocols for the rooting of cuttings, with capacity of application on a large commercial scale (Wendling & Brondani, 2015).

There are still few studies on *I. paraguariensis* improvement through the use of vegetative propagation and selection of superior mother trees (Wendling & Brondani, 2015). Both the survival of cuttings and their rooting are dependent on their genetic characteristics (Brondani et al., 2008). In searches done, there was no evidence of differences in rooting of cuttings between environments with a greater control of variables, such as temperature and irrigation, in relation to environments with lower control (Brondani et al., 2008, 2009). Currently, there are no precise recommendations for IBA dosage on cuttings of this species, and high concentrations (above 5,000 mg L^{-1}) are always recommended (Wendling & Brondani, 2015).

Therefore, the objective of this study was to observe the development of six genotypes of *I. paraguariensis* concerning the rooting of cuttings under two distinct environments: one with greater temperature and humidity control, and another simpler and of lower cost, also to evaluate the influence of different concentrations of IBA on the rooting of cuttings of this species, related to the time factor.

MATERIAL AND METHODS

Characteristics of the area of study

The field experiment was conducted in an experimental area in the municipality of Urupema, in Santa Catarina. The study area is located approximately 1,400 meters above sea level, at the coordinates are 28°17'38" S, 49°55'54" W.

This region has a humid temperate climate (Cfb), with annual mean precipitation of 1.650 mm. It is characteristic of the region that during winter there can be snow and minimum temperatures of up to -14 °C, considered to be the coldest in Brazil.

Cutting of different genotypes of *I. paraguariensis* in two rooting environments

In August 2017 six *I. paraguariensis* mother trees at least 20 m apart from each other, with an average diameter of 8.0 cm, were selected for collecting shoots from the canopy.

The shoots were collected, arranged in a Styrofoam box with water to avoid moisture loss and transported to the Forest Nursery of the State University of Santa Catarina (UDESC), in the municipality of Lages. There shoots were sectioned into cuttings of approximately 7 cm in length and having at least one leaf with 50% of its area removed.

The cuttings were put to root in tubes of 110 cm^3 with a mix of commercial substrate (mixture of *Pinus* bark and peat, pH 5.4) and thin vermiculite in the ratio 1:1, with the addition of 6 g L^{-1} slow release fertilizer (Osmocote® 15-9-12).

The experiment was conducted in a completely randomized design with 6x2 factorial arrangement, with factor A consisting of the six genotypes, composed of eight replicates of six cuttings each, and B the 2 rooting environments.

The trays containing the tubes and cuttings were conditioned in two different rooting environments (factor B), as:

I- In a mini tunnel, a simplified structure, inserted in a shade house, located in the Forest Nursery of UDESC, in Lages, Santa Catarina state, with automatic irrigation by micro sprinklers composed with four daily irrigations of five minutes each. According to the classification of Köppen, the region has a warm and temperate climate (Cfb), with average temperature of 16.6 °C and average annual precipitation of 1,441 mm.

II– In a greenhouse with intermittent nebulization (GWIN) of a forest company, in the municipality of Otacílio Costa, Santa Catarina state, with automatic temperature control (between 20 °C and 30 °C) by thermostat and humidity (always above 80.0%). Similar to the climate of Lages, Otacílio Costa has a warm and temperate climate (Cfb) according to the Köppen classification, with an average temperature of 16.3 °C and average precipitation of 1,519 mm.

The temperature and humidity data from both sites were obtained from a data logger, giving a reading every ten minutes (Figure 1).

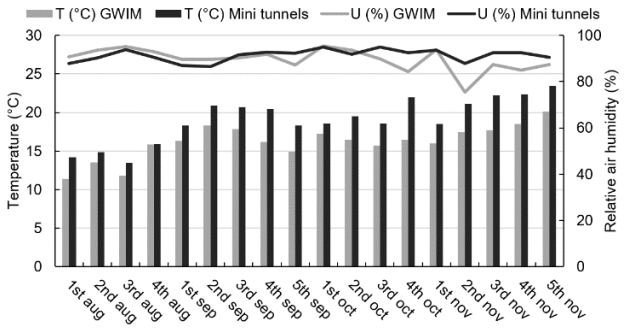


Figure 1. Weekly data of average temperature (°C) and relative air humidity (%) of the cutting experiment according of *I. paraguariensis* mother tree genotypes, for mini tunnels (Greenhouse) in Lages and greenhouse with intermittent misting (GWIN) in Otacílio Costa.

After 130 days (November 2017) survival (%), presence of new sprouts (%), average number of new leaves, callus formation (%), rooting (%), average number of roots and, average length of the three longest roots (cm) were evaluated.

IBA use in *I. paraguariensis* cuttings

Canopy sprouts from *I. paraguariensis* were randomly collected from ten mother trees in the study area in April 2017, under the same shading condition. We collected material from several mother trees to guarantee the genetic variability of the cuttings to be produced. The sprouts were arranged in a Styrofoam box with water to reduce transpiration and transported to the Forest Nursery of UDESC, in the municipality of Lages.

The sprouts were sectioned into cuttings, cut with pruning shears to a length of approximately 7 cm, with at least one leaf having removed 50% of its area.

The IBA solutions were prepared in hydro-alcoholic medium (50% ethanol) at concentrations: 0 mg L^{-1}, 2,500 mg L^{-1}, 5,000 mg L^{-1}, 7,500 mg L^{-1}, 10,000 mg L^{-1}. Each cutting went through a 15-second immersion of its base, approximately 3 cm, in the respective IBA solution.

After the immersion, the cuttings were put to root in tubes of 180 cm^3 with a mix of commercial substrate (mixture of *Pinus* bark and peat, pH 5.4) and thin vermiculite in the ratio 1:1, with addition of 6 g L^{-1} slow release fertilizer (Osmocote® 15-9-12). The trays, containing the tubes and cuttings, were placed into a mini tunnel, with micro sprinkler irrigation composed of four daily irrigations of five minutes each (Figure 2).

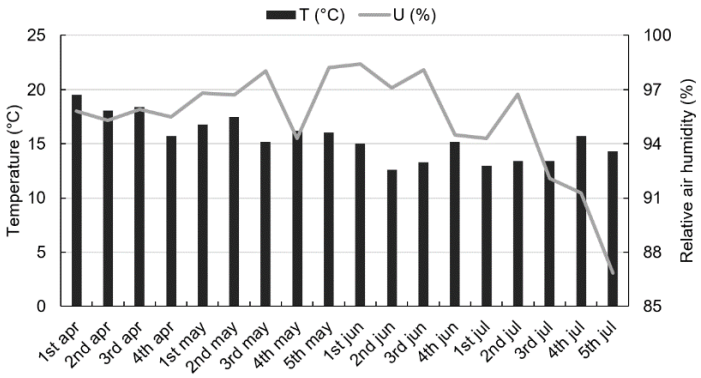


Figure 2. Weekly data of average temperature (°C) and relative air humidity (%) of the cutting experiment of *I. paraguariensis* using IBA, at the UDESC forest nursery in Lages.

After 50 days, the evaluation was started, occurring every 20 days until completing 130 days (five evaluations), for the following variables: survival (%), callus formation (%), rooting (%), mean number of roots and, average length of the three longest roots (cm). The cuttings evaluated at one time were no longer used in the later evaluations to avoid interference from their manipulation.

The experiment was carried out in a completely randomized design in a 5x5 factorial scheme, with factor A consisting of five treatments of IBA doses, and factor B being the five evaluations carried out (from March to July).

Statistical analysis

We evaluated the normality of the data from both experiments, where, with significance at 5% probability by analysis of variance (Two-Way ANOVA) and with normality Scott-Knott (for the genotypes experiment) and regression (for the IBA experiment), also with a 5% probability of error, were applied by the statistical program R software.

RESULTS AND DISCUSSION

Cutting of different genotypes of *I. paraguariensis* in two rooting environments

There was a significant interaction between rooting environments and the *I. paraguariensis* mother tree genotypes for survival (%), callus formation (%), rooting (%), mean number of roots and mean root length (cm) (Table 1).

Table 1. Survival (%), callus formation (%), rooting (%), mean number of roots and average length of the three largest roots (cm) in cuttings of *I. paraguariensis* according to

Rooting environment	Genotype						Averages
	G1	G2	G3	G4	G5	G6	
Survivor (%)							
GWIN	43.8 bB*	20.8 aC	25.0 bC	29.2 bC	50.0 bB	70.8 aA	39.9
Mini tunnels	72.9 aA	27.1 aB	68.8 aA	54.2 aB	72.9 aA	75.0 aA	61.8
Averages	58.3	23.9	46.9	41.7	61.4	72.9	50.9**
Callus formation (%)							
GWIN	41.7 bB	20.8 aC	20.8 bC	29.2 bC	41.7 bB	56.3 aA	35.1
Mini tunnels	62.5 aA	22.9 aC	60.4 aA	54.2 aA	72.9 aA	41.7 aB	52.4
Averages	52.1	21.9	40.6	41.7	57.3	48.9	43.8
Rooting (%)							
GWIN	6.3 aB	0.0 aB	0.0 aB	2.0 aB	12.5 aB	45.8 aA	11.1
Mini tunnels	2.0 aB	0.0 aB	2.0 aB	0.0 aB	2.0 bB	22.9 bA	4.9
Averages	4.2	0.0	1.0	1.0	7.3	34.4	8.0
Average number of roots							
GWIN	> 0.1 aB	NR	NR	> 0.1 aB	0.3 aB	4.3 aA	0.8
Mini tunnels	> 0.1 aA	NR	> 0.1 aA	NR	> 0.1 aA	1.3 bA	0.2
Averages	> 0.1	NR	> 0.1	> 0.1	0.2	2.8	0.5
Average length of the three longest roots (cm)							
GWIN	0.1 aB	NR	NR	> 0.1 aB	0.2 aB	1.5 aA	0.3
Mini tunnels	> 0.1 aA	NR	> 0.1 aA	NR	> 0.1 aA	0.3 bA	> 0.1
Averages	> 0.1	NR	> 0.1	> 0.1	0.1	0.9	0.2

*Averages followed by the same letter do not differ among themselves by the Scott-Knott averages test (P <0.05), Lowercase letters for rooting environments (column) and uppercase for genotypes (row), **Overall average of each variable, NR: not rooted.

There was a great variation of the survival averages for the genotypes considering both rooting environments, with higher or lower level of expression. The genotype with higher survival rate was G6 (70.8% for GWIN and 75.0% for mini tunnels) which also presented the lowest variation between rooting environments (4.2%). In a survival contrast, G2 had the lowest averages of survival in both environments (20.8% for GWIN and 27.1% for mini tunnels). Aside from genotype G6, the genotypes G1, G3 and G5 also had a high survival rate (72.9%, 68.8% and 72.9%, respectively), but only in the mini tunnel system. Some of these genotypes had differences of survival between rooting environments of more than 40.0% (G3).

Genetically superior *I. paraguariensis* clones can ensure a longer survival time in the rooting environment compared to clones not that well adapted (Brondani et al., 2008). Regardless of the rooting environment, genotypes G1, G5 and mainly G6 gave the highest percentages of live cuttings. The survival of propagules is directly linked to the genetics of the

mother plant used (Franzon et al., 2010), which is possible to be improved with the better environment, in this case with the mini tunnel system.

The higher mortality of cuttings in the GWIN, for most of the genotypes, may have been caused by the lower humidity observed in the first two weeks (up to 75.5%) of the last month of permanence in relation to the mini tunnels (constantly above 90.0%). Humidity must be kept as high as possible around the propagules (Xavier et al., 2013). It is possible that some genotypes (such as G6 and G5) are adapted to lower humidity conditions than the recommended, with cuttings able to withstand more severe conditions. In contrast, G1, G3 and G4 are probably more sensitive to sudden humidity decreases, considering the high differences of survival between environments (29.1%, 43.8% and 25.0%, respectively). As for G2, the low survival rate in both environments could be due to many factors, such as genetics and the ontogenetic age of the mother tree.

Different from the survival variable, a higher variation of callus formation was obtained between both factors. G5 (72.9%), G1 (62.5%), G3 (60.4%) and G4 (54.2%) gave the highest percentages for this variable for cuttings inside the mini tunnel. Only G6 (56.3%) achieved this statistical level inside GWIN, while G1 (41.7%) and G5 (41.7%) had intermediate results, and G2 (20.8%), G3 (20.8%) and G4 (29.2%) the lowest. The more profuse callus formation in some genotypes, mainly in the mini tunnel system, suggests that if these cuttings remained in this environment for longer periods, there might be a greater chance of rooting (Stuepp et al., 2017b). Data from works regarding *I. paraguariensis* vegetative propagation suggests that there is a tendency for primary rooting, i.e., root formation without the need of previous callus presence in this species (Stuepp et al., 2015, 2017a, 2017b).

When observing both callus formation and rooting, some genotypes with high percentages of callus had low percentages or even no root formation (Table 1), for example G1 (with high percentages of cutting survival in both environments), this genotype had a medium rate of callus formation in GWIN (41.7%) and high for mini tunnels (62.5%), and inverse results were obtained for rooting in GWIN (6.3%) and mini tunnels (2.0%). It is important to observe that the genotypes that yielded low percentages of callus formation (G2, G3 and G4) also had the lowest survival percentages.

As for rooting itself, the highest rooting average was obtained in G6 (45.8% in GWIN and 22.9% in mini tunnels) compared to the others, in which, there was no rooting or it was very low. G1 and G5 had a considerable root formation, but only in GWIN (6.3% and 12.5% respectively). G2 gave no rooted cuttings, while G3 and G4 had both one cutting with roots formed (inside the mini tunnels for G3 and inside GWIN for G4). Those results were examined for average number of roots formed and average length of the three longest roots, the highest results were also obtained by G6 (4.3 roots with 1.5 cm in GWIN, and 1.3 roots with 0.3 cm). Since the other genotypes presented low or no roots, such variables as number of roots and their lengths were also very low or nonexistent (Table 1).

As for rooting and its variables, the abrupt changes in temperature between the end of the penultimate month and the beginning of the last one in the mini tunnels (approximately 3.5 °C) may have hampered cell differentiation due to changes in metabolism and absorption capacity of nutrients (Cunha et al., 2009). Temperatures above 15 °C and below 35 °C provide the best rooting of propagules by maintaining high humidity, lower transpiration and metabolism at appropriate levels (Goulart & Xavier, 2008). This was observed for the experiment, in which there were similar averages, although mild, of temperatures between GWIN (16.2 °C) and the mini tunnels (19.1 °C). The probable environmental factor that affected the rooting of cuttings in the mini tunnels was the lower nocturnal temperatures, since in this environment this control did not occur. Still, the statistically equal results of rooting for both environments indicate that *I. paraguariensis* cuttings can be accommodated in simpler and cheaper structures, since the gains are similar.

The correlation between environments and genotypes can promote changes in rooting of cuttings, according to their adaptation. Environments with temperature control and irrigation by nebulization favors the survival and rooting of certain genotypes and negatively interfere with others (Brondani et al., 2008). In the present search, the G1, G3, G4 and G5

genotypes showed greater dependence on the environment for survival, while G1, G5 and G6 were more dependent on the rooting of their cuttings. In woody species, rooting of propagules is dependent on the genotype of the host plant, which may reflect on the environment, requiring different cultivation conditions for the same species (Mokotedi et al., 2000).

Vegetative propagation is an excellent method to obtain quick genetic improvement of forest tree species, allowing the multiplication of genetically superior plants in a short period of time (Wendling et al., 2014). However, all the variables influencing rooting in a single genotype called "C" factor which are attributed to the unique physiological or morphological characteristics of the propagules are still unknown and can be reflected in the rooting environment (Radosta et al., 1994). The success of rooting obtained for genotype G6 may have been caused by some factor outside the genetic proper or the conditioning environment, and may be associated with the variables related to the maturation of the mother tree, the propagule used for the cutting or the environment in which the tree was.

For sprouting variables and average number of new leaves there was no significant interaction between the rooting environments and the *I. paraguariensis* genotypes used.

Regarding the presence of new shoots, cuttings from G6 (65.6%), G5 (56.3%) and G1 (53.2%) presented at least twice as many shoots when compared to the G3 genotypes (25.0%), and G2 (11.4%). As for the conditioning environment, cuttings kept in the mini tunnels had a superior capacity for ($P < 0.05$) sprouting (46.5%) than in the GWIN (35.1%).

The new leaves were directly attached to the new sprouts, where G6 (3.4) and G1 (2.9) produced the highest average number of leaves per cutting. In contrast, G5 (1.9) was lower, but still better by six times than G2, which gave the lowest average (0.3). For the environments, both the mini tunnels (1.8) and the GWIN (1.7) were statistically the same for the production of new leaves ($P < 0.05$) (Figure 3).

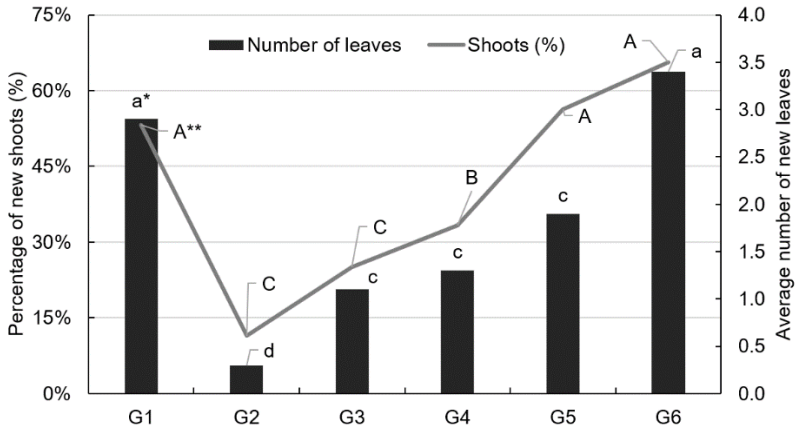


Figure 3. Averages of new sprouts (%) and number of leaves according to the cutting of different genotypes of *I. paraguariensis*. *Averages followed by the same letter do not differ among themselves by the Scott-Knott averages test ($P < 0.05$), Lowercase letters for number of leaves and uppercase for percentage of new sprouts.

New sprouts and the numbers of new leaves appear to be dependent only on the genotype, where a favorable environment would be responsible for the increase of these variables (Brondani et al., 2008, 2009). This was verified in the present study, since there was no interaction between environmental and genotypic factors for these variables.

IBA use in *I. paraguariensis* cuttings

There was no significant interaction between IBA concentrations and evaluation days. Considering the days of evaluation after cutting, survival decreased continuously from the first evaluation, at 50 days (90.6%) to 130 days (52.1%). The highest development of callus occurred between the 70th day (37.5%) and the 90th day (50.0%) evaluation, and gradually decreased

until the last evaluation (55.2%). Rooting was detected in the second evaluation with one rooted cutting (0.3%), increasing significantly between 90 days (6.3%) and 110 days (18.8%), which was maintained in the last evaluation (19.5%). As a consequence of rooting, the mean number of roots and their mean length were determined from the second evaluation (<0.1 and <0.1 cm), increasing according to the highest rooting percentage until the final evaluation (0.3 and 0.6 cm) (Figure 4).

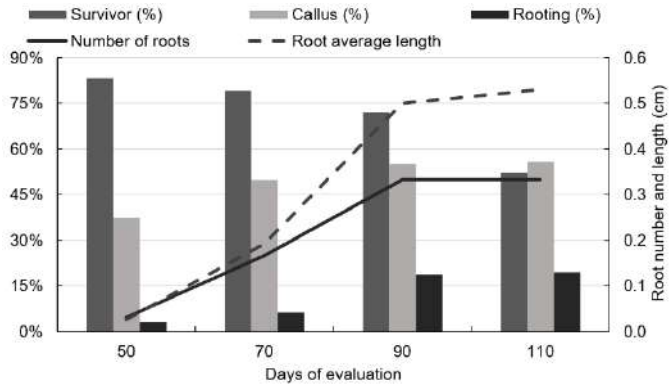


Figure 4. Survival (%), callus formation (%), rooting (%), mean number of roots formed and their average length (cm) of *I. paraguariensis* cuttings during the evaluation periods (50 to 130 days).

Regarding the evaluation periods, there was a difference of 39.0% in the survival of cuttings, considering the first evaluation (50 days) (91.0%) and the last (130 days) (52.0%). This relation can be considered normal, since there is the consumption of the energy reserves so that the cuttings are kept alive. At the end of the reserves, and without any efficient means of obtaining water and nutrients for the process of photosynthesis (roots) death of the cutting occurs. Survival did not change significantly related to the increase in IBA concentrations, varying at most 13.0% between no application (0 mg L^{-1}) and at the maximum doses (10,000 mg L^{-1}).

In relation to IBA concentrations, there were no significant changes in the survival of *I. paraguariensis* cuttings according to its increase, with the highest percentage detected at 2,500 mg L^{-1} (80.8%) and the lowest at 10,000 mg L^{-1} (65.0%). Callus formation decreased linearly, from 0 mg L^{-1} (55.0%) to the maximum concentration (30.0%) (Figure 5).

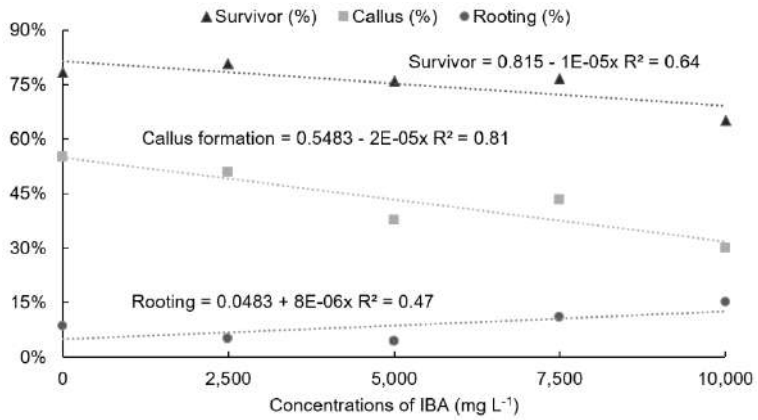


Figure 5. Survival (%), callus formation (%) and rooting (%) of *I. paraguariensis* cuttings according to the IBA doses during the evaluation periods (50 to 130 days). Average number of roots and length of roots were not significant for regression (P <0.05).

The rooting was best correlated linearly to the increase of concentration, especially at 7,500 mg L^{-1} (10.8%) and 10,000 mg L^{-1} (15.0%). On the other hand, cuttings without IBA application resulted in considerable rooting (8.3%) compared to the highest concentrations.

Due to the best rooting found in the concentrations of 0 mg L^{-1}, 7,500 mg L^{-1} and 10,000 of IBA, these concentrations gave the highest average number of roots with the highest average length. Although in similar numbers, root length at the highest concentration was 25% longer (0.5 cm) than 0 mg L^{-1} IBA (0.4 cm), while 5,000 mg L^{-1} had the shortest length (<0.1 cm).

The use of high concentrations of IBA can affect the survival of cuttings, presenting toxicity and its consequent death (Mantovani et al., 2017; Teixeira et al., 2017), but this was not detected in the present search. The higher survival of cuttings may be related to the time of collection of propagules, in which cold periods, such as autumn and winter, provide the greatest survival of cuttings of *I. paraguariensis*, regardless of IBA concentrations (Stuepp et al., 2017a). This is because the shoots collected in these periods have a good accumulation of reserves, providing more energy for the cuttings to grow, and consequently, extending their survival time (Oliveira et al., 2012).

According to the evaluation periods, there was a considerable amount of callus in cuttings of *I. paraguariensis* (18.0%), which could indicate a high maturation of the plant material used (Wendling et al., 2014; Stuepp et al., 2015). Considering the IBA concentrations, there was a strong decrease in callus formation according to IBA increase (up to 25.0% less) and consequently there was also an increase in rooting. The presence of callus in cuttings of *I. paraguariensis* hampers its rooting, indicating that the formation of roots for the species is best carried out directly (Stuepp et al., 2017a). This may indicate that, besides originating from a material with high maturation, the cuttings of this search did not have endogenous auxins at sufficient levels for efficient cell differentiation, necessitating an exogenous source (Wendling et al., 2015), which in this case were the highest concentrations (7,500 mg L^{-1} and 10,000 mg L^{-1}). However, plant materials, which in the presence of growth regulators produce little or no rooting, may have some inhibitory substance or deficiency of some compound necessary for that process (Hartmann et al., 2011).

The efficiency of IBA in the rooting of *I. paraguariensis* cuttings may be linked to the time of application in the experiment. With propagules collected in the winter and coming from aged mother trees, rooting tends to increase according to the increase of IBA concentrations, although it still occurs in low percentages (Stuepp et al., 2017a). This happened in the present study and rooting did not exceed 15.0% in the concentration of 10,000 mg L^{-1}, which would reinforce the hypothesis that the shoots used for the cutting were from mature mother trees (Ferreira et al., 2010; Wendling et al., 2014) and that only with high exogenous doses (Wendling et al., 2015) there is success in rooting. However, the shoots collected for this experiment come from plants with an average age of 15 years, considered to be young. Thus we can attribute another factor for their lower vegetative development, such as the environment. The city of Urupema is nationally known as being the coldest of Brazil, having a well adapted vegetation. It is possible that *I. paraguariensis* mother trees have a slower vegetative development under this condition, presenting a lower vegetative propagation capability.

As a consequence of the rooting, the average number of roots and the average length of the three longest roots were higher according to the periods of evaluation and the increase of IBA concentrations. Cuttings treated with growth regulators can start the rooting process before the untreated ones, which explains the greater number and length of roots (Stuepp et al., 2015), since the evaluations of the treatments were done at the same time.

CONCLUSIONS

Survival and rooting success of *I. paraguariensis* cuttings are directly linked to the conditioning environment and to the genetic characteristics of the mother trees which favors these variables,

Environments with micro sprinkler irrigation maintain a greater number of cuttings alive, while by nebulization they guarantee better rooting,

Although difficult due to selection criteria, the use of superior mother trees genotypes for cutting is essential for the success of its rooting in this species,

There were no significant results regarding the use of IBA in the rooting of cuttings in this study, even when considering the highest doses,

Further studies on the selection of *I. paraguariensis* mother trees are required for its vegetative propagation, determining selection criteria.

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Author's contributions: BN: Conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing – original draft, writing – review and editing, ACSS: Data curation, investigation, CM: Data curation, investigation, JCPS: Resources, MOP: Conceptualization, methodology, supervision, visualization, MCN: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation.



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Southern Forests: a Journal of Forest Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tsfs20

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To cite this article: Enéas Ricardo Konzen, Natália Saudade de Aguiar, Marcio Carlos Navroski, the late Clenilso Sehnen Mota, Leticia Miranda, Regiane Abjaud Estopa, Erasmo Luis Tonett & Mariane de Oliveira Pereira (2022): Artificial light improves productivity of mini-cuttings in a clonal minigarden of *Eucalyptus benthamii* and *E. dunnii*, Southern Forests: a Journal of Forest Science, DOI: 10.2989/20702620.2021.2000326

To link to this article: https://doi.org/10.2989/20702620.2021.2000326

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Southern Forests 2021, 83(4): 01–11
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SOUTHERN FORESTS
ISSN 2070-2620 EISSN 2070-2639
https://doi.org/10.2989/20702620.2021.2000326

Artificial light improves productivity of mini-cuttings in a clonal minigarden of *Eucalyptus benthamii* and *E. dunnii*

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Eucalypts are economically important worldwide, being largely propagated by clonal means, that is, frequently achieved by mini-cuttings. Regional climatic conditions, however, interfere with this strategy. Southern Brazil, especially in higher altitudes, has a considerable number of cloudy and cold days annually. It is a challenge for clonal propagation, even for cold-tolerant species such as *Eucalyptus benthami* and *E. dunnii*. The supplementation with artificial light has proved to be effective for improving growth in agricultural crops, but little work has been done with trees. Here we conducted a set of experiments for producing mini-cuttings of two commercial clones of *E. benthamii* and one clone of *E. dunnii* under the supplementation with artificial light, using red/far-red (660:730 nm) and red/blue (660:450 nm) light emitting diodes (LED), in comparison to a control (sunlight only). The supplementation with red/far-red and red/blue light increased the production of mini-cuttings in colder months, but more consistently positive impacts were observed with red/far-red lamps. The shoots produced under red/far-red light were more elongated and implicated in higher leaf canopy coverage. Overall, this work is a first step aimed at improving clonal propagation systems of *E. benthamii* and *E. dunnii* through mini-cuttings supplemented with artificial light.

Keywords: clonal propagation, leaf canopy coverage, light-emitting diodes, photosynthesis

Online supplementary material: Supplementary data for this article are available at https://doi.org/10.2989/20702620.2021.2000326

Introduction

Forest plantations in Brazil are largely composed of eucalypts, where they cover up to 5.67 million hectares (IBÁ 2019). However, southern areas of the country face significant challenges to growing eucalypts, as the prevailing climate is subtropical, with a considerable number of frost episodes, especially between fall (March to June) and winter (June to September) (southern hemisphere). This climate nearly impairs the cultivation of certain species on a commercial scale, especially those with low tolerance of cold and frosts (Konzen et al. 2017). Instead of *Eucalyptus grandis* W.Hill ex Maiden and *Eucalyptus urophylla* S.T. Blake, alternative species such as *Eucalyptus benthamii* Maiden & Cambage and *Eucalyptus dunnii* Maiden (Paludzyszyn-Filho et al. 2006) have been cultivated. *E. benthamii* originated from a limited area in New South Wales, Australia (Benson 1985). An adult tree attains a height up to 35 m and a diameter at breast height of approximately 1.5 m (Han et al. 2020). *E. dunnii*, also a tall tree, occurs in two disjunct populations in New South Wales and Queensland, Australia (Johnson and Arnold 2000). In Brazil, both species have adapted to the subtropical climates of the southern states, with considerable tolerance to frost episodes (Floriani et al. 2013; Baccarin et al. 2015; Konzen et al. 2017). Both species have also been cultivated in Uruguay (Resquin et al. 2020), which has a subtropical climate. Field trials with *E. benthamii* in North Carolina, USA, had high productivity, however, exceptionally low temperatures (below −10 °C) damaged plantations beyond recovery in 2014, demonstrating boundaries to the cold tolerance of the species (Butnor et al. 2019).

Clonal propagation of eucalypts by mini-cuttings is undoubtedly one of the most used techniques to achieve commercial-scale plantations (Brondani et al. 2012a). The viability of this technique relies on continuous collection of propagules aimed at rooting. Temperature, luminosity and photoperiod are critical factors in determining the production of new propagules (Konzen et al. 2018). Light is an essential component that affects the production of mini-cuttings in greenhouse systems. Diminished light incidence will probably reduce photosynthetic rates, limiting mini-stump growth and the production of mini-cuttings (Hartmann et al. 2011).

Studies have demonstrated that the production of mini-cuttings in a clonal minigarden and their rooting vary due the unstable meteorological conditions in southern Brazil (Brondani et al. 2010; Brondani et al. 2012b; Pereira Pires

et al. 2017). Our study was conducted in an area with high frequency of cloudy and foggy days over the year and therefore with more reduced levels of sunlight. An environment that artificially complements light radiation could optimise the performance of mini-stumps (Aguiar et al. 2018).

Artificial light supply to plants may be adjusted by setting irradiance, photoperiod and spectral quality of light (Hartmann et al. 2011). Light emitting diodes (LED) have been tested as alternative sources of light with potential to increase growth and development of plants, especially *in vitro*. As LED lamps emit light in specific spectral regions, with a range of wavelengths, it is possible to set the spectral quality and light intensity in plants. LED lamps may affect plant physiology, influencing photosynthesis, the synthesis of compounds such as chlorophyll, the emission of new shoots and their *in vitro* rooting and *ex vitro* growth (Nhut and Nam 2010; Dutta Gupta and Jatothu 2013; Batista et al. 2018).

LED lamps emitting light in red and blue ranges, alone or combined, have shown significant positive impact on plant growth (Dutta Gupta and Jatothu 2013). However, their responses vary according to the species (Nhut and Nam 2010). LED lamps emitting far-red (FR) radiation (wavelengths from 700 to 800 nm) combined with red (R) radiation (400 to 700 nm) has been tested with different ratios to evaluate growth of horticultural crops. Far-red radiation promoted seedling growth by increasing leaf expansion and net assimilation in shade-avoiding species (Park and Runkle 2017). After exposing lettuce to a 1.2 R/FR or higher ratios, higher shoot to root ratio, dry matter and stem length were found (Lee et al. 2015). Stem elongation is promoted by low red to far-red ratios during daytime or at the end of the day in several dicotyledonous species (Demotes-Mainard et al. 2016). The complementation of red light with blue lights, depending on the ratio, also promotes plant growth, such as in rice (Ohashi-Kaneko et al. 2006) and tomatoes (Fan et al. 2013). Blue light also stimulated *in vitro* shoot proliferation of *Corymbia torelliana* × *C. citriodora* and *C. citriodora* × *C. torelliana* hybrids (Souza et al. 2018) and *Vanilla planifolia* Jacks. ex Andrews (Bello-Bello et al. 2016).

Several studies have addressed the effect of artificial light, but few are available for woody trees only (Batista et al. 2018), such as for *Platanus orientalis* L. (Arena et al. 2016), *Pinus sylvestris* L., *Abies borisii-regis* Mattf. (Smirnakou et al. 2017a), *Quercus ithaburensis* var. *macrolepis* (Kotschy) Hedge & Yalt. (Smirnakou et al. 2017b), *Fagus sylvatica* L., *Quercus ilex* L. and *Prunus avium* L. (Astolfi et al. 2012). The experiments for those studies, however, were conducted in incubation chambers, not in greenhouses. Greenhouse experiments are scarce, such as that of Tombesi et al. (2018), that explored the potential of artificial light in leaf cutting rooting of hazelnut, obtaining an increase in the quality of propagules and their rooting.

In this work, we aimed at evaluating the production of mini-cuttings of three clones, two of *E. benthamii* and one of *E. dunnii*, supplemented with artificial light in a greenhouse environment. The study was developed under the hypothesis that artificial light enhanced the production of mini-cuttings, especially in a region where sunlight is frequently low due to cloudy and foggy weather. This enhancement could be achieved by augmenting the photosynthetic capacity under artificial light, as well as due to the genotypic differences that clones and species have among them.

Material and methods

Experimental environment and plant materials

This study was conducted at the greenhouse facilities of Klabin S. A., located at Otacílio Costa, Santa Catarina state, Brazil. The first set of experiments were evaluated from May to December 2017. The area has a temperate climate of type Cfb (Alvares et al. 2013). The region has a relatively high incidence of cloudy and foggy days, with reduced sunlight, especially in fall (March to June) and winter (June to September, Southern hemisphere). Klabin therefore uses greenhouses with transparent plastic mini-tunnels within the facilities, which are helpful in maintaining adequate temperatures during winter. Each plastic mini-tunnel is equipped with controlled irrigation and fertigation. Our experiment was conducted in a plastic mini-tunnel with mini-stumps of two clones of *E. benthamii* (referred to as A and B) and one clone of *E. dunnii* (clone C). The mini-tunnel was 16.6 m long, 0.8 m wide and 0.8 m high. Mini-stumps were grown in a substrate consisting on a layer of sand covering a narrow layer of crushed stone (total depth of 30 cm) (Figure 1).

The mini-stumps originated from cuttings of each of the clones evaluated. Mini-stumps were planted in consecutive lines spaced 10 cm × 10 cm apart (Figure 1c). Each clone was represented by eight lines, each one having eight mini-stumps planted in sand. Four plots per clone were planted in the mini-tunnel. Mini-stumps were adapted to the mini-tunnels, receiving daily irrigation with a balanced nutrient solution for continuous production of mini-cuttings. The nutrient solution was prepared with the same concentrations described by Aguiar et al. (2018). In winter, two events of fertigation were conducted per day, while in summer that frequency was increased to three or four events. Each fertigation lasted between two and four minutes.

Specification of the LED-lighting treatments

The mini-tunnel was equipped with LED lamps emitting light with a mixture (50% to 50%) of red and far-red light (R/FR) or a mixture of red and blue light (R/B: 66.7% to 33.3%). The specifications of each type of lamps were: (i) R/FR – 36″, 1W, AC85 – 265 V, red/far-red = 660:730 = 1:1, LED quantity: 36 pcs; and (ii) R/B – 36″, 1W, AC85 – 265 V, red/blue = 660:450 = 2:1, LED quantity: 36 pcs. The lamps were placed at the top of the mini-tunnel, at a height of 0.8 m. The mini-stumps were exposed to the artificial light for 14 h (between 6 am and 10 pm) daily. Photoperiod in this region varies from approximately 10 h in winter to 14 h in summer. Replicates of each clone not exposed to artificial light were set as the control treatment. To separate light-exposure treatments, eight lines of mini-stumps (borders) were planted in between lamps (in a space with no direct exposure to any lamp).

Experimental design and time-course evaluation

The experiment was designed with the combination of three clones (clones A and B of *E. benthamii*, and clone C of *E. dunnii*) and three light treatments (control with no artificial

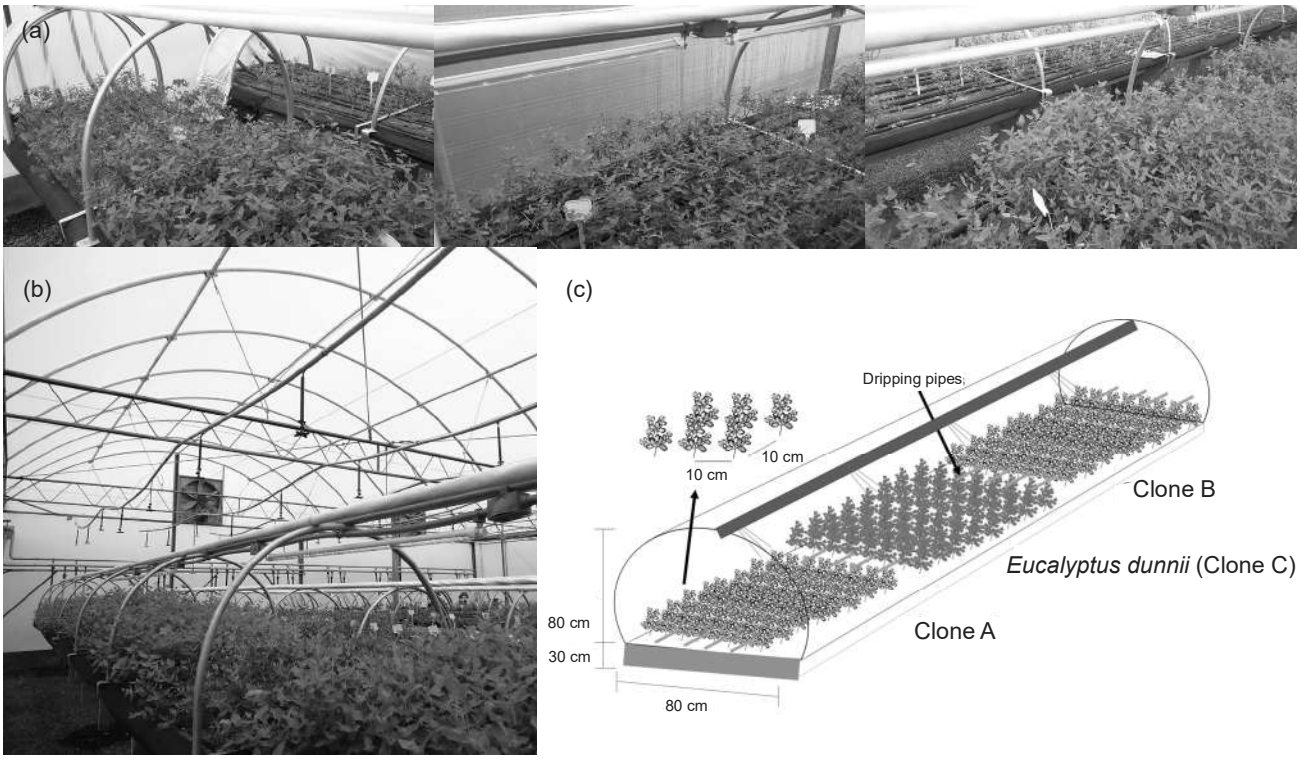


Figure 1: Experimental greenhouse and treatments. (a) Partial view of each light treatment: on the left, the control treatment, with no LED lamp. In the middle, the red/blue LED lamp, and on the right, the red/far-red lamp. (b) Partial view of the experimental greenhouse, showing an uncovered plastic mini-tunnel. (c) Schematic representation of one light treatment along with the distribution of mini-stumps of each clone

light; and red/far-red and red/blue lights emitted with LED lamps according to the previous descriptions). Eight lines of each clone (each one being considered a replicate) were exposed to each light treatment. In total, nine treatment combinations were evaluated in our study. All treatments were evaluated once a month, with at least six evaluation events. The first evaluation was conducted in May 2017.

Each evaluation consisted of two steps: (i) collection of data on leaf chlorophyll content and gas exchange variables (photosynthesis); and (ii) harvest of mini-cuttings (shoots) for rooting. The harvest of mini-cuttings was performed one day after the measurement of chlorophyll and photosynthetic variables. The time-points were also considered as a factor for statistical analyses.

Quantification of mini-cuttings

Mini-cuttings (shoots) were collected from each mini-stump from all treatments (clones × lights). One line with eight mini-stumps was considered as one replicate of mini-stumps. Therefore the number of shoots per line was considered a replicate, encompassing an area of 800 cm² (10 cm of spacing and eight consecutive mini-stumps). For the analyses, we adjusted the data to the number of shoots per square metre. After being collected, all mini-cuttings were immediately placed in the rooting environment.

Photosynthetic variables and chlorophyll content

Photosynthetic variables were evaluated through determination of gas exchanges using an Infrared Gas Analyzer (IRGA), model Li-6400 (LI-COR). Measurements were taken in completely expanded leaves after receiving at least 1 h of sunlight. The following variables were measured: transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), net assimilation rate (*A*, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (*Gs*, mmol H_2O m^{-2} s^{-1}) and the ratio between the intercellular concentration of CO_2 and the environmental concentration (*Ci*/*Ca*). Due to the reduced area of all leaves, they were all collected after measurements for further determination of their area. The data collected from IRGA were adjusted proportionally to the area of 6 cm². Four mini-stumps per treatment combination (among all nine described) were randomly chosen at each evaluation.

The chlorophyll content was measured in units of chlorophyll using ClorofiLOG (Falker) equipment. In total, 20 individual leaves were measured per treatment. At each collection event, 180 chlorophyll measurements were taken in the morning.

Leaf canopy coverage

As a variable that has been demonstrated to be correlated with the number of shoots produced in a clonal minigarden (Aguiar et al. 2018), we computed the leaf canopy coverage of each one of the treatments at all evaluation events. Photographs were taken using a Samsung Tab3 tablet at a height 30 cm above the canopy of each plot of mini-stumps. The leaf coverage was estimated with Easy Leaf Area (Easlom and Bloom 2014), following the recommendations of Aguiar et al. (2018) for the same eucalypt clones.

Leaf nutrient profiling

Fully expanded leaves were collected from the upper segment of shoots of each clone and under each light treatment in January 2018. Leaves were washed in a liquid detergent solution, followed by rinsing in deionised water. The samples were then dried in an oven (60–70 °C) until constant weight. Leaves were ground with a Willey mill (sieve with 40 mesh). Nutrient concentrations were determined according to Malavolta et al. (1997). Total nitrogen was determined through Microkleldahl digestion. For the percentage extraction of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S), wet nitric-perchloric digestion/inductively coupled plasma optical emission spectrometry (ICP-OES) was used. The determination of copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) was also conducted with ICP-OES. Conversely, total boron (B) was determined with dry digestion (ICP-OES).

Rooting experiment

A controlled experiment of rooting and further acclimation of the mini-cuttings was conducted after harvesting mini-cuttings. From each treatment (clone × light), 27 mini-cuttings were harvested per replicate, constituting 1 experimental block. The mini-cuttings were stored in closed plastic boxes and frequently sprayed with water until they were transplanted to Ellepot-type tubes (~50 cm^3) containing Carolina Soil II as substrate, fertilised with 1 500 kg m^{-3} (company standards for the preparation of large amount of substrate) of fertiliser (Osmocote — NPK at proportions 19:6:10). The experiment was arranged in randomised blocks, with four replicates. The experiment was installed in a greenhouse with fogging system (at least 90% humidity) and controlled temperature (25 to 30 °C) for rooting. The rooting experiment was conducted for 30 days. After that, all plants that survived were transferred to a shade house for acclimatisation. Survival and percentage of rooting were recorded from each treatment after the experimental period. Survival was recorded based on the number of propagules that emitted new sprouts, even if small in size, indicating some physiological activity. Adventitious rooting was recorded based on the number of propagules that presented roots at the bottom of each Ellepot tube.

Meteorological data

Meteorological variables were recorded daily from an automatic station available at Klabin. The meteorological variables were correlated with the amount of mini-cuttings produced and with the photosynthetic variables. The following variables were used: minimum, mean and maximum temperature (°C); relative humidity (%); precipitation (mm); radiation (M m^{-2} dia^{-1}); and photosynthetic active radiation (PAR) (M m^{-2} dia^{-1}).

Shoot productivity

One of the main variables analysed in our study was the number of shoots per square metre (NS m^{-2}). To better understand the effect of the use of artificial light, the variable Δ (difference between the number of shoots per square metre produced under artificial light and the number of shoots per square metre produced with no supplementation of light) was created. Therefore, two Δ were calculated (diffR_C: red/far-red to control; diffBR_C: red/blue to control).

Statistical analyses

To test the hypothesis that artificial light enhanced the production of mini-cuttings, we first checked if the number of shoots per square metre fitted normal distribution (Shapiro's test, $p < 0.05$) and variance homogeneity (Barttlett's test, $p < 0.05$). When necessary, the data were transformed with a Box Cox test. After rechecking the data for their fit to the basic requirements, we proceeded with analysis of variance (ANOVA) (F test, $p < 0.05$), followed by Tukey's mean comparisons test ($p < 0.05$). Not all factors encompassed data that could be adjusted to the requirements of ANOVA; therefore some data were analysed using the Kruskall–Wallis non-parametric test ($p < 0.05$), followed by a Dunn's post hoc test ($p < 0.05$), with Benjamini–Hochberg adjustment (Benjamini and Hochberg 1995). Data transformation was performed using the MASS package (Ripley et al. 2013). Tukey's mean comparisons were conducted using the laercio package (Silva 2015). Standard ANOVA and Kruskall–Wallis tests were performed in R environment as well (R Core Team 2020). Dunn's post hoc tests were conducted using the R package 'dunn.test' (Dinno and Dinno 2017).

The photosynthetic variables, chlorophyll content, leaf canopy coverage and nutrient concentrations were analysed following the same steps. Pearson's correlation coefficient was calculated and evaluated for significance ($p < 0.05$) among all variables, using PAST 4.0 (Hammer et al. 2001). The meteorological variables were included in the correlation analysis to better acknowledge the environmental variation and its influence on the data. Each Δ (diffR_C: R/FR to control; diffBR_C: R/FR to control) was quantified in absolute number of shoots per treatment and correlated (Pearson's, $p < 0.05$) with all the other variables analysed in this study.

Results

Artificial supply with red/far-red light enhances the production of mini-cuttings

Six data collection points were compiled in our study (between May and December), revealing seasonal effects on the production of mini-cuttings as well as the effects of clones and the supplementation with artificial light (Figure 2). Figure 2a shows mean temperature, radiation and PAR for the 30 days that preceded the data collection point. The number of shoots per square metre (NS m^{-2}) produced by mini-stumps varied over the months and for each light treatment (Figure 2b) and for the clones (see Supplementary Information Figure S1). The average NS m^{-2} was similar ($p > 0.05$) over the first three harvests, varying from approximately 138 to 210 shoots m^{-2}. A significant increase ($p < 0.05$) was observed in the next three harvests. At 150 days after the beginning of the supplementation with artificial light, an average of 366 shoots m^{-2} was obtained. This number increased ($p < 0.05$) to 481 after 180 days, and to 1 129 after 210 days.

Both supplementation with artificial light and clones had significant effects ($p < 0.05$) on the number of mini-cuttings

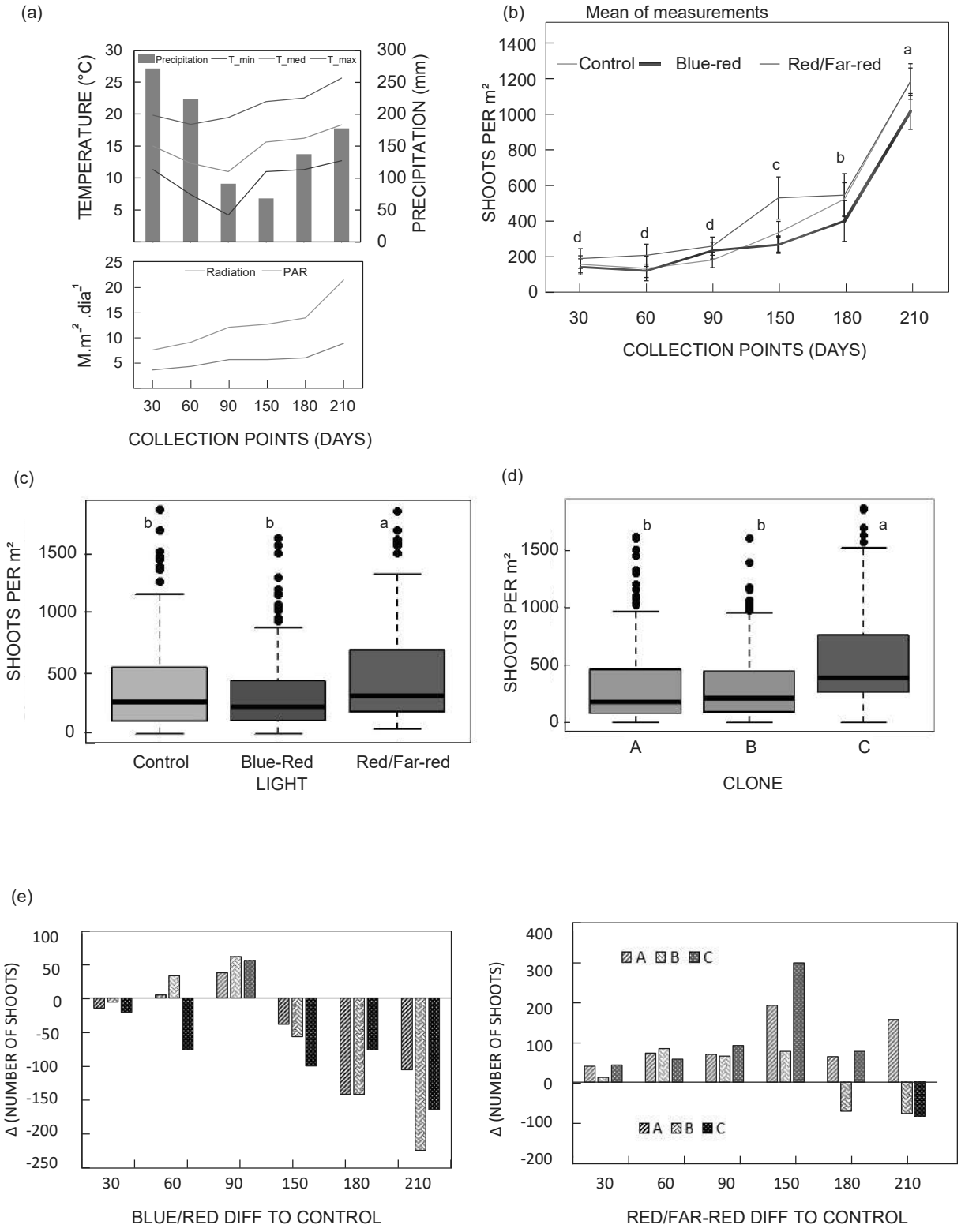


Figure 2: Shoots per square metre produced in at different time point collections (days after the beginning of the treatments with artificial light — LED). (a) Summary of meteorological variables of the month in that each harvesting of shoots was conducted. (b) Number of shoots per square metre at each time point. Bars represent the standard deviation of means. (c) Box plot graphs of the number of shoots per square metre for each LED light treatment. (d) Box plot graphs representing the same variable for each clone. (e) Absolute difference counting between the number of shoots per square metre produced under red/blue LED light and control (left graph) and under red/far-red light and control (right graph). Different letters mean statistical significance at $P < 0.05$, according to Tukey's test ($p < 0.05$) or Dunn's post hoc test (non-parametric test)

produced (Figure 2c and 2d). The mini-stumps supplemented with red/far-red light produced more mini-cuttings (477 shoots m^{-2}, $p < 0.05$) on average (mean of all the six harvests) than the mini-stumps submitted to red/blue light (352 shoots m^{-2}). That is, mini-stumps supplemented with red/far-red light produced 17.5% more mini-cuttings in comparison to the control. The control treatment (406 shoot m^{-2}) showed no difference from the red/blue based on statistical criteria, however, proportionally, the average production under red/blue light was 13.3% lower than the control. Clone C (*E. dunnii*) produced a higher average number of mini-cuttings (555 shoots m^{-2}, mean of all measurements, $p < 0.05$) than the clones of *E. benthamii* (Clone A = 343 shoots m^{-2}; Clone B = 337 shoots m^{-2}) (Figure 2d).

Overall, the supplementation with the mixture of red and blue light (2:1) implicated in a reduction of the total production of mini-cuttings in comparison to the control, as indicated by the Δ between the number of shoots produced between the two treatments (diffRB_C) (Figure 2e). However, after 60 and 90 days from the beginning of the experiment, a slight increase was observed, which was no longer the case in the following months. Conversely, the supplementation with red/far-red light had a positive effect in all collection points, although the response varied among clones.

In general, we detected a significant negative Pearson correlation between average temperatures (mean air temperatures of the preceding 30 days) and the Δ between the number of shoots produced under red/blue light and the control, for all clones and the mean of all clones (r between −0.90 and −0.98) (Supplementary Information, Figure S2). That is, higher Δ were observed when the average temperatures were lower, suggesting a positive difference to the exposure to red/blue light in those harvest points (60 and 90 days data collection points in red/blue treatment) (Figure 2e).

Photosynthetic activity was not significantly altered under red/far-red light

Mean net photosynthetic rate (*A*), as well as stomatal conductance (Gs) and transpiration (*E*) were influenced by the meteorological conditions at each day (Figure 3a), showing variable values over the data collection points. A considerable number of leaves were not well developed at this specific point. This may have influenced lower *A* values in the first collection point (Figure 3b).

Significant differences ($p < 0.05$) were encountered among the distinct measurements (time points) and among the light treatments for the net photosynthetic rate (*A*) (Figure 3b). Similar results were observed for G_s (Figure 3c). However, the net photosynthetic rate (*A*) was not different when comparing R/FR to the control (Figure 3d). The mean net photosynthetic rate of all measurements combined was lower under R/B light (13.1 µmol CO_2 m^{-2} s^{-1}) than the control and R/FR lights, according to the same statistical test ($p < 0.01$). No statistical difference was detected among the three clones for *A*.

Elongation of mini-cuttings is observed under supplementation with red/far-red light

An elongation of the mini-cuttings grown with supplementation of red/far-red light occurred with all clones, which is visually observed with more elongated shoots than with the other light treatments (Figure 4a). Shoots grown under R/FR light had mean length of 35.7 cm (±1.48, standard error) from the top leaf to their insertion on the main stem of the mini-stump, while shoots under red/blue and control had mean lengths of 23.1 cm (±1.75, standard error) and 26.0 cm (±2.51, standard error) respectively. In general, lower mean chlorophyll units were measured from more elongated shoots. Overall, mini-stumps with lower chlorophyll levels were observed with R/FR light, but that varied among clones (Figure 4b).

The higher number of shoots and their elongation under R/FR light implicated in higher values of leaf canopy coverage (Figure 4c), which is the percentage of a picture area (taken right above each plot of mini-stumps) occupied by leaves (Aguiar et al. 2018). The plots supplemented with R/FR light presented a mean leaf coverage of approximately 58%, while blue/red-supplemented and control plots had both approximately 30% of coverage only ($p < 0.05$ in comparison to the R/FR treatment).

Nutritional status of propagules and differential survival and rooting among light treatments

From the nutrient quantification of mini-cuttings, in general, significant differences were detected among the light treatments only for K, Ca and Mg, as macronutrients (Table I; for a complete list check Supplementary File 4). The only micronutrients that differed among the distinct light supplementation treatments were Mn and B. K, Ca, Mg and Mn had higher concentrations of nutrients under R/B and R/FR lights. The contrary was observed for B.

For the rooting experiment, five distinct harvests, beginning with the same number of propagules for each treatment were evaluated. By an analysis of all data combined, the survival rate was 63.9% of propagules that were previously treated with R/FR light, not different from the control (Figure 5). The mean survival of propagules treated with the R/B light, though, was lower (47.7%, $p < 0.05$). The performance of adventitious rooting was not significantly different among the light treatments, although the average rooting was numerically lower on average for the red/blue light-treated propagules (35.1%) in comparison to the red/far-red (43.6%) and control (43.9%) (Figure 5).

The performance of clones differed for survival ($p < 0.05$). Clone C of *E. dunnii* reached 73.4% of survival, while clones A and B of *E. benthamii* reached 48.6% and 50.1% respectively (Figure 5). Adventitious rooting percentages were not different according to statistical analyses. Clone C presented 42.7% of adventitious rooting, while clones A and B showed 39.5% and 40.4% respectively.

Discussion

Impact of LED-lighting on the yield of mini-cuttings

Our work is among a limited set of studies to test the supplementation of light with LED lamps in a greenhouse system. The supplementation with red/far-red light (~660 to 730 nm) with LED lamps increased the production of mini-cuttings in the clonal minigarden at Klabin facilities, revealing potential for improving results throughout the year. We highlight the importance of such results for designing strategies applied to the coldest months of the year (Southern

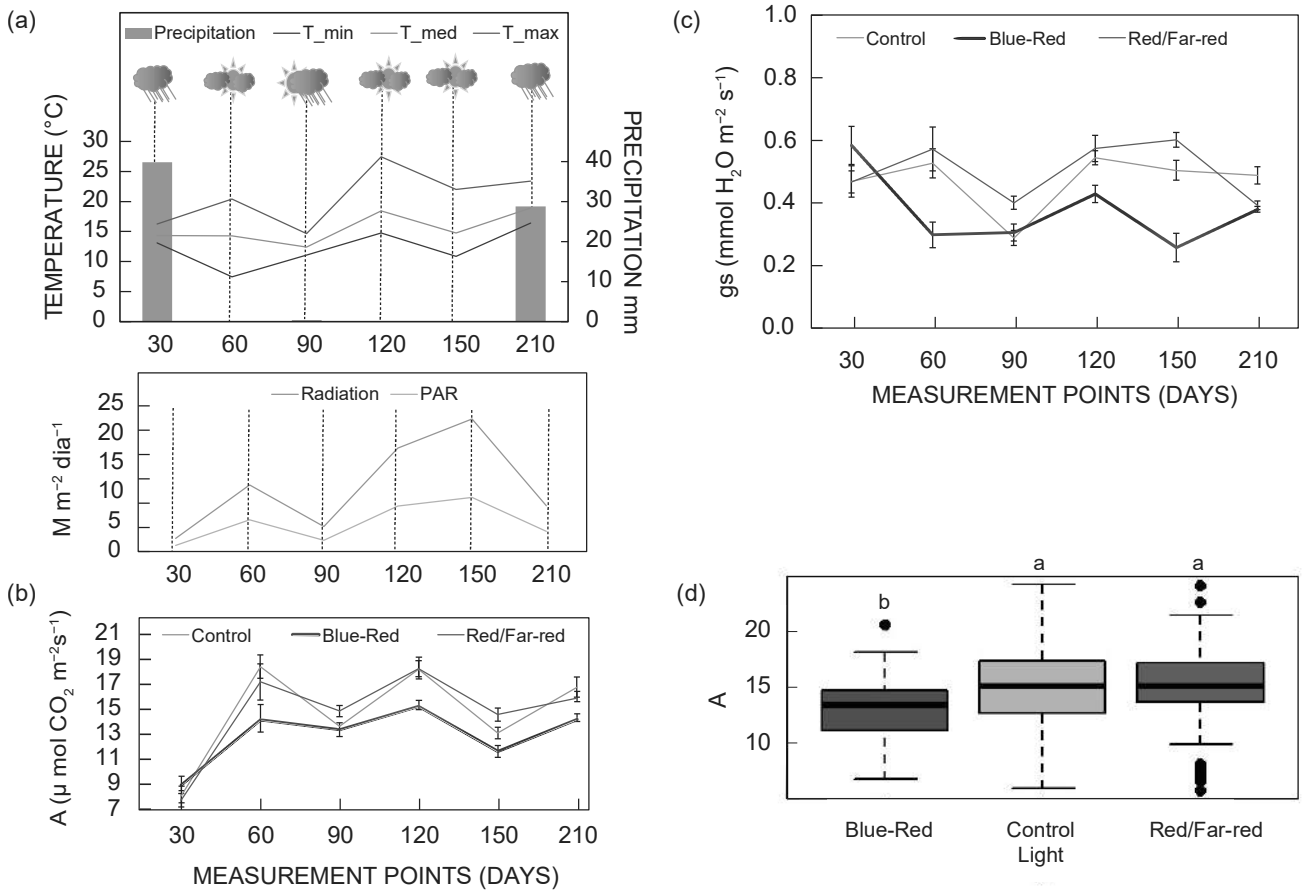


Figure 3: Photosynthetic variability among different time point collections (days after the beginning of the treatments with artificial light — LED), LED light and clones of *Eucalyptus benthamii* and *E. dunnii* evaluated in a clonal minigarden. (a) Summary of meteorological variables at each collection point. (b) Mean net assimilation rate (*A*) over the distinct time point on the course of the experiment. (c) Mean stomatal conductance (*Gs*). (d) Overall mean photosynthetic rates for the three light treatments. Different letters mean statistical significance at $P < 0.05$, according to Tukey's test ($p < 0.05$) or Dunn's post hoc test (non-parametric test)

hemisphere), between May and July, as they are usually the least productive months in southern Brazil.

By seeking comparisons to other studies in literature, we were restricted, in general, to *in vitro* tests or experiments conducted in growth chambers. With eucalypts, R/B light has been shown effective in *in vitro* cultivation of *Corymbia torelliana* × *C. citriodora* and *C. citriodora* × *C. torelliana* hybrids, improving shoot proliferation (Souza et al. 2018). In their study, no red LED lamps were assessed. In another experiment, red light improved plant growth in ornamental roses. The authors also used blue LED lamps, obtaining even better performance for the roses, but no mixture between red and blue light were tested (Azmi et al. 2016). *In vitro* shoot proliferation was improved in *Vanilla planifolia* after growing under LED lighting, especially under blue/red light (Bello-Bello et al. 2016). The use of 100% red LEDs improved *in vitro* growth of highbush blueberries by augmenting shoot proliferation. The use of 50% of each blue and red implicated in lower performance for the blueberries, in general, nearly proximal to fluorescent lamps, depending on the culture medium that was employed (Hung et al. 2016). Although those studies were conducted with other species, the results highlight the positive impact of red light in augmenting growth and shoot proliferation.

Morphological and physiological implications of the supplementation with artificial light

We observed no significant differences in photosynthetic rates between far-red treated plants and the control. The extent to which photosynthetic activity is translated into growth relies on several factors, including nutrient availability (Kirschbaum 2011). Red LEDs (650–665 nm) usually have a positive impact on plant growth as the wavelengths of these lamps fit the absorption peak of chlorophylls and phytochrome (Darko et al. 2014). One counterpart of the use of red light is etiolation, a phytochrome-dependent phenomenon observed in one of the first studies with LED-lighting in lettuce (Hoenecke et al. 1992). In our study, the shoots produced under R/FR light were more elongated than with R/B (~450 to 660 nm) LED lamps and control. An *in vitro* study with *Chrysanthemum* showed that red and far-red ranges of light stimulated excessive elongation, leading to a fragile stem, which negatively affected plant growth. The authors recommended an adjustment of the ratio between

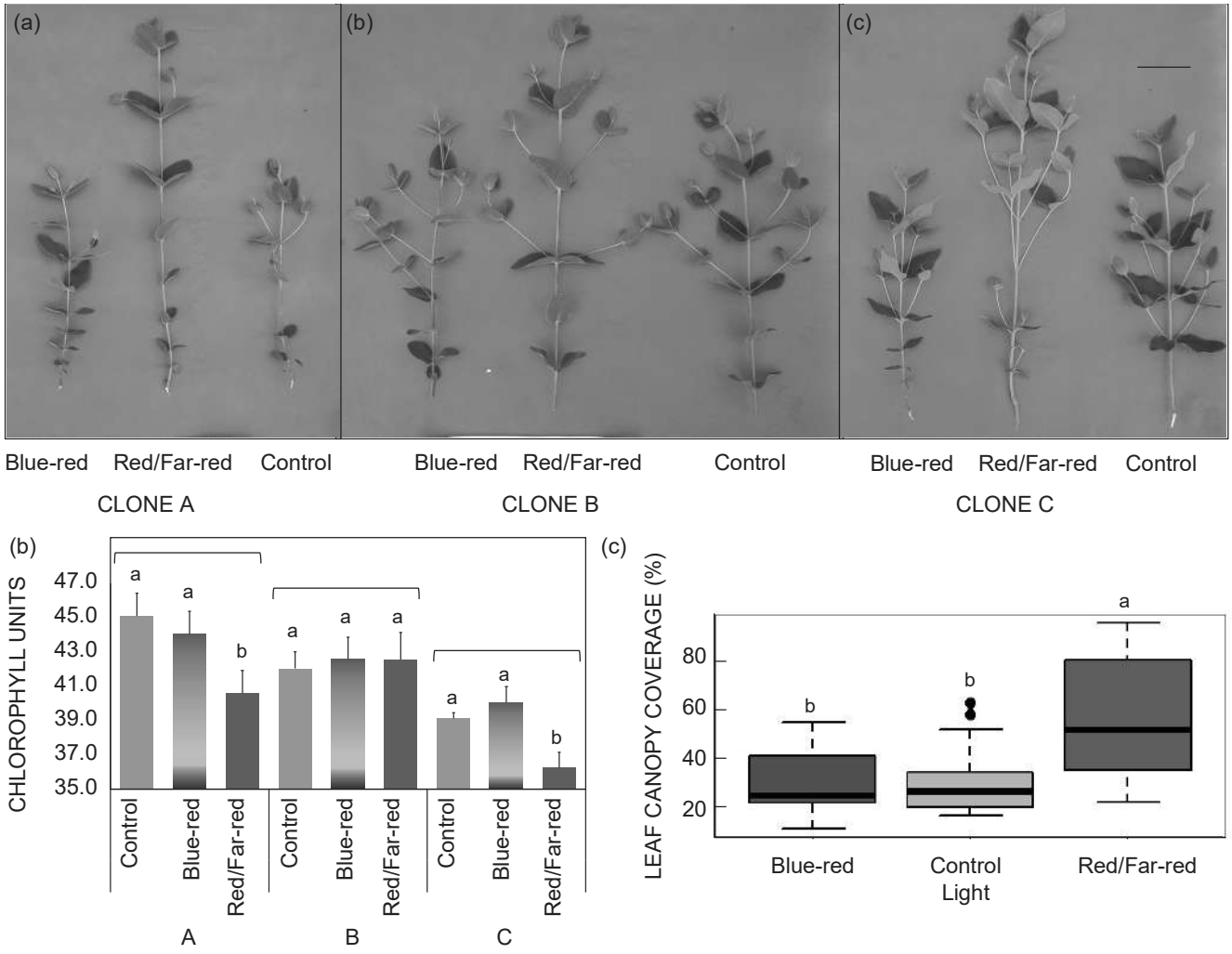


Figure 4: (a) Distinct elongation of shoots produced under supplementation with artificial light (LED). The bar represents 3 cm. (b) Average chlorophyll units measured from leaves of mini-stumps of two clones of *Eucalyptus benthamii* (A and B) and one of *E. dunnii* (C) supplemented with artificial light (red/blue or red/far-red). (c) Leaf Canopy coverage averages for each light treatment. Different letters mean statistical significance at $p < 0.05$, according to Tukey's test ($P < 0.05$) or Dunn's post hoc test (non-parametric test)

Table 1: Summary of the quantification of nutrients from mini-cuttings of *Eucalyptus* grown under distinct LED-lighting treatments. The control treatment refers to sunlight only. We showed here only those nutrients with significant differences among the light treatments. Different superscript letters mean statistical significance at $p < 0.05$, according to Tukey's test ($p < 0.05$) or Dunn's post hoc test (non-parametric test)

Light treatment	K (% m.m^{-1})	Ca (% m.m^{-1})	Mg (% m.m^{-1})	Mn (mg.kg^{-1})	B (mg.kg^{-1})
Control	2.0 (±0.14) ^{b}	0.46 (±0.03) ^{b}	0.15 (±0.016) ^{b}	94.8 (±10.51) ^{b}	74.1 (±3.89) ^{a}
Red	2.2 (±0.13) ^{ab}	0.54 (±0.06) ^{a}	0.17 (±0.020) ^{ab}	119.25 (±48.00) ^{a}	65.4 (±5.39) ^{b}
Red-blue	2.3 (±0.20) ^{a}	0.58 (±0.04) ^{a}	0.18 (±0.015) ^{a}	111.00 (±12.01) ^{a}	64.3 (±4.03) ^{b}

red and blue light to overcome elongation (Kim et al. 2004). Hoenecke et al. (1992) also supplemented the red light with blue fluorescent lamps in their study with lettuce, preventing extension and elongation from occurring. It seems that stem elongation can be promoted or inhibited by different synergistic interactions between blue/red light receptors and phytochrome according to species (Kim et al. 2004). Our experiments with the combination of red and blue in LED lamps was also implicated in no elongation.

Shoots exposed to R/FR light also showed lower chlorophyll content, although with considerable variation among clones. A reduction of chlorophyll was also observed in a previous study with LED lamps in beeches and holm oaks cultivated in growth chambers (Astolfi et al. 2012). Such

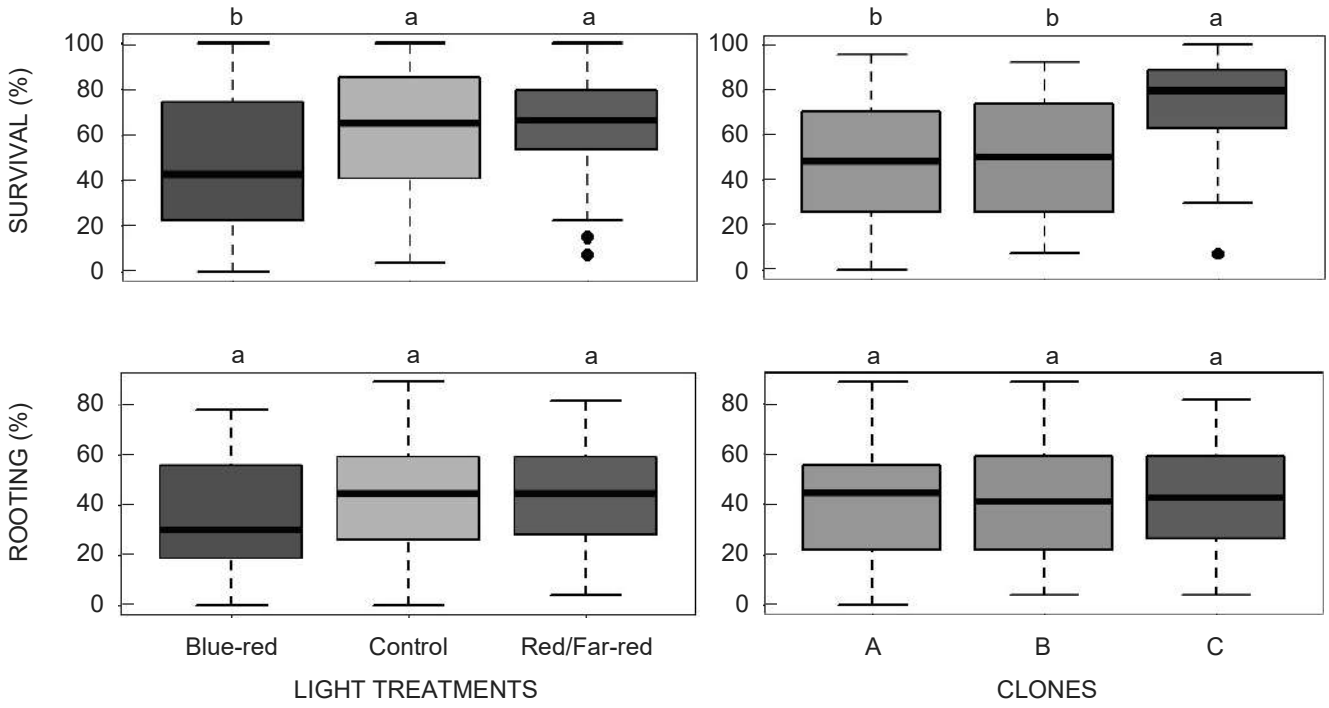


Figure 5: Mean differential survival and rooting of shoots collected from mini-stumps of three *Eucalyptus* clones supplemented with LED light. Clones A and B: *E. benthamii*. Clone C: *E. dunnii*. Different letters mean statistical significance at $p < 0.05$, according to Tukey's test ($p < 0.05$) or Dunn's post hoc test (non-parametric test)

reduction was accompanied by increased activity of ribulose-1,5-biphosphate carboxylase (RuBPC; EC 4.1.1.39) in their work, suggesting an improved light-use efficiency (Astolfi et al. 2012). Lower chlorophyll content was measured from *in vitro* grown plants of *Cymbidium* under red light, in comparison to blue and red/blue light (Tanaka et al. 1998). In another experiment, the supplementation with any LEDs in a greenhouse facility did not cause changes in the contents of chlorophyll *a* and *b* in *Dieffenbachia amoena* 'Camella' and *Ficus elastica* 'Melany' (Heo et al. 2010).

Higher production of shoots under R/FR light was also implicated in higher leaf canopy coverage as evaluated from the pictures taken over the mini-stumps. Previous work has shown that the leaf canopy coverage has a significant and high positive correlation with the number of shoots produced in clonal minigardens (Aguiar et al. 2018), which is consistent with the current data.

Nutrient profiling

In the quantification of the macro- and micronutrient concentration of the leaves, our study does not show much variation between the LED lamps used, with little superiority for a R/FR or R/B LEDs for K, Ca, Mg and Mn. The type and intensity of light also shows a nutritional difference in the leaves of Michaelmas daisies, where a light colour significantly changes the nutrition of plants with nitrogen, sodium, iron and manganese, while the type of light source influences the content of calcium, sodium and iron in parts above the plants. In an identical spectrum of light, also the type of bulb can have a significant effect on the nutritional status of plants (Schroeter-Zakrzewska and Kleiber 2014).

Survival and rooting of mini-cuttings harvested from the LED light-treated plants

In our study, although R/FR light was implicated in an overall higher yield of shoots, their survival and rooting is still limited, a challenge reported for *E. benthamii* (Brondani et al. 2012a, 2012b). Transplantation success is variable among other species as well, including agricultural crops. A study with lettuce showed enhanced growth of plantlets treated with LED lighting, but also attested to the variability in their transplant success. Interestingly, Bantis et al. (2016) found that red LED lighting accelerated root growth, which could be advantageous after transplantation. From an in vitro experiment with *Jatropha curcas* L., an exposure to white, red and blue LEDs were suitable for root induction, whereas the combination of red and blue lights completely impaired rootinig (Daud et al. 2013). Red light also stimulated adventitious rooting in grape (Poudel et al. 2008), *Ficus benjamina* L. (Gabryszewska and Rudnicki 1997), and *Morinda citrifolia* L. (Baque et al. 2010). In these species, the combination of red and blue light did not favour adventitious root induction. However, supplementation with artificial light occurred in the rooting environment. In our experiment, the supplementation of light was in the production of the mini-cuttings. Although our experiments showed no increase in rooting so far, no difference was encountered relative to sunlight only. That is, our results proved effective in augmenting the production of mini-cuttings, although rooting performance needs yet to be improved.

Clones also differed in their production of mini-cuttings, as previously shown (Aguiar et al. 2018), but also responded to the light treatments. Distinct responses of clones are due to

genotypic effects (Brondani et al. 2012a) and, in our case, also distinct species. E. benthamii is a species that is difficult to cultivate due to low seed production and low adventitious rooting with cuttings or mini-cuttings (Brondani et al. 2012b).

Perspectives and recommendations

In general, LED lamps have been proven more effective in *in vitro* systems or growth chambers than fluorescent lights. Our greenhouse system showed the same potential in improving growth performance and yield of mini-cuttings. This recommendation has been made in several studies (such as Hung et al. 2016) that highlighted their application for the rapid propagation of plants, also suggesting the replacement of traditional fluorescent lamps. An advantage of the LED system resides on the peak emissions of red and blue, that are close to the absorption peaks of chlorophylls and the wavelength of maximum photosynthetic activity. LEDs have a longer life, diminishing labour cost, and generating little heating, which are positive aspects for their use (Tanaka et al. 1998). Overall, LED technologies present an opportunity for improving plant growth and making systems more sustainable (Darko et al. 2014).

Conclusion

Our study demonstrated the potential of LED lighting in augmenting the production of mini-cuttings, especially with red/far-red LED lamps. The combination of blue and red had a slight positive effect in the coldest months only. A positive aspect of the mixture of red and blue light is a reduced elongation of shoots. Further assessments need to be performed to test distinct quantity combinations of each red and blue LEDs in order to achieve a higher yield of propagules and with sufficient quality for obtaining better performances in the rooting environment. LED-lighting technologies combined with efficient strategies for rooting and acclimation certainly will assist in obtaining high quality plants for the field, sustaining positive impacts on species that are adapted to subtropical climates.

Acknowledgments — We dedicate this article to the memory of our colleague, the late Professor Clenilso Sehnen Mota. This study was funded by Klabin Florestal S.A. The author NSA also received a scholarship from the same company. ERK received a post-doctoral fellowship from the CAPES/PNPD program for the execution of this project. We also thank the staff of Klabin Florestal that operated the harvests of propagules and managed the implementation of the rooting stage of the experiments.

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Ciência Florestal

ISSN 1980-5098
Acesso aberto

Ci. Fl., Santa Maria, v. 31, n. 3, p. 1258-1277, jul./set. 2021 • https://doi.org/10.5902/1980509836857
Submissão: 12/02/2019 • Aprovação: 03/01/2021 • Publicação: 06/09/2021

Artigos

Enraizamento de *Sequoia sempervirens* (Cupressaceae) em função do padrão de miniestacas, substratos e regulador de crescimento

Rooting of *Sequoia sempervirens* in function of the pattern of minicuttings, substrates and use of growth regulator

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RESUMO

Sequoia sempervirens (sequoia) é uma espécie conhecida pela qualidade superior de sua madeira, sendo a produção de mudas por miniestaquia uma alternativa viável. O objetivo do trabalho foi avaliar a posição de coleta, presença ou ausência de folhas aciculares em miniestacas e a produção de mudas cultivadas em diferentes substratos e utilização de AIB. Foram utilizadas miniestacas de diferentes clones, oriundas de minijardim clonal. Para o padrão de miniestacas utilizou-se a porção lignificada, com média lignificação e pouca lignificação, na presença ou ausência de folhas aciculares, avaliando-se um clone. Em relação aos substratos, observou-se o comportamento de três clones e quatro substratos de marcas comerciais. Para o emprego de regulador de crescimento utilizou-se um clone e diferentes concentrações de AIB (0,0 a 5000 mg L-1). As miniestacas foram colocadas para enraizar em estufim e avaliadas aos 90 dias. Todos os experimentos foram instalados em delineamento inteiramente casualizado, com posterior avaliação da porcentagem de sobrevivência; porcentagem de miniestacas com calos; porcentagem de miniestacas enraizadas e número de raízes emitidas por miniestaca enraizada. Miniestacas pouco lignificadas (ápice) com folhas aciculares apresentaram os melhores resultados para sobrevivência (96%) e enraizamento (94%); miniestacas muito lignificadas sem folhas aciculares obtiveram baixo enraizamento (10%). Houve considerável variação de enraizamento entre os substratos (66 a 100%) e o número de raízes foi mais influenciado pelo clone utilizado. Os melhores resultados foram observados naqueles com componentes mais orgânicos. O emprego de AIB na miniestaquia não foi significativo para nenhuma das variáveis analisadas. Miniestacas coletadas em porções apicais e com presença de folhas aciculares mostraram-se mais indicadas para trabalhos com a espécie. Para a seleção do melhor substrato deve-se observar o efeito clonal, priorizando-se substratos com composição mais orgânica. A utilização de AIB não influenciou positivamente o enraizamento de miniestacas, não sendo recomendado o seu uso.

Palavras-chave: Gymnospermae; Propagação vegetativa de conífera; Enraizamento adventício; AIB; Silvicultura clonal

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ABSTRACT

Sequoia sempervirens (sequoia) is a species known for the superior quality of its wood, making plants production by minicuttings a viable alternative. The objective of the work was to evaluate the position of collection, presence or absence of acicular leaves in minicuttings and production of plants grown on different substrates and use of IBA. Minicuttings from different clones, from a clonal minigarden, were used. For the minicuttings pattern, use a lignified portion, with medium lignification and little lignification, in the presence or absence of acicular leaves, available if a clone. Regarding substrates, the behavior of three clones and four substrates of commercial brands. For the growth regulator, was used a clone and different concentrations of IBA (0.0 to 5000 mg L^{-1}). Minicuttings were placed to root in mini-tunnel and evaluated at 90 days. All experiments were installed in completely randomized design, with subsequent assessment of the percentage of survival; percentage of minicuttings with calluses; percentage of rooted minicuttings and number of roots emitted by rooted minicuttings. Little lignified minicuttings (apex) with acicular leaves, showed the best results for survival (96%) and rooting (94%); very lignified minicuttings without acicular leaves obtained low rooting (10%). There was considerable variation in rooting between substrates (66 to 100%) and the number of roots was more influenced by the clone used. The best results were observed in those with more organic components. The use of IBA in the minicutting was not significant for any of the variables analyzed. Minicuttings collected in apical portions and with the presence of acicular leaves are more suitable for work with a species. To select the best substrate, must be observe the clonal effect, prioritizing substrates with a more organic composition. The use of IBA did not positively influence or reduce minicuttings, it was not recommended or its use.

Keywords: Gymnospermae; Vegetative propagation of conifers; Adventitious rooting; IBA; Clonal forestry

1 INTRODUÇÃO

As atuais demandas produtivas, concentradas em algumas espécies florestais, revelam oportunidades para o trabalho com espécies alternativas, visando a um aumento da produção de madeira e abertura para novos mercados. *Sequoia sempervirens* (D. Don) Endl. (Cupressaceae), conhecida popularmente como sequoia, pode ser uma opção para trabalhos em que há exigência de qualidade superior da madeira. É uma conífera nativa da costa oeste do estado da Califórnia e costa sudoeste do estado do Oregon, nos Estados Unidos (OLSON; ROY; WALTERS, 1990).

Sequoia está entre as madeiras mais duradouras dos EUA, possuindo longa durabilidade natural, fácil de ser trabalhada e grã direita com baixa variação dimensional (retração e inchamento). A espécie possui um cerne de grandes proporções e seus extrativos tornam a madeira resistente ao ataque de fungos e insetos (UNITED STATES, 2010).

A espécie apresenta ciclo longo e sua propagação por sementes demonstra baixas taxas de germinação (média de 10%) e baixa viabilidade de plântulas (BOE, 1974). A propagação vegetativa pode ser uma alternativa para a obtenção de mudas de sequoia, por meio da utilização de técnicas variadas. Nos últimos anos, houve grandes avanços no desenvolvimento de sistemas operacionais de propagação vegetativa para espécies de coníferas. A estaquia é considerada o meio mais efetivo e operacionalmente disponível para multiplicar indivíduos específicos que possuem características desejáveis (LUNA, 2008).

A técnica de miniestaquia pode ser utilizada para várias espécies lenhosas de interesse florestal, pois, além dos incrementos em enraizamento, as miniestacas desenvolvem um sistema radicial de melhor qualidade em termos de vigor, uniformidade e volume, o que reflete positivamente na sobrevivência e desempenho da planta no campo (ALCANTARA *et al*., 2007).

Alguns fatores podem aumentar a eficiência dessa técnica, como a utilização de reguladores de crescimento vegetal (ALCANTARA *et al*., 2007), a escolha do tipo de miniestaca e do substrato empregado (PIMENTEL *et al*., 2016). Vários fatores afetam a formação de raízes, estando diretamente relacionados ao sucesso da produção de mudas via propagação vegetativa, como: condições fisiológicas da planta-matriz, balanço hormonal e a posição do propágulo na brotação (OLIVEIRA *et al*., 2016).

Conforme a posição de coleta das miniestacas no ramo é possível classificá-las como apicais, intermediárias ou basais. O uso de diferentes tipos de miniestacas implica maior aproveitamento das brotações colhidas, aumentando a produtividade do minijardim (OLIVEIRA *et al*., 2016). O tipo de miniestaca utilizado vai influenciar no enraizamento, devendo-se observar a presença e o número de folhas, que constituem fonte de auxinas e carboidratos importantes no processo de enraizamento (HARTMANN *et al*., 2011).

Além da preocupação com a posição de coleta e do tipo de miniestaca utilizado, também é importante considerar a utilização de um substrato adequado. O substrato utilizado para o enraizamento de estacas é um fator de grande importância na propagação vegetativa, sendo variável de acordo com a espécie a ser propagada e devendo permitir bom suprimento de oxigênio e água para a base da estaca. O substrato mais adequado deve ser inerte, poroso, com boa drenagem e capaz de manter a aeração e a umidade, permitindo o bom desenvolvimento do sistema radicial (OLIVEIRA *et al*., 2012).

Outro aspecto importante relacionado à composição do substrato é que as espécies arbóreas têm necessidades nutricionais e fisiológicas específicas, tornando importante a realização de estudos para definir o melhor substrato para propagação de cada espécie. Diversas são as opções de tipos de substrato, sendo os mais comuns nos viveiros de produção de mudas: areia, turfa, serragem semidecomposta, casca de arroz carbonizada, composto orgânico, terra de subsolo, fibras de coco, vermiculita e mistura entre eles (XAVIER; WENDLING; SILVA, 2013).

A utilização de um promotor de enraizamento também é um fator importante a ser considerado. À medida que a idade da planta doadora aumenta, a aplicação de auxinas pode maximizar o sucesso no enraizamento (LUNA, 2008). Aplicações de reguladores de crescimento aos propágulos vegetativos, principalmente auxinas, proporcionam maior porcentual, velocidade e qualidade de enraizamento. A auxina sintética mais utilizada para o enraizamento de estacas ou miniestacas é o ácido indolbutírico (AIB), apresentando diferentes resultados conforme a espécie, época do ano, concentração e condições ambientais (HARTMANN *et al*., 2011).

A partir disso, o objetivo deste trabalho foi avaliar a posição de coleta e presença ou ausência de folhas aciculares em miniestacas de *Sequoia sempervirens*, além da produção de mudas sob cultivo em diferentes substratos e utilização de AIB.

2 MATERIAIS E MÉTODOS

2.1 Fonte de material reprodutivo

Como fontes de propágulos para a miniestaquia foram utilizadas minicepas de matrizes selecionadas de sequoia de aproximadamente 40 anos de idade, localizadas em São Francisco de Paula - RS, as quais foram propagadas pelo processo de estaquia convencional após 120 dias do anelamento parcial ou completo da árvore matriz. Esse material foi coletado nas árvores-matrizes e transportado até o Viveiro Florestal do Centro de Ciências Agroveterinárias (CAV) da Universidade do Estado de Santa Catarina – UDESC. Após, confeccionaram-se estacas com tamanho de 10 cm, contendo um par de folhas aciculares reduzidas a 50 % da área foliar e imersas em solução hidroalcóolica de AIB (6.000 mg L^{-1}) durante 10 segundos. Em seguida, foram inseridas em tubetes (180 cm^{-3}) contendo vermiculita de granulometria média e substrato comercial (1:1 v/v) à base de turfa, casca de arroz carbonizada e calcário calcítico e dispostas em casa de vegetação para o enraizamento.

Após a estaquia, mudas enraizadas de cinco meses de idade foram aclimatadas em casa de sombra por 30 dias, sendo em seguida transferidas para casa de vegetação para completar a aclimatação por mais 30 dias. Posteriormente, mudas com aproximadamente 20 cm de altura foram transferidas para vasos com capacidade de 5L, preenchidos com substrato comercial (50%) e vermiculita de granulometria média (50%). Quinze dias após a instalação, tempo necessário para adaptação das mudas ao sistema, efetuou-se a poda do ápice da brotação principal das mudas a uma altura de 10 cm (± 2 cm), formando-se assim as minicepas para o estabelecimento do minijardim clonal. Mensalmente, durante quatro meses, realizou-se poda de formação das minicepas.

A solução nutritiva utilizada no minijardim clonal foi à base do fertilizante comercial Forth Solúvel Inicial®, composto por 10% N (solúvel em água); 42 % P_2O_5

(solúvel em água); 10% K_2O (solúvel em água); 0,6% Mg; 0,1% Fe e 0,02 Br. A fertirrigação era realizada duas vezes por semana, tendo cada minicepa recebido 50 mL da solução composta pela diluição de 1,5 gramas do fertilizante por litro de água. Após o período de formação das minicepas efetuou-se a coleta das brotações para a realização dos experimentos.

2.2 Experimento I – padrão de miniestacas

O experimento foi conduzido em delineamento inteiramente casualizado (DIC), com seis tratamentos (porção lignificada, com média lignificação e pouca lignificação; com ou sem folhas aciculares) (Figura 1), compostos por 13 repetições de seis miniestacas cada. Para isso, foi utilizando o clone A138, obtido do minijardim clonal, o qual foi produzido e conduzido conforme descrito anteriormente. Considerou-se como miniestacas lignificadas as localizadas nas porções basais que apresentam idades superiores, média lignificação em porções intermediárias e pouca lignificação em porções apicais.

Foram preparadas miniestacas com aproximadamente 10 cm de comprimento, sendo a porção basal cortada em bisel e a porção superior incisada transversalmente, com presença ou ausência de folhas aciculares. Nos tratamentos em que havia a presença de folhas, estas foram cortadas pela metade, à exceção das folhas presentes na parte apical das miniestacas pouco lignificadas.

Após preparadas, as miniestacas foram dispostas para enraizar em tubetes de 180 cm^3 contendo vermiculita de granulometria média e substrato comercial (1:1 v/v), com a adição de 6 g L^{-1} de fertilizante de liberação controlada (18-05-09) com 5-6 meses de liberação. O substrato comercial foi composto por turfa, casca de arroz carbonizada, calcário calcítico, aditivado com N (0,04%), P_2O_5 (0,04%) e K_2O (0,05%) (característica físicas e químicas descritas em substrato I – Tabela 1).

Figura 1 – Miniestacas na presença e ausência de folhas aciculares conforme posições estabelecidas, sendo: A. pouco lignificada com presença de folhas; B. média lignificação com presença de folhas; C. lignificada com presença de folhas; D. pouco lignificada com ausência de folhas; E. média lignificação com ausência de folhas; F. lignificada com ausência de folhas



Fonte: Autores (2017)

Em que: *As barras representam 2 cm de comprimento.

2.3 Experimento II – avaliação de diferentes clones e substratos

O experimento foi instalado em delineamento inteiramente casualizado (DIC), em esquema fatorial 3 x 4, utilizando-se nove repetições com seis miniestacas cada. O fator A foi composto por três diferentes clones (A113, A140 e A228) e o fator D por quatro substratos (marcas comerciais) denominadas no presente trabalho como substrato I, II, III e IV. A caracterização física e química do substrato (Tabela 1) foi determinada no Laboratório de Substratos do Departamento de Horticultura e Silvicultura da Universidade Federal do Rio Grande do Sul, conforme a Instrução Normativa nº 17 do Ministério da Agricultura, Pecuária e Abastecimento.

As composições de cada substrato, conforme especificação dos fabricantes, são as seguintes: substrato I - turfa, casca de arroz carbonizada, calcário calcítico, aditivado com N (0,04%), P_2O_5 (0,04%) e K_2O (0,05%); substrato II - casca de pinus compostada, casca de arroz carbonizada, fibra de coco, vermiculita, aditivado com calcário (0,2%) e

superfosfato triplo (0,1%); substrato III - turfa, vermiculita expandida, casca de pinus e carvão vegetal; substrato IV - turfa de Sphagnum, vermiculita, resíduo orgânico agroindustrial classe A, calcário dolomítico, gesso agrícola e fertilizante NPK (traços).

Tabela 1 – Análise física e química dos substratos utilizados na miniestaquia de *Sequoia sempervirens*

Atributos analisados	Substrato I	Substrato II	Substrato III	Substrato IV
DU (kg m^{-3})	614,8	515,8	651,1	282,86
DS (kg m^{-3})	292,8	226,4	392,7	133,1
UA (%)	52,4	56,10	39,7	52,9
PT (%)	84,2	89,3	78,9	87,7
EA (%)	20,0	39,4	25,2	31,2
AFD (%)	21,3	13,02	14,20	21,2
AT (%)	4,7	2,0	3,7	4,4
AR (%)	38,2	34,84	35,9	30,9
CRA10 (%)	64,2	49,8	53,8	56,5
CRA50 (%)	42,9	36,8	39,6	35,3
CRA100 (%)	38,2	34,8	35,9	31,0
pH	5,6	5,69	6,46	6,26
CE (mS cm^{-1})	1,39	0,26	0,27	0,11

Fonte: Autores (2017)

Em que: DU = densidade úmida; DS = densidade seca; UA = umidade atual; PT = porosidade total; EA = espaço de aeração; AFD = água facilmente disponível; AT = água tamponante; AR = água remanescente; CRA10, 50 e 100 = capacidade de retenção de água sob sucção a 10, 50 e 100 cm de coluna de água; pH = potencial hidrogeniônico; CE = condutividade elétrica.

Os clones selecionados para o estudo tiveram suas brotações seccionadas e procedeu-se o preparo das miniestacas, cada uma com aproximadamente dez cm de comprimento. A porção basal foi cortada em bisel e a porção superior incisada transversalmente, mantendo-se um par de folhas aciculares cortadas pela metade. Estas foram dispostas para enraizar em tubetes de 180 cm^3, contendo o substrato definido conforme o tratamento, adicionando-se 6 g L^{-1} de fertilizante de liberação controlada (18-05-09) com 5-6 meses de liberação.

2.4 Experimento III - concentrações de AIB no enraizamento de miniestacas

Foi utilizado o clone A228, sendo o experimento conduzido em delineamento inteiramente casualizado (DIC), com seis tratamentos, compostos pelas diferentes concentrações de AIB em mg L^{-1} (0,0; 1000; 2000; 3000; 4000 e 5000). Foram utilizadas 11 repetições de 10 miniestacas cada. As miniestacas tiveram sua base imersa em soluções hidroalcoólicas (50% v/v) com diferentes concentrações de AIB, permanecendo em contato com a solução por um período de 20 segundos. Foi utilizado o substrato comercial à base de turfa, casca de arroz carbonizada, calcário calcítico - substrato I (Tabela 1) adicionando-se 6 g L^{-1} de fertilizante de liberação controlada (18-05-09) com 5-6 meses de liberação.

2.5 Condições de cultivo, variáveis e análise estatística

As bandejas contendo os tubetes com as miniestacas foram acondicionadas em estufim de cobertura plástica (estrutura apresentando 8,0 m de comprimento x 1,20 m de largura x 0,9 m de altura). A temperatura no interior do estufim variou entre 20-32ºC e a umidade relativa do ar manteve-se superior a 80%, sendo a irrigação realizada por microaspersão durante cinco minutos, cinco vezes por dia. As miniestacas permaneceram nesta condição por 90 dias, momento em que foi realizada a avaliação do experimento.

Nos três experimentos, avaliou-se a porcentagem de sobrevivência; porcentagem de miniestacas que apresentavam calos; porcentagem de miniestacas enraizadas e número de raízes emitidas por miniestaca enraizada. Foram consideradas sobreviventes as miniestacas que apresentavam lenho vivo, folhas velhas ou brotações jovens, enraizadas ou não. Para calos foram consideradas as miniestacas vivas, com formação de massa celular indiferenciada na base, enraizadas ou não. A porcentagem de enraizamento foi considerada sobre o total, não somente sobre as miniestacas sobreviventes. Consideraram-se como enraizadas aquelas com indução de primórdios radiculares de no mínimo 1,0 mm de comprimento.

Após a verificação da normalidade dos dados por meio do teste de Kolmogorov-Smirnov e da homogeneidade pelo teste de Bartlett, realizou-se a análise de variância. Quando necessário, os dados foram transformados pela função $(x + 0,5)^{0,5}$ e as médias foram comparadas pelo teste de Scott-Knott, a 5% de probabilidade. Para as análises foi utilizado o pacote estatístico SISVAR (FERREIRA, 2011).

3 RESULTADOS E DISCUSSÃO

3.1 Experimento I - padrão de miniestacas

Miniestacas pouco lignificadas (obtidas do ápice) e com presença de folhas aciculares apresentaram maior percentagem de sobrevivência (95,7%) (Tabela 2), havendo interação entre o grau de lignificação e a presença de folhas para esta variável. Já para a formação de calos, não houve efeito significativo para nenhum dos fatores, sendo a média geral de 89%.

O enraizamento seguiu o mesmo padrão da sobrevivência, em que miniestacas obtidas de tecidos menos lignificados e com presença de folhas aciculares apresentaram melhores resultados. Na utilização de miniestacas de tecidos muito lignificados e sem folhas, houve um enraizamento inferior a 10%. Miniestacas coletadas da porção intermediária apresentaram grande variação, obtendo-se enraizamento de 74,1% na presença de folhas e resultado de 16,1% na ausência destas. Essa mesma tendência foi observada para o número de raízes. Miniestacas originárias do ápice ou da posição intermediária e com a presença de folhas aciculares apresentaram o maior número de raízes, com valores entre 7 a 9 raízes.

Para o padrão das miniestacas de sequoia é visível a importância da manutenção das folhas aciculares, principalmente com média ou baixa lignificação (miniestacas apicais). A maior porcentagem de sobrevivência e enraizamento destas miniestacas pode ser explicada pela maior concentração de auxinas endógenas neste

local (DANTAS *et al*., 2016), uma vez que este fitormônio é produzido por tecidos meristemáticos e transportado de forma basípeta pelas células parenquimáticas para a base das miniestacas (COSTA *et al*., 2013). Do mesmo modo, a menor sobrevivência e enraizamento das miniestacas na ausência de folhas aciculares provavelmente se deve à falta de produção de carboidratos resultantes da fotossíntese e das auxinas produzidas nas folhas e gemas apicais, que são essenciais para o enraizamento e, consequentemente, sua sobrevivência (XAVIER; WENDLING; SILVA, 2013). Muitas vezes essas auxinas são suficientes para estimular o enraizamento de estacas apicais, dispensando o uso exógeno.

Tabela 2 – Sobrevivência (%), enraizamento (%) e número de raízes de miniestacas de S*equoia sempervirens* em função de diferentes padrões de miniestacas (posição/grau de lignificação e presença ou ausência de folhas) obtidas do clone A138

Variável	**Posição/grau de lignificação**	**Folhas aciculares**		
		Presença	**Ausência**	**Média**
Sobrevivência (%)	Base (lignificada)	56,1 cA	20,1 bB	38,1
	Intermediário (média lignificação)	80,4 bA	24,0 bB	52,2
	Ápice (pouco lignificada)	95,7 aA	71,3 aB	83,5
	Média	77,4	38,5	
Enraizamento (%)	Base (lignificada)	39,5 cA	8,7 bB	24,1
	Intermediário (média lignificação)	74,1 bA	16,1 bB	45,1
	Ápice (pouco lignificada)	94,3 aA	71,3 aB	82,8
	Média	69,3	32,0	
Nº de raízes	Base (lignificada)	3,1 bA	1,6 bB	2,3
	Intermediário (média lignificação)	7,4 aA	2,6 bB	5,0
	Ápice (pouco lignificada)	8,8 aA	6,4 aB	7,6
	Média	6,4	3,5	

Fonte: Autores (2017)

Em que: * Médias seguidas pela mesma letra maiúscula na linha e minúscula na coluna não diferem entre si pelo teste de Scott-Knott, a 5% de probabilidade de erro. p- valor da interação para sobrevivência: 0,002. CV (%): 26,6; p- valor da interação para enraizamento: 0,001. CV (%): 32,2; p- valor da interação para nº de raízes: 0,0017. CV (%): 22,1.

Na ausência de folhas aciculares, observou-se uma sobrevivência de 71,3% utilizando-se miniestacas apicais, porém com valores inferiores à presença de folhas (94,3%). A utilização de miniestacas muito lignificadas provocou elevada mortalidade, principalmente na ausência de folhas aciculares. Dessa forma, não se recomenda a utilização de miniestacas de galhos muito lignificados, geralmente obtidas da base dos galhos das minicepas. Esse resultado também favorece a operacionalização na confecção das miniestacas, na qual a manutenção das folhas aciculares acelera o processo.

Resultados superiores para o enraizamento de miniestacas apicais ou menos lignificadas também foram obtidos em outras espécies, como em *Araucaria angustifolia* (WENDLING; STUEPP; ZUFFELLATO-RIBAS, 2016), *Cariniana estrellensis* (HERNÁNDES *et al*., 2013), *Peltophorum dubium* (MANTOVANI *et al*., 2017) e *Piptadenia gonoacantha* (HERNÁNDES *et al*., 2012). Ferreira *et al*. (2008) verificaram maior enraizamento de estacas apicais de atemoia "Gefner" (*Annona cherimola* x *A. squamosa*), relacionando esses resultados com a menor lignificação de ramos apicais, maior quantidade de células meristemáticas e um metabolismo mais ativo em comparação com outros tipos de estacas.

Considerando-se a preparação das miniestacas em um sistema de propagação clonal, o uso de miniestacas apicais é preferível em relação às basais ou intermediárias, principalmente por não haver necessidade de remover o ápice. Essa prática também reduz a possibilidade de formação de brotos múltiplos, reduzindo a necessidade de trabalho na preparação dos propágulos e manejo das miniestacas para formar as mudas (WENDLING; BRONDANI, 2015). Neste estudo também foi possível observar esse comportamento, em que miniestacas apicais originaram mudas com características ortotrópicas e sem brotos múltiplos (Figura 2a) em relação às miniestacas de origem basal ou intermediárias do ramo (Figura 2b).

Figura 2 – a) Miniestaca obtida da porção apical, apresentando crescimento ortotrópico; b) miniestaca obtida da parte basal, apresentando múltiplas brotações



Fonte: Autores (2017)

3.2 Experimento II – avaliação de diferentes substratos e clones

Na miniestaquia de sequoia em função do uso de diferentes substratos e clones não houve diferença significativa para nenhum dos fatores relacionados à sobrevivência (média: 97,9%) e à formação de calos (média: 95,6%). Já para o enraizamento (p=0,016) e número de raízes (p=0,0001) houve interação entre os fatores.

Considerando-se todos os clones indistintamente, houve variação nas percentagens de enraizamento para os substratos utilizados, com valores entre 66 e 100% (Tabela 3). Para os clones A113 e A140, as diferenças entre os substratos não foram significativas, variando entre 84,5 a 100%. Miniestacas oriundas do clone A228 apresentaram maiores percentagens de enraizamento quando cultivadas nos substratos com maior densidade e menor porosidade, que apresentam composição

mais orgânica (turfa, casca de pinus, carvão vegetal) (substrato I e III), não havendo diferenças significativas. Os substratos II e IV apresentaram aproximadamente 50% menos raízes. Silva, Simões e Silva (2012) também destacam os efeitos de diferentes composições de substratos e de suas propriedades físicas e químicas sobre o enraizamento e a qualidade de mudas clonadas, principalmente em clones e/ou espécies difíceis de se propagarem vegetativamente.

O número de raízes foi mais influenciado pelo clone do que pelo substrato utilizado. A média geral para o clone A113 foi 57% menor que para o clone A140 e 43% menor que para o clone A228. Para o clone A113, não houve diferença significativa entre os substratos; já para os clones A140 e A228, o número de raízes mostrou diferenças estatísticas entre os substratos. Independentemente dos clones estudados, o substrato III apresentou a maior formação de raízes, sendo 35% melhor que o substrato II, que apresentou os menores valores.

Tabela 3 – Enraizamento (%) e número de raízes em miniestacas de S*equoia sempervirens* em função dos diferentes clones e substratos utilizados

Variável	Substrato	Clone			Média
		A113	A140	A228	
Enraizamento (%)	Substrato I	90,0 aA*	100,0 aA	93,3 aA	94,4
	Substrato II	84,5 aA	97,8 aA	67,8 bB	76,1
	Substrato III	94,4 aA	98,9 aA	97,8 aA	97,0
	Substrato IV	92,1 aA	90,0 aA	66,7 bB	82,9
	Média	90,2	96,6	81,4	
Número de raízes	Substrato I	4,7 aB	10,5 bA	9,8 bA	8,3
	Substrato II	4,0 aB	11,4 aA	4,8 cB	6,7
	Substrato III	5,2 aB	13,1 aA	12,5 aA	10,3
	Substrato IV	4,9 aB	9,5 bA	6,1 cB	6,8
	Média	4,7	11,1	8,3	

Fonte: Autores (2017)

Em que: * Médias seguidas pela mesma letra minúscula na coluna e maiúscula na linha não diferem entre si pelo teste de Scott-Knott, a 5% de probabilidade de erro. ^{ns} - não significativo a 5% de probabilidade de erro. CV% enraizamento: 17,3; CV% número de raízes: 28,9.

Ao contrário do que é recomendado na miniestaquia para várias espécies florestais, como *Piptocarpha angustifolia (*STUEPP *et al*., 2016); *Eucalyptus urophylla* x *E. grandis* (SILVA; SIMÕES; SILVA, 2012) e *Ilex paraguariensis* (KRATZ *et al*., 2015), o clone A228 da *Sequoia sempervirens* não se adaptou (Tabela 3) a compostos leves e com elevada porosidade, com a presença de fibra de coco, vermiculita e turfa de Sphagnum (Tabela 1). Esses componentes têm sido cada vez mais utilizados na composição de substratos para propagação vegetativa de plantas, proporcionando excelente drenagem, boa oxigenação de raízes, estabilidade estrutural relativa e baixa densidade (CALDEIRA *et al*., 2013). Contudo, diferenças podem ocorrer entre as espécies e clones, como observado neste estudo. Para *Piptadenia gonoacantha*, as maiores médias de sobrevivência e enraizamento de estacas foram obtidas quando cultivadas no substrato composto por material orgânico (HERNÁNDES *et al*., 2012), corroborando os resultados obtidos para o clone A228.

Outro fator facilmente observado é o efeito clonal na formação de raízes, no qual o clone A113 apresentou resultados bem inferiores aos demais clones, independentemente do substrato utilizado. Esse resultado confirma a hipótese de Assis e Teixeira (1999) de que o genótipo é um dos fatores que influenciam o enraizamento e que há uma grande variação entre espécies, cultivares e clones em relação à maior ou menor habilidade natural para formar raízes. Resultados semelhantes foram encontrados em *Castanea sativa* (DANTAS *et al*., 2016) e *Acacia mearnsii* (ENGEL *et al*., 2017), cujo enraizamento é variável em função de clones ou genótipos.

O substrato tem papel fundamental no enraizamento adventício, não tendo apenas a função de suporte, devendo também apresentar características que favoreçam o desenvolvimento radicular. Contudo, o sucesso da propagação por miniestaquia não é determinado apenas pelo substrato utilizado, dependendo também de diversos outros fatores, tais como: controle das condições ambientais no local de propagação, tipos de miniestacas, nutrição das minicepas (DIAS *et al*., 2015) e resposta do material genético ao enraizamento adventício.

3.3 Experimento III - concentrações de AIB no enraizamento de miniestacas

A utilização de AIB na miniestaquia de sequoia não apresentou diferença significativa em nenhuma das variáveis avaliadas. A sobrevivência das miniestacas apresentou média de 92%, variando entre 88% e 95%. O enraizamento apresentou média de 89%, com baixa variação entre os tratamentos, ficando entre 87% e 91%. A formação de calos foi elevada, com média de 95%. Foram encontradas em média 5,6 raízes por miniestaca.

A ausência de efeito significativo da auxina AIB no enraizamento das miniestacas pode indicar que esta espécie apresenta um elevado potencial rizogênico natural. Condições fisiológicas adequadas para a iniciação radicial também podem ter sido induzidas pelo sistema de cultivo em minijardim clonal, que favorece a formação de brotos rejuvenescidos e revigorados e pela nutrição fornecida. Resultados semelhantes foram observados por Mantovani *et al*. (2017), trabalhando com *Peltophorum dubium* e em outras coníferas, como: *Pinus patula* (APARICIO-RENTERÍA *et al*., 2014) *Pinus pinaster* (MAJADA *et al*., 2011), e *Taxodium distichum* (KING; ARNOLD; WELSH, 2011). Em muitos casos, a aplicação de AIB pode ter uma influência indireta, aumentando a velocidade de translocação e movimento de carboidratos para a base de estacas e, consequentemente, estimulando o enraizamento.

4 CONCLUSÕES

Miniestacas apicais e com presença de folhas aciculares apresentaram maior sobrevivência, enraizamento e número de raízes, além de uma melhor formação das mudas.

Os clones A113 e A140 não apresentaram diferença na porcentagem de enraizamento com os substratos testados. O clone A228 obteve maior porcentagem de enraizamento e número de raízes, utilizando-se substratos com maior densidade e composição mais orgânica.

A utilização de AIB não proporcionou um acréscimo no enraizamento das miniestacas para o clone testado, não sendo recomendado o seu uso.

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Como citar este artigo

Pereira, M. O.; Navroski, M. C.; Ângelo, A. C.; Schafer, G.; Andrade, R. S.; Moraes, C.; Souza, G. Enraizamento de Sequoia sempervirens (Cupressaceae) em função do padrão de miniestacas, substratos e regulador de crescimento. Ciência Florestal, Santa Maria, v. 31, n. 3, p. 1258-1277, 2021. DOI 10.5902/1980509836857. Disponível em: https://doi.org/10.5902/1980509836857. Acesso em: xx mês-abreviado. 2021.

GENETIC VARIABILITY IN JUVENILE CHARACTERS OF PROGENIES OF *Apuleia leiocarpa*

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Received for publication: 29/09/2019 – Accepted for publication: 19/03/2021

Resumo

Variabilidade genética em caracteres juvenis de progênies de Apuleia leiocarpa. O objetivo desta pesquisa foi quantificar a variabilidade genética em progênies oriundas de matrizes de grápia em populações naturais, para caracteres do crescimento inicial de mudas. Coletou-se sementes de 13 matrizes, de quatro procedências, localizadas nos municípios de Pareci Novo, São José do Sul e Aratiba no RS, e de Seara em SC, assim como os dados das plantas e do local de origem. Foi realizada a biometria em um lote de 100 sementes de cada matriz, e instalado um experimento para avaliar as progênies, em delineamento inteiramente casualizado. Avaliou-se a velocidade de emergência das plântulas e a porcentagem de emergência, o diâmetro do coleto e altura das mudas, assim como a porcentagem final de sobrevivência. Foi encontrada diferença significativa para todas as variáveis observadas para sementes e mudas das matrizes de grápia. Comprimento e largura de sementes demonstraram valores altos para herdabilidade individual no sentido restrito (acima de 0,6), sendo possível utilizar o tamanho da semente para a análise de variabilidade genética entre plantas da espécie, mas não como variável indicativa de qualidade fisiológica e vigor. A análise de correlação indicou que a porcentagem de emergência das plântulas, tem relação com as características da planta matriz (altura e diâmetro). Assim, é adequado o uso das mudas para áreas de restauração florestal, pois apresentam indicativos de diversidade genética.

Palavras-chave: Grápia. Biometria de sementes. Parâmetros genéticos.

Abstract

The objective of this search was to quantify the genetic variability in progenies from grápia mother trees in natural populations, for initial growth characters of the seedlings. Seeds were collected from 13 mother trees, from four origins, located in the municipalities of Pareci Novo, São José do Sul and Aratiba in RS, and Seara in SC, as well as data from plants and place of origin. Biometrics were performed on 100 seeds lot from each mother tree, and an experiment was installed to evaluate progenies in a completely randomized design. It was evaluated the seedling emergence speed and the emergence percentage, the stem diameter and seedlings height, as well as the final survival percentage. A significative difference was found for all variables observed for seeds and seedlings of grápia mother trees. Seed length and width demonstrated high values for individual heritability in the narrow sense (above 0.6), being possible to use the seed size for an analysis of genetic variability between plants of the species, but not as an indicative variable of physiological quality and vigor. The correlation analysis indicated that the seedling emergence percentage is related to the characteristics of the mother plant plant (height and diameter). Thus, the use of seedlings for forest restoration areas is appropriate, as they have indications of genetic diversity.

Keywords: Grápia. Seed biometrics. Genetic parameters.

INTRODUCTION

Apuleia leiocarpa (Vogel) J. F. Macbride (2*n*=26) is a species of the Fabaceae family native to Brazil, vulnerable to extinction and known as the queen of forests in the southern region of the country. Popularly called grápia, it grows in the Deciduous Seasonal Forest and reaches large dimensions, with an average height of 25-35 meters and a diameter of 60-90 centimeters (LORENZI, 2008). The species has wood potential due to its dimensions and wood characteristics, with a density of 0.75 to 1.00 g cm^{-3}, yellowish color and high durability. It also has apiculture potential, landscaping, medicinal use in areas of forest restoration and use in the tanning industry, considering that its bark reaches up to 24% of tannins (MARCHIORI, 1997).

The species has phenological stages at similar times in the southern states, blooming during spring and bearing fruit during summer (FELIPPI *et al*, 2012). Monoecious plant, it has white or cream colored flowers, masculine and hermaphroditic, predominantly allogamous and with pollination mainly by bees. It has the formation of inflorescences arranged in panicles, the fruit being an ovoid pod, indehiscent and flat, dispersed by wind, having only one seed per fruit, hard, flat and dark brown in color (BACKES; IRGANG, 2002).

The collection of seeds and the production of grápia seedlings is affected by fruit productivity in irregular periods, taking two or more years to obtain a satisfactory harvest (FELIPPI *et al*., 2012). Brancalion *et al.* (2009), mention that forest restoration plantations rarely include endangered species, such as grápia, especially due to the difficulty in obtaining seeds and seedlings from these individuals. This variation in seed production is linked to environmental conditions, as well as the variability that exists naturally within populations and between individuals. Within a population, individuals who have favorable genotypes (to that situation) are more likely to perpetuate themselves, having reproductive and adaptive success. Tree species generally have a relatively high degree of genetic variation, when compared to other plants and animal species (HAMRICK; GODT, 1989).

The limitation between the gene flow of plant populations and the selection pressures suffered over time give population groups distinct genetic characteristics, verifying that the genetic variation of the species is associated with their geographic distribution. The gene flow via pollen and seeds has been shown to be associated with the genetic structure of populations of tree species, in which the flight distance of pollinators and dispersers is indicative of the spatial structure of the genetic diversity of populations (KAGEYAMA; LEPSCH-CUNHA, 2001).

Knowledge about the ecology and genetic structure of natural populations of tree species in tropical regions is scarce, but essential to define strategies for their conservation, genetic improvement and sustainable management (KONZEN, 2014; KUBOTA *et al*., 2015a). Knowing the genetic variability of native species, especially those that are vulnerable to extinction, such as grápia, become of relevance, for the production of seedlings and use of the species in forest restoration (SANT'ANA *et al*., 2013). Thus, the objective of this study was to quantify the existence of genetic variability of grápia progenies for characters of the initial growth of seedlings.

MATERIAL AND METHODS

Seed collection area

The pre-marked progenies of grápia come from the Germplasm Rescue and Bank Formation Project of the Transmission Line (LT) 525 kV Salto Santiago – Itá – Nova Santa Rita. Among the 40 mother trees marked in the project, 13 were used in this study due to the time of collection and production of seeds. For the collection of open pollination seeds, the minimum distance of 100 meters between the trees was respected, in order to minimize the risk of sampling related individuals, seeking genetic and physical-physiological quality of the seeds.

Tabela 1. Altura e diâmetro à altura do peito (DAP) das matrizes de *Apuleia leiocarpa* (AL) e municípios onde estão localizadas.

Table 1. Height and diameter at breast height (DBH) of *Apuleia leiocarpa* (AL) mother trees and municipalities where they are located.

Mother tree	Height (m)	DBH (cm)	Origin
AL 7	19.0	44.9	Pareci Novo, RS
AL 8	15.0	32.2	Pareci Novo, RS
AL 9	23.0	28.3	São José do Sul, RS
AL 14	21.0	71.0	São José do Sul, RS
AL 29	28.0	69.4	São José do Sul, RS
AL 30	21.0	59.8	São José do Sul, RS
AL 31	27.0	58.3	São José do Sul, RS
AL 32	28.0	61.8	São José do Sul, RS
AL 33	20.0	33.1	São José do Sul, RS
AL 34	24.0	36.6	São José do Sul, RS
AL 36	12.0	48.4	São José do Sul, RS
AL 37	35.0	88.5	Aratiba, RS
AL 38	37.0	82.8	Seara, SC

The mother trees marked in the project are visibly healthy, without attack by pests and mechanical damage to the trunk. Dendrometric data were collected from each mother tree (Table 1), as well as climatic data from the

municipality they are located: Aratiba (RS) - average temperature 19.4 ºC, altitude 423 m and 1,833 mm of annual precipitation; Pareci Novo (RS) - average temperature 19.6 ºC, altitude 33 m and 1,418 mm of annual precipitation; São José do Sul (RS) - average temperature 19.4 ºC, altitude 104 m and 1,442 mm of annual precipitation; Seara (SC) - average temperature 18.8 ºC, altitude 551 m and 1,981 mm of annual precipitation.

In the areas the mother trees are located soil collection was carried out at a depth of 0-10 cm with a Dutch type auger, composing representative composite samples, in order to carry out the chemical characterization of the soil in each area. For the mother trees AL 07, AL 08, AL 09, AL 14, AL 37 and AL 38, samples were collected close to the individual, with a sample for each mother tree. A sample was carried out in the area where the mother trees AL 29, AL 30, AL 31 and AL 32 are located, and another sample in the area where the mother trees AL 33, AL 34 and AL 36 are located.

Fruit collection and processing

The collections were carried out in January/2018 and March/2018. The brown colored fruits were collected, stored in plastic bags, identified and taken to the Forest Nursery of the University of the State of Santa Catarina (UDESC), in the municipality of Lages, SC. They remained in a fresh and ventilated place for drying for 20 days. The extraction of seeds occurred manually, opening the pod for their removal. The wilted, attacked by fungi or predated seeds were discarded, and the visually healthy seeds were stored in a paper container, in a refrigerator at 8 ºC, until April/2018. According to the Köppen classification, the climate in the municipality of Lages/SC is humid mesothermal with mild summer (Cfb-temperado). The average annual temperature is 15 °C and the average annual precipitation varies between 1,300 to 1,500 mm.

A sample of 100 seeds from each mother tree was randomly selected for biometric measurements. The measures of length and width of the seeds were obtained with the use of a digital caliper (0.01 mm).

Dormancy overcome and experimental design

The grápia seeds were submitted to mechanical scarification manually with sandpaper No. 120, to overcome integumentary dormancy. After this process, the seeds were washed in distilled water and dried on paper towels. In order to standardize and favor the process of soaking in water, it was decided to place the seeds in Gerboxes in a germinator, before sowing in the nursery. The seeds were put in the Gerbox box under Germitest® paper, spaced and then covered with another sheet of Germitest® paper, both moistened with distilled water. The boxes, with 50 seeds each were deposited in a BOD (*Biochemical Oxigen Demand*) germinator for 30 hours, regulated at 25 ºC and a photoperiod of 12 h. During this period, the humidity of the Germitest® paper was checked every 8 h to avoid complete drying.

After 30 hours in BOD, the boxes with the seeds were taken to the Forest Nursery - CAV. Sowing was done in 175 cm³ polyethylene tubes, using commercial substrate based on composted pine bark, vermiculite and basic fertilization, enriched with 6 g L^{-1} of controlled release fertilizer (15-09-12, 5 M). The seeds were put on the substrate and covered with 15 ml of medium-sized expanded vermiculite. All seeds received equal coverage to not influence the emergence and evaluation process. The grids with the tubes remained in a greenhouse with a temperature of 20 to 30 ºC, irrigation by micro sprinkler with four irrigations a day for 10 minutes, reduced to 3 times a day in the winter period for 5 minutes. The experimental design was completely randomized with eight replications of 17 plants each, with 13 treatments (mother trees).

In addition to seed biometrics, it was evaluated as well the seedling emergence speed, emergence percentage, seed diameter and seedling height and the final percentage of survival was. The emergence evaluation was performed daily, considering as emerged seedlings those that presented the cotyledonary loop above the substrate.

The calculation of the emergence speed index (ESI) was performed according to the formula proposed by Maguire (1962):

$$\mathrm{ESI} = \left(\frac{\mathrm{E1}}{\mathrm{N1}}\right) + \left(\frac{\mathrm{E2}}{\mathrm{N2}}\right) + \cdots + \left(\frac{\mathrm{En}}{\mathrm{Nn}}\right)$$

As for: ESI = emergence speed index; E = number of seedlings emerged each day, and; N = number of days elapsed from sowing to the last germination count.

The seedling emergence percentage was evaluated in conjuction with the last emergence count, on the 18th after sowing. Normal seedlings were considered for this calculation. At 210 days, the height and diameter at neck height of the seedlings was measured. For height, a 60 cm ruler was used, and for the diameter, a digital caliper (0.01 mm). Afterwards, the height/diameter at the neck height (H/D) and the percentage of seedling survival were calculated.

Statistical analysis

After analyzing the data of the observed variables, confirming normality using the Kolmogorov-Smirnov test (p>0.05) and homogeneity using the Bartlett test (p>0.05), the data were submitted to analysis of ANOVA variance and when significant, the Scott-Knott averages test (p<0.05) was performed, with the aid of the Sisvar statistical software.

Pearson's correlation analysis was performed for the variables: mother tree height, mother tree diameter, average temperature and soil characteristics (pH, Ca, Mg, Al, MO, P, K and CEC) of the mother tree location, seed width and length, ESI, percentage of emergence, percentage of survival, diameter at the neck height and height of seedlings.

The genetic analysis was performed with the aid of the *software* Statistical System and Computerized Genetic Selection (SELEGEN) (RESENDE, 2007). For the seed length and width variables, the statistical model used was: $y = Xu + Za + e$, as y is the data vector, u is the scalar referring to the general mean (fixed effect), a is the vector of the effects individual genetic additives (assumed to be random), e is the vector of errors or residues (random).

For the estimation of the genetic parameters of the variables ESI, percentage of emergence, diameter of the collection and height of the seedlings, the statistical model used was: $y = Xu + Za + Wp + e$, as y is the data vector, u is the general average effect (fixed), a is the vector of the individual additive genetic effects (assumed to be random), p is the vector of the parcel effects, e is the vector of errors or residues (random). In both models, the capital letters represent the incidence matrices for the referred effects.

The mixed model equations for BLUP prediction of individual genetic values are equivalent to:

$$\begin{bmatrix} X'X & X'Z & Z'W & X'T \\ Z'X & Z'Z + I\lambda_1 & Z'W & Z'T \\ W'X & W'Z & W'W + I\lambda_2 & W'T \\ T'X & T'Z & T'W & T'T + I\lambda_3 \end{bmatrix} x \begin{bmatrix} r \\ a \\ p \\ b \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \\ T'y \end{bmatrix}$$

As for:

$$\lambda_1 = \frac{\hat{\sigma}_e^2}{\hat{\sigma}_a^2} = \frac{1 - h^2 - C_p^2 - C_{dic}^2}{h^2} \; ; \; \lambda_2 = \frac{\hat{\sigma}_e^2}{\hat{\sigma}_p^2} = \frac{1 - h^2 - C_p^2 - C_{dic}^2}{C_p^2} \; ; \; \lambda_3 = \frac{\hat{\sigma}_e^2}{\hat{\sigma}_{dic}^2} = \frac{1 - h^2 - C_p^2 - C_{dic}^2}{C_{dic}^2}$$

$h^2 = \hat{\sigma}_a^2 / \hat{\sigma}_a^2 + \hat{\sigma}_p^2 + \hat{\sigma}_{dic}^2 + \hat{\sigma}_e^2$ is individual heritability in the narrow sense;

$C_p^2 = \hat{\sigma}_p^2 / \hat{\sigma}_a^2 + \hat{\sigma}_a^2 + \hat{\sigma}_{dic}^2 + \hat{\sigma}_e^2$ is the correlation, due to the common environment of the plot; and

$C_{dic}^2 = \hat{\sigma}_{dic}^2 / \hat{\sigma}_a^2 + \hat{\sigma}_p^2 + \hat{\sigma}_{dic}^2 + \sigma_e^2$ is the correlation, due to the common environment of the completely randomized design.

In order to detect the genetic variability of the characters in the population, the index ($\hat{b}$) was calculated, according to the formula: $\hat{b} = CV_{gi}/CV_e$, which it represents the ratio between the coefficient of genetic variation and the coefficient of experimental variation, not influenced by the character average.

RESULTS

For the seeds biometrics it was possible to observe that the seeds had different sizes (Table 2). There was a greater difference between seeds width than between the length. The two mother trees with the largest seeds were not the longest, just as the two of the longest were not the largest. The mother tree AL 33 presented the largest seed size, considering the dimensions of width and length, followed by the mother trees AL 38, AL 34, AL 07 and AL 37. The smallest seeds were from the mother trees AL 32, AL 30, AL 29 and AL 09.

Tabela 2. Variáveis analisadas para sementes (LS, CS, IVE e E) e para as mudas (SB, H, DC, H/D) de *Apuleia leiocarpa*.

Table 2. Variables analyzed from seeds (SW, SL, ESI and E) and seedlings (SV, H, ND, H/D) of *Apuleia leiocarpa*.

M. Tree	SW (mm)	SL (mm)	ESI	E (%)	SV (%)	H (cm)	ND (mm)	H/D
AL 9	5.56 f	7.11 d	1.29 a	93 a	47 a	9.69 b	1.75 b	5.53 b
AL 8	6.55 d	7.56 c	1.60 a	84 a	61 a	11.12 a	1.96 a	5.68 a
AL 36	7.04 b	7.56 c	1.07 b	69 b	60 a	10.54 b	1.99 a	5.33 b
AL 32	6.27 e	7.53 c	1.37 a	65 b	55 a	10.00 b	1.84 b	5.48 b
AL 29	6.13 e	7.64 c	0.99 b	65 b	59 a	10.18 b	1.84 b	5.52 b
AL 33	7.39 a	7.89 b	1.20 a	65 b	52 a	12.36 a	2.02 a	6.15 a
AL 14	6.28 e	7.78 b	0.92 b	63 b	48 a	9.65 b	1.76 b	5.49 b
AL 31	6.51 d	7.78 b	1.06 b	62 b	56 a	11.12 a	2.05 a	5.49 b
AL 38	6.89 b	8.19 a	0.80 b	61 b	49 a	9.76 b	2.00 a	4.68 c
AL 34	6.70 c	8.13 a	0.91 b	57 b	60 a	11.93 a	2.03 a	5.87 a
AL 30	6.26 e	7.68 c	1.05 b	57 b	48 a	10.47 b	1.94 a	5.41 b
AL 7	7.24 a	7.45 c	0.56 b	38 c	38 a	11.30 a	1.90 b	5.89 a
AL 37	6.79 c	7.93 b	0.57 b	32 c	42 a	9.28 b	1.86 b	5.03 c
	Aver.: 6.58	Aver.: 7.71	Aver.: 1.03	Aver.: 62.5	Aver.: 51.79	Aver.: 10.61	Aver.: 1.92	Aver.: 5.54
	CV % 4.18	CV% 4.67	CV% 41.33	CV% 27.9	CV% 38.72	CV% 25.83	CV% 20.22	CV% 18.10

*Data followed by the same lowercase letter in the column do not differ statistically from each other by the Scott-Knott Test ($p < 0.05$).
As for: SW – seed width, SL – seed length, ESI – emergence speed index, E – emergence, SV – survival, H – seedling height, ND – seedling neck diameter (diameter at neck height), H/D – height and diameter ratio.

The emergence of grápia seedlings started on the 5th day after sowing (counted from the moment the seeds were placed in the BOD germinator). The evaluation and counting of the emergence occurred every day until stabilization, a fact stated on the 18th day after sowing. The largest number of seedlings emerged was observed between the 8th and the 10th day. For the calculation of the ESI, data from the 5th to the 10th day were used. This limitation in the use of the evaluation days was adopted in order not to favor the results for any mother tree.

Four mother trees had a higher ESI and differed statistically from the others (Table 2). The highest ESI was assigned to mother tree AL 08, followed by AL 32, AL 09 and AL 33. The emergence presented variation between mother trees (Table 2). The seeds of AL 09 and AL 08 stood out from the others with 93% and 84% emergence, respectively. These two mother trees were also among the four with the highest recorded ESI. The lowest indices were with AL 07 and AL 37 with 38% and 32% of emergence, respectively, coinciding with the lowest rates of ESI among the thirteen mother trees evaluated. The general average of emergence was 62%.

For the height, diameter at the neck height and percentage of survival of the seedlings there were also differences between the mother trees (Table 2). The observed values for survival were considered low, due to the loss of many seedlings throughout the experiment. The percentage of survival of the seedlings did not differ statistically between the mother trees (Table 2). The general survival average was 52%, with a decrease in seedlings until the evaluation (7 months). For height and diameter at the neck height, it can be observed that the values found are considered low for seedlings of 7 months old of the species, as the average height was 10.6 cm and the diameter was 1.92 mm. The seedlings of the mother trees that presented the greatest growth (height and ND) were: AL 33, AL 34, AL 07, AL 31 and AL 08. Among these, only AL 08 showed a higher percentage of emergence than the others, while the seeds of AL 07 revealed low seedling emergence power. The seeds of the other mother trees obtained average rates of emergence.

Correlation analysis

With Person's correlation analysis (Table 3), it was noticed for seed biometry that the width did not show any correlation greater than 0.5. The seeds length, on the other hand, presented a significant and positive correlation close to 0.5 with the height and diameter of the mother trees and negative with the temperature of the collection areas. The width and length of the seeds also showed values close to 0.5. The emergence and the ESI presented a strong correlation (0.85), confirming what was observed in the averages test, with the highest rates of ESI coincided with the highest percentages of emergence. The emergence also revealed a negative correlation close to 0.5 in relation to the length and width of seeds.

The diameter and height of the mother trees correlate with each other and furthermore with most of the soil nutrients. The height of the cultivated seedlings showed 0.69 correlation with the phosphorus (P) from the place of origin of the mother trees, just as the seedlings survival had a correlation of 0.64 with the aluminum (Al) from the collection area.

Tabela 3. Resultado da correlação de Pearson para as 18 variáveis analisadas, nas progênies de *Apuleia leiocarpa.*
Table 3. Pearson correlation results for the 18 variables analyzed in the progenies of *Apuleia leiocarpa*.

	H	**DBH**	**T*a***	**pH**	**Ca**	**Mg**	**Al**	**MO**	**P**	**K**	**CEC**	**SW**	**SL**	**ESI**	**E**	**SV**	**H*a***	**ND*a***
H	1.00	0.72	**0.65***	0.10	0.20	**0.02**	**0.15**	0.57	**0.80**	0.08	0.32	**0.13**	0.49	**0.42**	**0.37**	**0.29**	**0.47**	**0.09**
DBH		1.00	**0.52**	0.09	0.10	0.03	**0.12**	0.47	**0.70**	0.04	0.25	**0.01**	0.46	**0.55**	**0.55**	**0.30**	**0.67**	**0.18**
T*a*			1.00	**0.06**	**0.14**	0.02	0.16	**0.57**	0.60	0.02	**0.16**	**0.05**	**0.56**	0.23	0.01	0.05	0.34	**0.19**
pH				1.00	0.95	0.92	**0.95**	0.69	**0.08**	0.98	0.86	**0.04**	**0.20**	**0.43**	**0.14**	**0.73**	**0.40**	**0.53**
Ca					1.00	0.93	**0.88**	0.69	**0.20**	0.94	0.95	**0.26**	**0.29**	**0.35**	0.00	**0.72**	**0.52**	**0.63**
Mg						1.00	**0.80**	0.49	0.06	0.90	0.89	**0.25**	**0.32**	**0.30**	0.02	**0.64**	**0.41**	**0.68**
Al							1.00	**0.79**	0.20	**0.89**	**0.73**	**0.08**	0.04	0.39	0.13	0.64	0.37	0.39
MO								1.00	**0.54**	0.59	0.58	0.18	0.33	**0.60**	**0.35**	**0.60**	**0.50**	**0.18**
P									1.00	**0.03**	**0.32**	0.30	**0.29**	0.12	0.09	0.18	0.69	0.15
K										1.00	0.87	**0.07**	**0.32**	**0.43**	**0.15**	**0.77**	**0.36**	**0.55**
CEC											1.00	**0.45**	**0.34**	**0.32**	**0.01**	**0.72**	**0.65**	**0.73**
SW												1.00	0.48	**0.39**	**0.54**	**0.10**	0.54	0.64
SL													1.00	**0.41**	**0.47**	0.09	0.17	0.56
ESI															0.85	0.63	0.18	0.01
E															1.00	0.55	0.01	**0.14**
SV																1.00	0.33	0.40
H*a*																	1.00	0.69
ND*a*																		1.00

As for: H – mother's tree height; DBH – diameter at breast height; T*a* –average temperature of the mother's tree location; pH, Ca, Mg, Al, MO, P, K, CEC – chemical soil characteristics of mother's tree location; SW – seeds width; SL – seeds length; ESI – emergence speed index; E – emergence percentage; SV – survival percentage; H*a* – average seedlings height; ND*a* – average seedling neck diameter (diameter at the neck height).
* Numbers highlighted in bold present negative correlation.

Genetic parameters estimative

Among the characters analyzed (Table 4), seed length (*SL*) and seed width (*SW*) presented the highest values for individual heritability in the strict sense (h^2_a) of 0.67 and 0.69, respectively, considered high values.

Tabela 4. Componentes da variância para as variáveis *CS* (comprimento de sementes), LS (largura de sementes), *IVE* (índice de velocidade de emergência), *E* (emergência), *DC* (diâmetro do coleto) e *H* (altura das mudas) para as sementes e mudas de *Apuleia leiocarpa*.
Table 4. Variation components for variables *SL* (seed length), *SW* (seed width), *ESI* (emergence speed index), *E* (emergence), *ND* (neck diameter) and *H* (seedling height) for seeds and seedlings of *Apuleia leiocarpa*.

Components	**SL (mm)**	**SW (mm)**	**ESI**	**E(%)**	**ND (mm)**	**H (cm)**
$\hat{\sigma}^2_a$	0.31	0.97	0.05	133.72	0.03	2.72
$\hat{\sigma}^2_{parc}$	-	-	0.04	175.84	0.02	0.86
$\hat{\sigma}^2_e$	0.15	0.40	0.12	201.94	0.11	4.79
$\hat{\sigma}^2_{fi}$	0.47	0.57	0.22	511.50	0.16	8.38
h^2_a	0.67 ± 0.12	0.69 ± 0.20	0.24 ± 0.27	0.26 ± 0.28	0.18 ± 0.09	0.32 ± 0.13
h^2_{aj}	-	-	0.3054	0.3983	0.2165	0.3625
C^2_{parc}	-	-	0.19	0.34	0.14	0.1
h^2_{ad}	0.6102	2.2133	0.2480	0.3318	0.1717	0.2990
$CV_{gi}(\%)$	7.28	14.93	22.08	18.51	9.05	15.74
$CV_{gp}(\%)$	3.64	7.46	11.04	9.26	4.53	7.87
$CV_e(\%)$	8.06	8.69	23.80	23.40	9.46	11.09
$\hat{b}$	0.90	1.72	0.93	0.79	0.96	1.42
General average	7.71	6.59	1.05	62	1.92	10.61

As for: $\hat{\sigma}^2_a$ = additive genetic variance; $\hat{\sigma}^2_{parc}$= environmental variance between plots; $\hat{\sigma}^2_e$= residual variance (environmental + non additive); $\hat{\sigma}^2_{fi}$= individual phenotypic variance; h^2_a= individual heritability in the strict sense; h^2_{aj}= individual heritability in the strict sense, adjusted for the plot effects; C^2_{parc} = coefficient for determining the plot effects; h^2_{ad}= additive heritability within the plot; $CV_{gi}(\%)$ = individual additive genetic coefficient of variation; $CV_{gp}(\%)$= genotypic coefficient of variation between progenies; $CV_e(\%)$= residual coefficient of variation; $\hat{b}$= ratio between the genetic coefficient of variation and the experimental coefficient of variation.

SW, in relation to *SL*, presented higher values of important genetic parameter estimates such as additive genetic variance ($\sigma^{\wedge 2}_a$), individual phenotypic variance ($\hat{\sigma}^2_{fi}$), individual additive genetic coefficient of variation ($CV_{gi}(\%)$) and genotypic coefficient of variation between progenies ($CV_{gp}(\%)$). The emergence speed index (ESI) and the emergence percentage (E) showed heritability close to 0.25 (low) and individual additive genetic coefficient of variation ($CV_{gi}(\%)$) between 18.51 and 22.08. These two variables presented a and genotypic coefficient of variation between progenies ($CV_{gp}(\%)$) of approximately 10%. The values of the residual coefficient of variation ($CV_e(\%)$) for these variables were higher than 20%. The $\hat{b}$ index, *SW* and *H* obtained values greater than 1.

In relation to the variables diameter at the neck diameter (*ND*) and height of the seedlings (*H*) there was a greater heritability for *H* (0.32) as well as for the additive genetic variance ($\hat{\sigma}^2_a$), individual phenotypic variance ($\hat{\sigma}^2_{fi}$), individual additive genetic coefficient of variation ($CV_{gi}(\%)$) and genotypic coefficient of variation between progenies ($CV_{gp}(\%)$)

DISCUSSION

Grápia seeds showed variation in size between mother trees. The seeds size is related to the amount of reserves, influencing germination and emergence (PÁDUA *et al.*, 2010). The variation found in terms of size probably happened due to genetic variability, since the heritability values for *SL* and *SW* were high, above 0.6, manifesting a high genetic effect for this character. The fruits and seeds size can vary between plants of the same species, between reproductive years and on the same plant. The difference in the size for both fruits and seeds between individuals, in the same population, constitutes one of the most important sources of variability available (SANTOS *et al*., 2009), also helping in the study of dispersion and dispersing agents. The observed values were between 0.71 and 0.79 cm for *SL* and 0.50 to 0.70 cm for *SW*, smaller than other experiments in which the seeds were 1.2 to 2.1 cm long and 0.60 to 0.95 cm wide (FELIPPI *et al*., 2012).

Searches consider that the classification of seeds by size and weight has the objective of standardizing the speed of emergence (PÁDUA *et al.*, 2010). In this study, the greatest results for ESI were observed in the mother trees AL 08, AL 32 and AL 09; being attributed the seeds considered small by biometrics. The correlation of ESI with length and width of seed was low and negative (-0.41 and -0.39 respectively), suggesting that the size of the seed has little influence on ESI and germination. This result limits the use of seed size as an indicator of physiological seed quality in grápia. Similar results were obtained by Santos *et al*. (2009), who found no influence on the size of the seeds of *Tabebuia chrysotricha* Mart. Ex. A. on the germination test characteristics.

With the different rates of emergence, the average among all mother trees was 62%. Felippi *et al*. (2012) obtained a final percentage of emerged seeds ranging from 26.38% to 87.54% without using any methods to overcome dormancy, and Nicoloso *et al*. (1997) found a germination potential from 90% to 100% using sulfuric acid. The results obtained suggest that the potential for germination as well as vigor is associated with the mother tree individually and that overcoming dormancy is important for homogenization of germination, mainly for the production of seedlings from a several mother trees lot.

For the production of seedlings of the species it is important to select mother trees that produce seeds with high germination power, considering that this factor can be quite variable. The mother tree AL 37 has a high height and a large diameter when compared to the others and can be considered an older plant, justifying the low germination rate of the seeds. The correlation found between emergence and DBH of the mother trees was negative, of -0.55, confirming this hypothesis. Still considering the diameter of the plants, the seeds of the mother trees AL 09 and AL 08, which had the highest germination rates, came from mother trees of smaller diameters, possibly the youngest plants and with greater vegetative vigor. Dendrochronology studies are important to confirm the results found.

As for survival, the mortality rate of the plants was high, even with some mother trees showing a greater tolerance to adverse factors. What can be used as a guide for future experiments with grápia seedlings is the care with irrigation, especially in winter and with high relative humidity. Irrigation is one of the key points in the production of seedlings, and the excess of irrigation can cause mortality in many native species, which often have low water demand (THEBALDI *et al*., 2016). The temperature inside the greenhouse can also damage the leaves. High mortality of seedlings of the species was observed in the winter period (June and July) due to the amount of irrigation received, standard for the other species of the Forest Nursery. Because it is a species found in Santa Catarina in the Seasonal Deciduous Forest (FONTANA; SEVEGNANI, 2012), low temperatures probably contributed to mortality. The first symptoms of mortality appeared on the leaves, which turned a light green color and later on the complete fall of the seedlings. After mortality, the seedlings started to receive irrigation less frequently.

The height and diameter at the neck height evaluation showed a low growth of seedlings. In other studies with the species, four months after germination, the average height was 18 cm and the diameter at the neck

height was around 3 mm (FELIPPI *et al.*, 2012) and 10 cm at 100 days (NICOLOSO et al., 1997). However, this difference in growth rates may be due to the climatic conditions where each study was carried out and at the time when sowing occurred. In this experiment, sowing was carried out in the month of April and in the second month the plants were already subjected to winter conditions, when growth is generally slower.

Regarding the genetic parameters, the size of the seeds proved to be an efficient criterion to verify the genetic diversity among mother trees. Heritability was high for both evaluated criteria (width and length). Heritabilities are classified according to the interpretation proposed by Resende (1995) from 0.01 to 0.15 as low; 0.15 to 0.50 are medians and; above 0.50 high. Based on this classification, the emergence speed index (ESI) and emergency speed (E %), presented median heritabilities (0.24 and 0.26 respectively). These variations are important indicators of genetic variability to be explored in breeding programs, enabling the selection of promising materials, because with the knowledge of the heritability coefficient of a trait and the study of the population to be improved it is possible to estimate the expected genetic gain with the selection even before it is carried out (ROSADO *et al*., 2010).

It should also be noted that the heritability coefficient in the narrow sense reflects how much of the phenotypic variation observed in a quantitative character is inheritable due to the effects of additive genes, transferred from parental individuals to their descendants (COSTA *et al*., 2010). It is an important genetic parameter because it allows knowing the potential or the power to change the mean of a character in a population by artificial selection (KUBOTA *et al*., 2015b).

As for the genetic parameters in relation to the variables diameter at the neck height (*ND*) and height of the seedlings (*H*), *H* was shown to be a variable that has greater facility for selecting superior and contrasting materials. Generally, the heritability values for quantitative characteristics such as height and diameter are between 0.2 and 0.3. However, it can present high variation, mainly in younger stages of plant development, as observed in the study by Menegatti *et al*. (2016), who found average heritability values of *Mimosa scabrella* Benth. progenies at 12 months of age ranging from 0.11 to 0.54 for the DBH character and from 0.01 to 0.82 for the height character.

In addition to having greater heritability, the height of the seedlings (*H*) presented a higher genetic coefficient of variation, which favors selection. The genetic coefficients of variation, both at the individual level ($CV_{gi(\%)}$) and at the progeny level ($CV_{gp(\%)}$) are admitted as essential indicators of the existing variation, allowing to estimate genetic gains in provenance and progeny tests (AGUIAR *et al*., 2010). According to Sebbenn *et al*. (1998) a coefficient of genetic variation greater than 7% is considered high. Thus, all parameters of seed biometrics, germination and initial growth of seedlings can be used as indicative of success in the selection of genetic materials from grápia mother trees.

The values of the experimental coefficient of variation ($CV_{e(\%)}$) were considered median for all evaluated variables, with values between 8.06% and 11.09%, with the exception of ESI and E, which presented (>20%). However, in general, the experimental control was satisfactory, and one can expect good precision in the estimates of genetic parameters for the evaluated characters. Only the variables SW (1.72) and H (1.42) presented values of the index $\hat{b}$, greater than 1. The use of these characters may result in greater gains in the selection, since the values of the index greater than 1 are due to their own high genetic variance, probably little influenced by the environment, indicating great possibility and success in the selection (VENCOVSKY, 1978).

Early characters such as those used in this study can serve as indicators of genetic diversity (KUBOTA *et al*., 2015b). The relative contribution of each character to the genetic divergence is of great importance to identify the characters with the greatest contribution and assist in the disposal of those with the least contribution. Therefore, in order to predict genetic gains with selection, it is essential to estimate genetic parameters (BERTI *et al*, 2011).

Regarding the collection of seeds, the definition of zones for the collection and use of seeds can be carried out based on biophysical information (altitude, latitude, climate, soil, etc.), which are considered important causes of selection pressures and which probably lead to genetic differentiation between populations (BIERNASKI *et al*., 2012). From the correlation analysis, there seems to be no established trend in the pattern of seed size, germination and initial growth of seedlings as a function of location, climate and soil characteristics. But, instead as a function of the mother tree characteristic itself, such as height and diameter , which may indicate younger age of the plant in which the collection was made.

CONCLUSIONS

- It was found the existence of genetic variability among the thirteen mother trees of the study as well as significant differences for all the evaluated characteristics, being the use of the seedlings in areas of forest restoration favorable.

- Length and width of seeds showed a high value for heritability (above 0.6) and can be used as an indicator of genetic variability of the species.

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Ciência Florestal

ISSN 1980-5098
Open access

Ci. Fl., Santa Maria, v. 31, n. 4, p. 1569-1590, Oct./Dec. 2021 • https://doi.org/10.5902/1980509836889
Submitted: 14th/02/2019 • Approved: 30th/03/2021 • Published: 17th/11/2021

Artigos

Hydrogel and irrigation frequencies in survival, growth and gas exchanges in *Eucalyptus urograndis*

Hidrogel e frequências de irrigação na sobrevivência, crescimento e trocas gasosas em *Eucalyptus urograndis*

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ABSTRACT

The objective of this study was to evaluate the effect of the hydrogel on the planting of clonal *Eucalyptus urograndis* seedlings in pots, related to different water management, to provide information about the hydrogel use efficiency. The experiment was carried out in a forest nursery in a factorial scheme, as factor "A" referred to the absence and presence of hydrogel and factor "B" to water management (0 - irrigation only at planting, 1 - daily, 4 - every 4 days, and 8 - every 8 days). Evaluations of the water stress symptomatology, morphological characteristics, chlorophyll content, soil water content, gas exchange, increase in diameter and height, and dry biomass were performed. The hydrogel provided an increase in water content in the soil and a delay in the symptoms of water stress, increasing the survival rate. Water stress was a limiting factor for stomatal opening, contributing to the decrease in carbon assimilation by photosynthesis.

Keywords: Water-insoluble polymer; Intermittent irrigation; Water deficit

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RESUMO

O objetivo do trabalho foi avaliar o efeito do hidrogel no plantio de mudas clonais de *Eucalyptus urograndis* em vasos, relacionados com diferentes manejos hídricos, buscando fornecer informações sobre a eficiência do uso do hidrogel. O experimento foi conduzido em viveiro florestal, esquema fatorial, em que o fator "A" se referiu à ausência e à presença de hidrogel e o fator "B" ao manejo hídrico (irrigação somente no plantio, 8 dias, 4 dias e diariamente). Foram realizadas avaliações da sintomatologia do estresse hídrico, características morfológicas, teores de clorofila, teor de água no solo, trocas gasosas, incremento em diâmetro e altura e biomassa seca. O hidrogel proporcionou um aumento no teor de água no solo e um atraso nos sintomas de estresse hídrico, aumentando a taxa de sobrevivência. O estresse hídrico foi um fator limitante para a abertura estomática, contribuindo para a queda na assimilação de carbono pela fotossíntese.

Palavras-chave: Polímero hidrorretentor; Irrigação intermitente; *Deficit* hídrico

1 INTRODUCTION

Among the most cultivated forest species in Brazil, stands out the *Eucalyptus* genus, occupying 5.7 million hectares of the planted trees area, representing 72.7% of total (INDÚSTRIA BRASILEIRA DE ÁRVORES, 2017). *Eucalyptus* is known to be a fast grow tree genus, and for that, it has been increasing its prominence in reforestation programs. This genus is often selected due to the ease of manipulation, diversity of species, fast growth, and mainly for its wide range of industrial purposes.

The influence of environmental variables on the growth of *Eucalyptus* stands notorious, as the growth and development of the plants are affected by environmental factors (PFAUTSCH *et al*., 2016). The introduction of new species from other regions is always a process that requires studies aiming to test their adaptability and survival, as well as their economic and productivity effects (OTTO *et al*., 2013).

Variations in productivity of *Eucalyptus* are related to differences in water supply, nutrients, soil aeration and mechanical restriction to root growth. Water is considered the factor that most influences plant growth (CASTRO *el al*., 2017). The effect of water deficiency on plants is complex, as there are mechanisms of resistance to drought, plants can respond through various adaptive processes to water scarcity, such as through the ability to reduce water potential, allied to physiological, anatomical and morphological adaptations (NOGUEIRA; ALBUQUERQUE; SILVA, 2005).

As a possible aid in the survival of seedlings after transplantation is the use of hydroretent polymers (hydrogels) as soil moisture conditioners, assisting mainly in the retention and availability of water for newly planted plants (MENDONÇA *et al*., 2013). It is important to note that the use of adequate irrigation systems is a key factor in the reduction of irrigation frequency, improving silvicultural techniques, contributing to the viability of planting throughout the year, and not becoming dependent on the climatic variations observed during the seasons (NAVROSKI *et al*., 2014; 2016).

The main objective of this search was to verify if the hydrogel presents a reduction possibility in the irrigation frequency of *Eucalyptus* seedlings, using visual characterization of wilt in seedlings submitted to water stress and the use of physiological parameters under these conditions, combining the determination of water content in the soil. In this way, this study evaluated the effect of the hydrogel on *Eucalyptus urograndis* planting, relating different water managements, seeking to provide information about the efficiency of hydrogel use in a controlled environment.

2 MATERIALS AND METHODS

The study was carried out in forest nursery of the University of Santa Catarina State, located in Lages, southern Brazil (coordinates 27°47'33"S and 50°18'4"W), with an altitude of approximately 900 meters. The experiment was conducted in a greenhouse with a 150-micron plastic cover, with an average temperature of 25-30 ºC and humidity between 65 and 90%. According to the Köeppen classification, the climate of Lages/SC is humid mesothermal with mild summer (temperate Cfb). The average annual temperature is 15 °C and the average annual rainfall is 1300 to 1500 mm (ALVARES *et al*., 2013). The study occurred during the end of September until the end of November 2015.

Rooted mini cuttings of *Eucalyptus urograndis* were purchased in a Forestry Nursery located in Anchieta-SC, with approximately 120 days. The plants were produced in polypropylene tubes with a capacity of 55 cm³, using substrate based on

peat and pine bark. A total of 6 g L^{-1} of controlled release fertilizer was added to the substrate. The mini cuttings were placed to root in a greenhouse with humidity above 80% and temperature close to 28ºC for 40 days. After this period, the seedlings were placed for another 60 days in a shade house and last 20 days in a rustification area. For the experiment, seedlings of 25 cm in height, 2 mm in stem diameter, between 4-5 pairs of leaves, reddish stem, well lignified and good phytosanitary condition were standardized.

The soil used for the filling of the polyethylene pots (10 L) was composed of soil collected at the depth of 0 to 50 cm of a clayey Humic Cambisol. (World Reference Base - WRB/FAO systems). The physical and chemical soil analysis are: pH = 4.7 H_2O^2; SMP Index = 4.90; $Ca^{(2)}$ = 2.2 $cmol_c\ dm^{-3}$; $Mg^{(2)}$ = 1.2 $cmol_c\ dm^{-3}$; $Al^{(2)}$ = 4.9 $cmol_c\ dm^{-3}$; P Mehlich$^{(2)}$ = 3.7 mg dm^{-3}; $K^{(2)}$ = 48 mg dm^{-3}; organic matter – OM = 2.9%; Effective CEC = 6.46 $cmol_c\ dm^{-3}$; CEC pH 7.0 = 13.1 $cmol_c\ dm^{-3}$; Base saturation = 26.8%; Al saturation = 58%; clay = 47%, silte = 24% and sand = 28%. The soil was homogenized in 10 mm sieves in order to avoid clods. No fertilization or liming was applied.

For the reduction of experimental errors, a uniform scale of a soil mass of 8 kg was standardized through a digital scale and water was subsequently added to the soil until it reached the field capacity, and its weight was measured. To determine the field capacity, the θ-t observable curve procedure was used, according to the methodology of Casaroli and Van Lier (2008). For the transplanting, a manual hole of approximately 300 cm^3 was opened in the center of each pot for the planting. The treatments were composed of four irrigation frequencies, besides the presence and absence of hydrogel. Water management for hydrogel treatment was exactly the same as that established in the absence of hydrogel treatment.

In the treatments using the hydrogel, 300 mL in the already hydrated form was added before the planting. The hydration occurred thirty minutes before in a dosage of 3 g L^{-1} of hydrogel, which was a granulated synthetic polymer of the brand Hidroplan-EB®. After the opening of the hole for planting, whether or not the hydrogel was filled,

the seedlings (former mini cuttings) were planted. After transplantation, in addition to the 1,500 mL to moisten the soil, 300 mL of water was added to each pot for the treatments without the hydrogel, in order to compensate the volume of water to be added by the polymer. For the irrigation treatments, 300 mL of water was used in each pot, as the water replacement was based according to the frequency determined in each treatment.

The experiment was conducted in a completely randomized design, with 8 replications (one seedling each), in a 2 x 4 factorial arrangement. The hydrogel factor was composed of two levels (presence and absence) and the frequency of irrigation was composed of four levels (0 - irrigation only at planting, 1 - daily, 4 - every 4 days and 8 - every 8 days).

2.1 Evaluation of visual symptomatology, morphological evaluations and gas exchange

Evaluations of the dry stress symptomatology were carried out daily until the 47th day. For the visual symptomatology it was adopted the criterion of analysis according to Navroski *et al*. (2014), as the number of days in which the plant remained in each condition was noted: DWS - days without symptoms (turgid plant, visually vigorous, no evidence of water deficit; DMWS - days with mild wilt symptoms; DMS - days with moderate symptoms (permanent wilt point with darkened and curved apex); DSS - days of severe wilt symptoms (dry leaves/in abscission) and DPRA - number of days the plant remained alive.

The gas exchange evaluations were performed with the aid of a LI-6400XT Portable Photosynthesis System, using in 4 replicates. The values of photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*), relation between intercellular and atmospheric concentration of CO_2 (Ci/Ca) and water use efficiency (WUE) were determined. The photosynthetically active radiation used during the measurements were 700 µmol of photons $m^{-2}s^{-1}$. Photosynthetically active radiation was provided by

an artificial light source (LI-6400-40), percentage of blue light used was 10% of the total photosynthetically active radiation (PAR). The atmospheric CO_2 concentration ranged from 390 to 400 μmol mol^{-1} during measurements. The evaluations were performed every seven days and started immediately after transplantation, always taking place between 8:00 am and 11:30 am. The same leaf was used throughout the experimental period. The evaluations were stable within 2-3 minutes when recorded by the equipment. The water use efficiency (WUE) was calculated by dividing *A* by *E*.

Biometric height and diameter evaluations were performed in two steps. In the first one, data were collected on the day of the experiment, and the last one at the end of the experimental period (47 days after implantation). The height of the plants (cm) was determined with millimeter ruler, measuring from the base to the insertion of the highest leaf. The diameter of the stem - DS (mm) was determined with digital caliper ruler.

At the end of the experimental period (47 days), when the plants without irrigation were permanently wilted, the total biomass of the plants was manually collected by means of a destructive method. The samples were separated in root, stem and leaves and conditioned in paper bags kept in forced air circulation (65±3 °C) until reaching a constant mass (g). Afterwards, weighing was carried out to determine the contents of matter of the components in a precision balance (0,01 g). For the determination of the individual leaf area, all the leaves of each plant were collected, separately in paper bags and taken to the laboratory. The leaf area was quantified with Li-Cor leaf area integrator model LI3000A.

2.2 Water content in soil

In order to follow the moisture content of the vessels, it was decided to determine the initial water content of the soil, which makes it possible to monitor the soil water content during the experimental period. Before planting the cuttings, all pots were saturated and allowed to drain for two hours, then a sample of approximately 100

grams of soil was collected in each pot, so that all the containers were analyzed. The samples were separated and packed in aluminum foil cups. The determination of the wet mass of the pots occurred at the experiment site after their collection, using a precision scale, thus avoiding water lose from the samples until the arrival at the laboratory.

After determination of the wet mass, the samples were dried in an oven. This occurred until the moment of stabilization of their masses, being later re-weighed, for which the dry mass was obtained. The monitoring of pots' weight occurred every 4 days, through a semi analytical balance, always performed before irrigation. With the obtaining of these values, through equations, the soil water content was established by the graviometric method.

2.3 Statistical analysis

The treatment variances were tested for homogeneity by the Bartlett test and normality by Shapiro-Wilk test. With homogeneity of variances and normality, the data was subjected to analysis of variance and with significant difference by the F test, the Scott-Knott test was applied at the 5% probability level. When necessary, the data were transformed by the boxcox test. The analyzes were performed with the Sisvar statistical software (FERREIRA, 2019).

3 RESULTS AND DISCUSSION

3.1 Visual symptomatology and morphological evaluations

In relation to the symptoms of the *Eucalyptus urograndis* clones affected by the water deficit, there was interaction ($p = 0.0001$) between irrigation frequency and the use of the hydrogel for all variables evaluated. The polymer had influence on all classes of stress, regardless of the frequency of irrigation (Table 1).

Table 1 – Duration of water stress symptoms in plants, in days, evaluated from planting until the 47th day after planting *Eucalyptus urograndis* clones in pots, depending on the presence or absence of hydrogel and different irrigation frequency

Variable	Hydrogel	Irrigation Frequency				Average
		planting (0)	8 days	4 days	daily (1)	
		Appearance of stress symptoms (days)				
DWS ** CV%I 2,6	Presence	16.7 (2.0)II Da*	38.0 (0.5) Ba	44.0 (1.0) Ba	47.0 (0) Aa	36.4
	Absence	15.4 (1.5) Cb	35.0 (1.5) Ba	42.0 (1.5) Ba	47.0 (0) Aa	34.8
	Average	16.0	36.5	43.0	47.0	
DMWS CV% 4,0	Presence	24.7 (3.5) Ba	41.0 (2.0) Aa	47.0 (0) Aa	47.0 (0) Aa	39.9
	Absence	19.2 (4.5) Bb	39.0 (3.5) Aa	47.0 (0) Aa	47.0 (0) Aa	38.0
	Average	21.9	40.0	47.0	47.0	
DMS CV% 3,3	Presence	30.0 Ba (3.5)	47.0 (0) Aa	47.0 (0) Aa	47.0 (0) Aa	42.7
	Absence	24.0 Bb (4.0)	47.0 (0) Aa	47.0 (0) Aa	47.0 (0) Aa	41.2
	Average	27.2	47.0	47.0	47.0	
DSS CV% 3,9	Presence	36.2 (3.0) Ba	47.0 (0) Aa	47.0 (0) Aa	47.0 (0) Aa	44.3
	Absence	31.3 (4.5) Bb	47.0 (0) Aa	47.0 (0) Aa	47.0 (0) Aa	43.1
	Average	33.8	47.0	47.0	47.0	
DPRA CV% 3,9	Presence	38.2 (4.5) Ba	47.0 (0) Aa	47.0 (0) Aa	47.0 (0) Aa	44.8
	Absence	33.3 (5.0) Bb	47.0 (0) Aa	47.0 (0) Aa	47.0 (0) Aa	43.5
	Average	35.7	47.0	47.0	47.0	
		Morphological variables				
DS (mm) CV% 9,7	Presence	0.77 (0.31)	0.97 (0.22)	1.77 (0.39)	3.13 (0.45)	1.66 a
	Absence	0.73 (0.27)	0.78 (0.18)	1.56 (0.34)	2.59 (0.51)	1.41 b
	Average	0.75 C	0.87 C	1.66 B	2.86 A	
IH (cm) CV% 12,5	Presence	6.60 (1.4)	7.80 (1.7)	9.10 (2.1)	14.8 (2.3)	9.57 a
	Absence	5.38 (1.8)	5.16 (1.6)	7.98 (1.9)	9.50 (2.0)	7.00 b
	Average	5.99 C	6.48 C	8.54 B	12.15 A	
DLB (g) CV% 9,2	Presence	1.71 (0.32) Ca	2.30 (0.29) Ca	4.92 (0.64) Ba	5.81(0.53) Aa	3.68
	Absence	1.62 (0.23) Ca	2.08 (0.22) Cb	3,71 (0.52)Bb	5.79(0.67) Aa	3.3
	Average	1.67	2.19	4.31	5.8	
DRB (g) CV% 15,2	Presence	1.92 (0.45)	2.01 (0.34)	3.21 (1.23)	5.56 (2.02)	3.17
	Absence	1.86 (0.67)	2.25 (0.29)	3.13 (1.34)	4.32 (1.94)	2.89
	Average	1.89 C	2.13 C	3.17 B	4.94 A	
DSB (g) CV% 9,7	Presence	1.72 (0.32)	2.19 (0.34)	3.37 (0.94)	4.10 (1.43)	2.87
	Absence	1.50 (0.21)	2.05 (0.49)	2.87 (0.73)	2.90 (1.23)	2.33
	Average	1.61 C	2.12 C	3.17 B	3.50 A	

To be continued ...

Table 1 – Conclusion

Variable	Hydrogel	Irrigation Frequency				Average
		planting (0)	8 days	4 days	daily (1)	
		morphological variables				
DTB (g) CV% 14,2	Presence	6.40 (1.33)	7.10 (1.44)	10.91 (3.42)	16.20 (3.97)	10.16
	Absence	4.00 (0.98)	6.74 (0.97)	12.44 (4.11)	12.44 (4.13)	8.30
	Average	5.20 D	6.74 C	10.65 B	14.32 A	
LA (cm^2) CV% 14,8	Presence	189.7 (54)	294.2 (75)	662.1 (121)	796.3 (132)	485.5 a
	Absence	132.7 (45)	233.4 (69)	635.5 (99)	781.8 (143)	436.1 b
	Average	161.2 C	263.8 B	636.5 A	781.8 A	

Source: Authors (2017)

In where: * Averages followed by the same capital letter in the row and lowercase in the column do not differ by Scott-Knott's test, 5% probability of error. ** DWS - days without symptoms; DMWS - days with mild wilt symptoms; DMS - days with moderate symptoms; DSS - days of severe wilt symptoms; DPRA - days the plant remained alive; DS - increase in diameter of the stem (mm); IH - increase in height (cm); DLB - dry leaf biomass (g); DRB - dry root biomass (g); DSB - dry stem biomass (g); DTB - dry total biomass (g); LA - leaf area (cm^2); [I] - coefficient of variation; [II] - standard deviation.

For classification on DWS the presence of hydrogel was efficient only at planting. The DMWS had a delay when the hydrogel was used at the frequency of irrigation at planting. The use of the hydrogel provided a reduction of 5 days in the mild wilt symptoms. For treatments in which irrigation was performed, there was no difference. For the DMS, DSS and DPRA, there was a behavior similar to the mild symptom, characterized by a delay in stress when the hydrogel was used only in the planting. This difference in the use of the hydrogel was not observed in the other irrigation frequencies. In both (presence and absence) of the polymer, the treatments that received irrigations (every day, every 4 and 8 days) did not verify mortality of the plants until the 47th day of evaluation, only specific symptoms of wilt.

Using this information for field planting, it can be assumed that in cases where irrigation occurs frequently, or in cases where rainfall frequency is higher, the use of the polymer could be ruled out (NAVROSKI *et al*., 2015). However, under less frequent rainfall conditions, or by making irrigations more spaced, the use of the polymer may be recommended.

In the implantation of *Eucalyptus* forests, it is generally observed that the most critical period for loss of seedlings due to lack of water is in the first 20 days, when the plant is being established and with a root system still in formation (FERNÁNDEZ; TAPIAS; ALESSEO, 2010). The first physiological consequence for plants subjected to water stress is the reduction or interruption of growth rates, due to the reduction in cell differentiation, influenced by water deficiency. The water deficit can directly affect the growth in diameter, height, leaf area and consequently biomass as observed in the treatments with less frequency of irrigation and no use of hydrogel. In these cases, there is a reduction in cell expansion and cell wall formation and, indirectly, a reduction in the availability of carbohydrates or influence the production of growth regulators (GONÇALVES; PASSOS, 2000). It is also important to highlight that the largest root biomass was observed in plants with hydrogel.

In the evaluation of the number of days the plants remained alive, it was noticed that the use of the hydrogel caused an increase of approximately 5 days. This result may indicate that the use of the polymer could imply the reduction of replanting resulting in more homogeneous stands and cost reduction. In this sense, Lopes *et al*. (2010) observed that hydrogel delayed the symptoms of water stress in *Eucalyptus urograndis*, increasing survival. According to the authors, the hydrogel allowed the plants to remain alive without symptoms of lack of water for up to 20 days, up to seven days longer than in the absence of the same in the planting pits.

Similar results to those found in this study were also found by Buzetto, Bizon and Seixas (2002), in which studying the efficiency of the polymer in the water supply to *Eucalyptus urophylla*. The polymer retained irrigation water for a longer period, making it available gradually to the plants, which resulted in a decrease of the mortality of seedlings cultivated with the polymer. In the same sense, beneficial effects of the hydrogel (prolonged tree survival compared to the controls) were found in works by Agaba *et al*. (2010) with *Eucalyptus grandis, Eucalyptus citriodora, Pinus caribaea, Araucaria cunninghamii, Melia volkensii, Grevillea robusta, Azadirachta indica, Maesopsis*

eminii and Terminalia superba. Furthermore, Zonta *et al*. (2009) with *Coffea canephora*, in which they reported that when incorporated into the post-planting substrate the hydrogel favored seedling growth.

For the increase in diameter of the stem – mm (DS) and increment of height – cm (IH) significant effects were observed in the isolated factors (hydrogel and the irrigation frequencies). The DS and IH increased as the irrigation frequency enhanced. The plants that received irrigation daily presented between two to three times greater growth than the moistened ones only in the planting or every 8 days. The use of hydrogel also caused a greater increase, in average among all the frequencies.

When evaluating the biomass produced by *Eucalyptus urograndis* clones, 47 days after the planting, the results obtained by variables analyzed, such as root dry biomass, dry stem biomass and total dry biomass, showed no interaction between the use of hydrogel with irrigation frequencies ($p > 0.05$). However, there was a significant effect for the isolated form factors. For the leaf dry biomass (DLB) there was a significant interaction between hydrogel use and irrigation frequencies ($p < 0.05$).

For the DLB variable, it was observed that as water availability decreased, both in the presence and in the absence of hydrogel, the leaf biomass significantly decreased. In general, the use of hydrogel, in the frequency of irrigation every day, provided higher values of dry matter accumulated in the leaves, which did not differ statistically from the absence (Table 1). Regarding DRB, the plants that received irrigation every day, presented higher averages, differing from the other frequencies. The frequency of irrigation every 4 days also showed good results when compared to the lower frequencies. The biomass results of the stem (DSB), and consequently for DTB showed similar results to those of the root, that the higher the frequency of irrigation, the greater the biomass production of the plants. These results prove the great importance of water for crops, that according to Kerbauy (2009) of all the resources that plants need to grow and function, water is the most abundant and, at the same time, often the most limiting. In this sense, irrigation practice reflects the fact that water is a key resource that limits the productivity of forest plantations.

The leaf area – cm² (LA) varied according to the presence and absence of the polymer, as the presence obtained the highest average in relation to the absence. For irrigation frequencies analysis of variance showed a statistical difference between the highest frequencies and the lowest frequencies. Irrigations every day and every 4 days, presented the highest averages, differing from the irrigation only at planting and every 8 days. This difference in leaf area between the frequency differences reflects the importance of water availability for crop productivity, especially in forest plantations. Many morphological characteristics of plants are genetically determined, but others, such as leaf size, can be strongly influenced by the climate conditions in which they are inserted. The limitation of the leaf area can be considered the first line of defense against water deficiency (CHAVES *et al*., 2004).

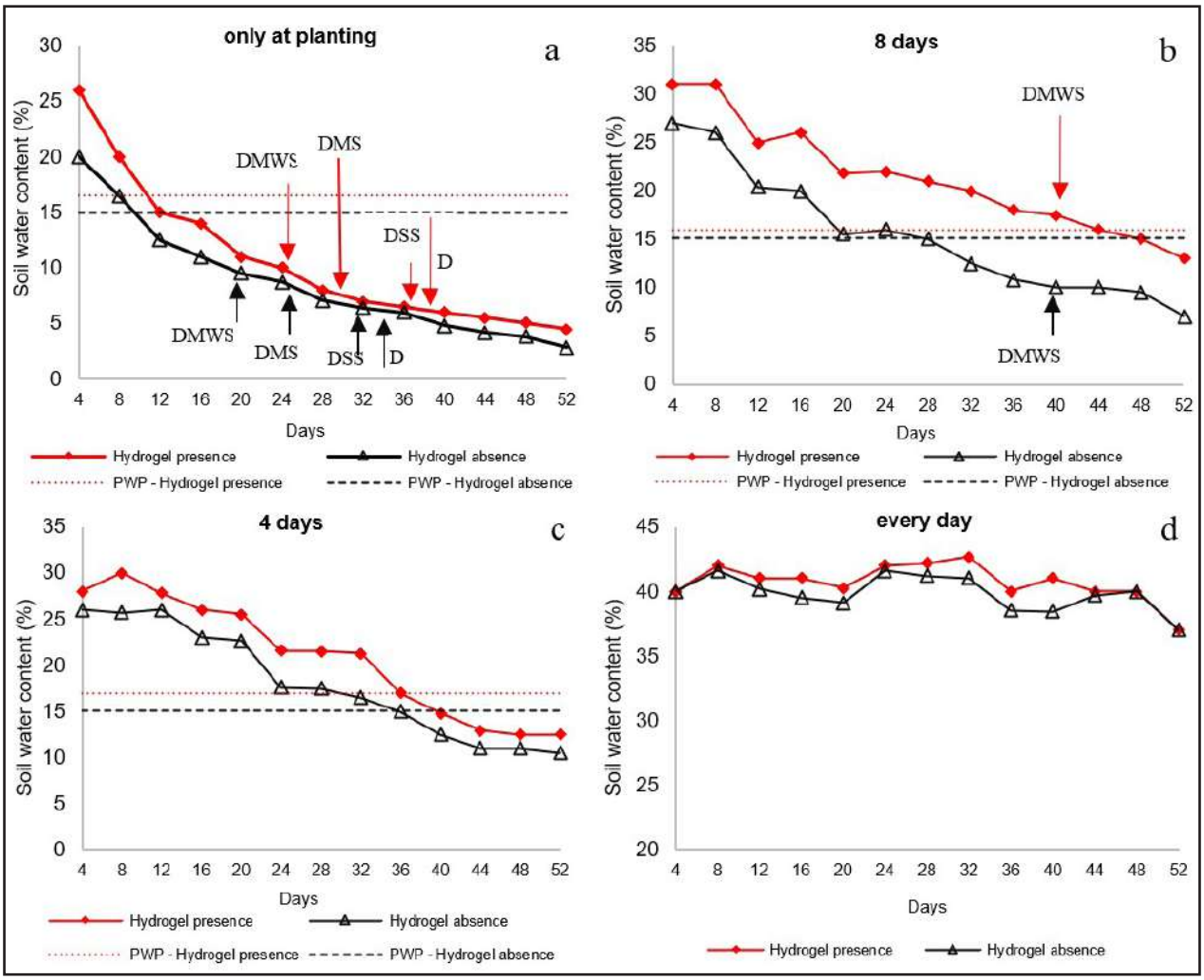
3.2 Soil water content

In general, soil moisture values varied according to the frequency of irrigation. The soil water content increased when the polymer was used, presenting higher values when compared to its absence, which may be closely related to the presence of the hydrogel as a conditioning of the soil moisture. Regardless of the frequency used to the presence of the hydrogel, it maintained the soil with the highest amount of water (Figure 1). The frequencies of 0 (planting only) and 8 days (Figure 1a and 1b) presented more pronounced curves in relation to the others, and therefore, a decreasing tendency in the loss of water is inferred as the frequency of irrigation decreases. Also, it is verified that the variations in the soil water content throughout the experiment were higher when the polymer was not used.

When the irrigation occurred only at planting (Figure 1a), there was a delay in the days when the visual symptoms of the evaluated water deficit appeared in the presence of the hydrogel. The mild wilt symptoms (DMWS) appeared approximately 25 days after planting, when soil water content reached 10%. When compared to its

absence, this symptom appeared five days before with approximately 9% humidity. The presence of moderate wilt symptoms (DMS) in the presence of hydrogel occurred at 30 days after planting when the water content was 7.5% and in the absence of water at 24 days after planting with a humidity of 9%.

Figure 1 – Soil water content related to the visual symptomatology of water deficit in *Eucalyptus urograndis* clones in function of soil water content relationship between of irrigation and presence or absence of hydrogel. a) irrigation only at planting; b) irrigation every 8 days; c) Irrigation every 4 days and d) irrigation every day



Source: Authors (2017)

In where: DMWS: mild symptoms of wilt, DMS: moderate symptoms of wilt. DSS: severe symptoms of wilt and D: dead plant. PWP - permanent wilt point.

Considering the severe symptoms of wilt (DSS), it was observed that in the same way as the other evaluated symptoms. In the presence of hydrogel there was a delay of approximately five days in the appearance of these symptoms, occurring when the water content reached 6.5% (36 days after planting). In the absence, this symptom occurred at 31 days and at a soil water content of 7%. When the water retention polymer was used, it was verified that the death of the plants occurred approximately 38 days after planting when the soil water content reached 6.3%. With the non-use of hydrogel, these symptoms were observed at 33 days with 6.3% humidity.

For the 8 days frequency (Figure 1b), the onset of mild symptoms (DMWS) occurred two days later in the presence of the hydrogel. For the other irrigation frequencies, there were no symptoms of wilt. Water content in soil was always higher in the presence of the hydrogel at lower irrigation frequencies (only at planting, 8 and 4 days). This higher soil water content delayed the onset of stress symptoms in plants. There was no difference in water content in soil when irrigation was performed every day (Figure 1d), with a content close to 40% throughout the evaluations.

In general, the presence of the hydrogel promoted greater humidity. The onset of symptoms occurred when soil volumetric moisture exceeded the PWP - permanent wilt point (15.7%), whereas in the absence of symptoms appeared before reaching PWP, which in this condition was 15%. In the same sense, Felippe *et al*. (2016) state that hydrogel can be used to increase water storage capacity, minimizing the problems associated with irregular or deficient availability of water and poor structuring, being an alternative for low water availability in the ground. The increase in soil water storage capacity was also reported by Mendonça *et al*. (2013). The treatment of 4 g pot^{-1} (8L) increased the storage capacity of water in the soil in relation to the control by 12%, while, applying double the product (8 g pot^{-1}), the increase in relation to the control was 13%.

3.3 Gas exchange

In overall, the plants were affected by the irrigation frequencies, easily seen in the photosynthetic rate - *A* - (μmol m^{-2} s^{-1}) (Figure 2a). From the planting until the 14th day, the photosynthesis showed an increase of both in the absence and in the presence of the polymer, with no statistical difference between the treatments. During this period, water was not the limiting factor since there was enough soil water content in all treatments. The differences between irrigation frequencies were observed from the 21st day after planting, both in the treatments with the presence or absence of the polymer, thus occurring a rapid decrease of the photosynthetic rate. It should be noted that in this period (21 days) the plants that received irrigation only at planting with the presence of hydrogel already exhibited mild wilt symptoms, while in the absence of the polymer, mild and moderate wilt symptoms have already occurred. Among all frequencies, the highest photosynthetic rate was observed in the presence of hydrogel at 21 days after planting when irrigation occurred every day. In the absence of hydrogel the reduction was more pronounced reaching zero at 46 days.

Water deficit stress is produced both by water limitation in the soil and by more aerial transpiration than the roots can absorb. These processes are influenced by environmental factors and plant characteristics (KLIPPEL *et al*., 2014). Lawlor (2002) states that during water stress, photosynthetic metabolism is more sensitive to changes in cellular water content in some types of plants than in others, although the causes of these differences are still poorly understood. Some plants show decreases in the photosynthetic rate in a parallel and linear manner to the decrease in the relative water content, while others show a decrease in the photosynthetic rate only when the decrease in water content exceeds between 20 to 30%.

Analyzing the transpiration data - *E* - (mmol m^{-2} s^{-1}) (Figure 2b), it was verified that there was an independent variation of the irrigation frequency used. In general, there was an increase in perspiration rate up to the 14th day. After this period there was reduction, except when irrigation was performed more frequently. When irrigation occurred only at planting and every 8 days, the transpiration presented the lowest

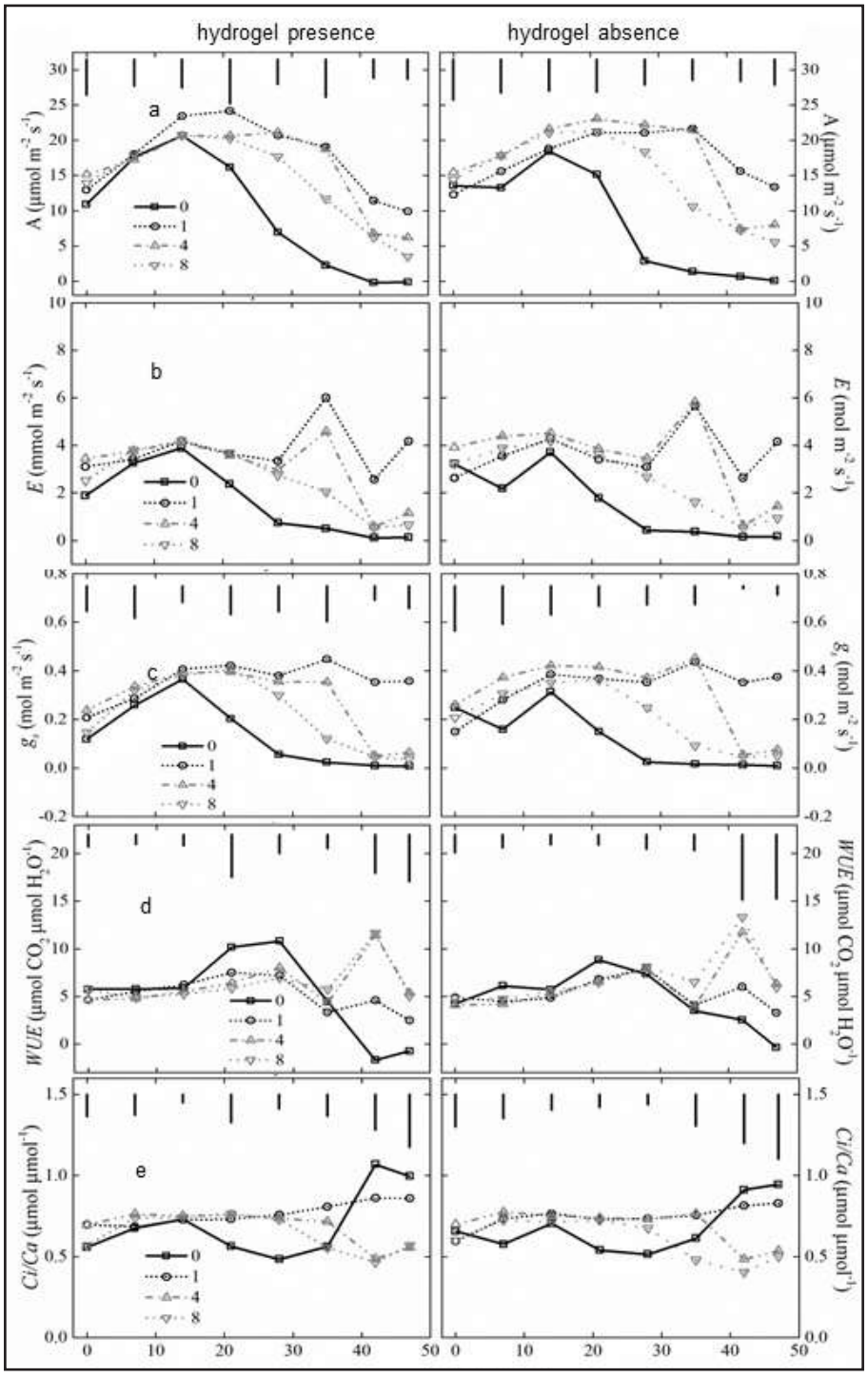
values, decreasing rapidly. In the presence of the hydrogel, the transpiration presented higher rates than in the absence of the polymer.

In the presence of the hydrogel, the transpiration (*E*) presented higher rates than in the absence of the polymer. This reduction in transpiration is because water deficit was responsible for decreasing leaf transpiration due to stomatal closure. Since the opening of stomata depends on the degree of water saturation of stomatal cells, there may be great restriction of transpiration when the water deficit in the plant is very large. The partial closure of the stomata is a strategy of plant tolerance to water deficiency because it reduces transpiration rate, maintains leaf water content, reduces the risk of dehydration and eventual death by desiccation (NASCIMENTO *et al*., 2011). In this work the results showed that, with a decline in the relative water content in the soil, i.e., as the frequency of irrigation decreases, there is also a decrease in the rate of transpiration.

The decline of stomatal conductance in the *Eucalyptus urograndis* clone used in the present study coincides with the behavior observed in irrigated and non-irrigated *Eucalyptus* sp. under field conditions, observed by Otto *et al*. (2013). This demonstrates that *Eucalyptus* exerts efficient stomatal control under conditions of low water availability in the soil.

The water use efficiency - *WUE* (μmol CO^2 μmol H_2O^{-1}) (Figure 2d) also obtained a behavior close as transpiration. When irrigation occurred only at planting, water use was similar until 14th, and there was no statistical difference between treatments regardless of presence or absence of the polymer. From the 14th to the 35th day in the presence of hydrogel, a higher *WUE* was detected. However, a more efficient use of water in the 35th day treatment did not use the polymer. It is likely that this efficiency, in this case, is linked to the lower rate of plant transpiration. The ecological advantage that plants obtain when regulating transpiration is to maintain a linear relation with the *WUE*, thus optimizing the gas exchanges. The efficiency of water use for crop productivity depends on the species, stage of development, plant density, environmental conditions, and above all, water availability and air evaporation capacity (LARCHER, 2006).

Figure 2 – a) photosynthetic rate (*A*); b) transpiration (*E*); c) stomatal conductance (g); d) water use efficiency (*WUE*) and e) relation between the intercellular and atmospheric concentrations of CO_2 (*Ci/Ca*) in *Eucalyptus urograndis* clone as a function of the presence or absence of hydrogel submitted to different irrigation frequencies



Source: Authors (2017)

In where: The floating bars indicate the minimum significant difference between the irrigation frequencies by the Scott-Knott's test ($p < 0.05$).

For a general perspective of the relation between the intercellular and atmospheric concentrations of CO_2 (*Ci/Ca*) (μmol^{-1} μmol^{-1}), there was only difference between the highest frequencies and the lowest ones of irrigation (Figure 2e). Both in the presence and in absence of hydrogel the values increased due to the water deficit, especially when irrigation occurred at planting. At this frequency of irrigation, during 14 to 28 days, there was a decrease in the *Ci/Ca*. These values are justified due to the stomatal closure, which generally reduced the exchange of gases between the environment and the interior of the leaf, but there was still the assimilation of CO_2 by the enzymes. From the 35th day, there was an increase consequently to the enzymes, mainly rubisco, assimilating CO_2. Thus, the limitation of *A* ceased to be strictly stomatal and became biochemically.

The understanding of the factors influencing the physiological responses of *Eucalyptus* plantations can help in the planning and location of the plantations and the selection of genotypes, as well as in the choice of the appropriate management for plantations installed in environments with water restrictions. In addition, obtaining data on the photosynthetic capacity of plants is an important tool for models based on processes that require physiological information to predict the growth and use of water of different genotypes (OTTO *et al*., 2013).

4 CONCLUSION

The hydroretent polymer allowed the addition of approximately 5 days in the survival of *Eucalyptus urograndis* clones, providing an improvement in the morphological variables.

The hydrogel effect positively influenced the storage and availability of water to the plant in the soil, especially when the irrigations occurred less frequently.

The gas exchanges were affected by the soil water content, which varied according to the frequency of irrigation. The water deficit was a limiting factor for the stomatal opening and contributed to the decrease in carbon assimilation by photosynthesis,

because of the lower was the water availability, the lower was the photosynthetic rate (*A*), transpiration (*E*) and stomatal conductance (*g*) values. Furthermore, the water use efficiency (*WUE*) increased when the availability of water decreased.

ACKNOWLEDGMENTS

The authors would like to thank Research and Innovation Support Foundation of Santa Catarina State (FAPESC) for the scholarship awarded.

The authors pay tribute to the co-author of the article and Professor Clenilso Sehnen Mota, who died before the publication of the work. Professor Clenilso was a research enthusiast and a lover of plant physiology.

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How to quote this article

Felippe, D.; Navroski, M. C.; Sampietro, J. A.; Mota, C. S.; Pereira, M. O.; Albuquerque, J. A.; Andrade, R. S.; Moraes, C. Hydrogel and irrigation frequencies in survival, growth and gas exchanges in Eucalyptus urograndis. Ciência Florestal, Santa Maria, v. 31, n. 4, p. 1569-1590, 2021. DOI 10.5902/1980509836889. Available from: https://doi.org/10.5902/1980509836889.

Ambiente & Água - An Interdisciplinary Journal of Applied Science
ISSN 1980-993X – doi:10.4136/1980-993X
www.ambi-agua.net
E-mail: ambi.agua@gmail.com

Hydrogel in the seedling growth of *Eucalyptus dunnii Maiden* under different irrigation management

ARTICLES doi:10.4136/ambi-agua.2582

Received: 29 Apr. 2020; Accepted: 03 Dec. 2020

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ABSTRACT

This study evaluated the effect of using hydrogel in the planting of *Eucalyptus dunnii* seedlings in pots, under different water-management regimens. Seedlings of approximately 25 cm height were planted in pots filled with 4.5 L of soil. The experiment was of Completely Randomized Design, in a 2x3 factorial, with absence and presence of hydrogel related to different water-management regimens (irrigation only in planting, every 3 to 6 days). From planting until the 47th day the seedlings were evaluated for the symptoms of water stress, chlorophyll content and gas exchange. Finally, the development of the plants was evaluated using the root dry weights and shoots, as well as by determining the water retention curve. As a result, the use of the polymer yielded an increase in water retention and a delay in the symptoms of drought stress, consequently increasing chlorophyll levels. One can observe the direct effect gas exchange of this kind, where recovery of the variables analyzed 24 h after irrigation was visible. Reviews of dry biomass demonstrated that treatment with the use of hydrogel showed the best development. However, the use of hydro retainer polymer increased the quality of *Eucalyptus dunnii* seedlings, besides being an alternative planting method in dry periods of the year.

Keywords: hydro retainer polymer, symptoms of water stress, water management.

Hidrogel no crescimento de mudas de *Eucalyptus dunnii Maiden* Submetidas a diferentes manejos de irrigação

RESUMO

Objetivou-se avaliar o efeito do hidrogel no plantio de mudas de *Eucalyptus dunnii* em vasos, relacionados com diferentes manejos hídricos. Utilizou-se mudas de aproximadamente 25 cm de altura plantadas em vasos preenchidos com 4,5 L de solo. O experimento foi realizado em DIC, esquema fatorial 2x3, com ausência e presença de hidrogel relacionados aos manejos hídricos (irrigação somente no plantio, a cada três e seis dias). Do plantio até o 47° dia foram realizadas avaliações da sintomatologia do estresse hídrico, teores de clorofila e trocas gasosas. Avaliou-se o desenvolvimento das mudas através da biomassa seca radicular e da parte aérea, além da determinação da curva de retenção de água. Como resultado, o uso do polímero proporcionou um aumento na retenção de água, e um atraso nos sintomas de estresse hídrico,

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aumentando assim os níveis de clorofila. Houve efeito direto nas trocas gasosas, sendo visível a recuperação das variáveis analisadas, 24 h após a irrigação. Avaliações da biomassa demonstraram que os tratamentos com o uso do hidrogel, apresentaram melhor desenvolvimento. O uso do polímero aumentou a qualidade das mudas, sendo uma alternativa de plantio em épocas secas do ano.

Palavras-chave: manejo hídrico, polímero hidroretentor, sintomatologia do estresse hídrico.

1. INTRODUCTION

A combination of several factors is necessary for the plantation of productive forests, such as site-adapted genetic materials, favorable edaphoclimatic conditions, proper use of management techniques and high-standard seedlings (Davide and Faria, 2008). Among the most widely cultivated forestry species in Brazil, the genus *Eucalyptus stands out,* currently occupying 6.97 million hectares of planted forest area, representing 71.9% of the total (IBA, 2020).

Soil and humidity conditions are dominant factors in the forest establishment, because they affect both planting and regeneration of plants (Sarvas *et al*., 2007). However, one of the main causes of mortality rates of forestry seedlings species, just after transplant, is their inability to stay properly hydrated (Thomas, 2008).

The use of hydro retainer polymers as soil humidity conditioners may therefore be beneficial. According to Mendonça *et al*. (2013), a "hydro retainer polymers" is a polymer that helps mainly in water retention for newly planted seedlings, increasing water availability for crops and local productivity, and minimizing production costs, contributing to the viability of planting throughout the year.

Based on this, the objective of this experiment was to evaluate the effects of hydrogel on *Eucalyptus dunnii* seedlings with different water-management regimens.

2. MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Santa Catarina State University Forest Nursery, located at coordinates 27°47'33.62" S e 50°18'4.60" W, with an approximate altitude of 900 meters above sea level.

According to the Köppen classification, the climate of the municipality of Lages (Santa Catarina) is humid mesothermal with a mild summer (Cfb-temperate). The annual average temperature is 15°C and the annual average precipitation stands between 1300 and 1500 mm.

The *Eucalyptus dunnii* seedlings used were grown from seed, and after being properly developed for planting, with a height close to 25 cm and at least 2 mm of stump diameter, were transferred to vases containing soil classified as Aluminium Humic Cambisol. Analysis (0-40 cm) showed the soil had the following chemical assignments: pH: 4.7; SMP: 4.9; Ca: 2.2 (cmolc/dm³); Mg: 1.2 (cmolc/dm³); Al: 4.9 (cmolc/dm³); H+Al: 9.6 (cmolc/dm³); effective CEC (Cation Exchange Capacity): 13.1 (cmolc/dm³); Aluminium saturation: 58.2%; base saturation: 26.8%; Clay: 47.6%; P (Mehlich): 3.7 (mg/dm³); K: 48.0 (mg/dm³); Cu (Mehlich): 1.8 (mg/dm³) and Zn (Mehlich): 3.4.

To simulate field conditions, we used polyethylene pots with 5-liter capacity and 4.5 liters of previously unsealed and dry soil. After filling with soil, the pots were watered until they reached field capacity (approximately 1.5 liters). Then a hole of approximately 300 cm³ was manually opened in the center of each pot for planting. In the treatments using hydrogel, 250 mL were added in the hydrated form before seedling planting. Hydration was performed with running well water 30 minutes before the planting; the hydrogel dosage used was 3 g L^{-1}. After the hole opening, with or without hydrogel, began the planting of *Eucalyptus dunnii* seedlings.

After planting, besides the 1500 mL to moisten the soil, 250 mL of water were added to each pot for the treatments without hydrogel, to compensate for the volume added by the hydrogel to the other treatments. For the treatments with irrigation, 250 mL of water were added in each pot, following the previously set frequency, according to each treatment. All irrigation treatments received 250 mL of water during the course of the experiment. This value was obtained by the average field capacity of the experiment by Felippe *et al*. (2016). The irrigation of the pots was carried out manually, with the amount of water measured in a beaker, and after carefully pouring over the soil (pot), an attempt was made to spread it as much as possible over the entire surface.

The experimental design was the Completely Randomized, in bifactorial scheme, where the "A" factor levels referred to presence or absence of hydrogel, and the "B" factor levels are referred to water management (only at planting, intervals of three and six days between each irrigation). Ten replicates composed of one plant from each replication were used.

2.1. Water retention curve

To determine the water retention curve, we used five soil samples with the presence of hydrogel incorporated to the substrate and five samples with the absence of the hydrogel, totaling ten samples, collected in cylindrical rings of approximately 50 cm^3, in order to preserve the maximum original soil structure. The available soil water content was calculated by subtracting the soil volumetric humidity value corresponding to the field capacity (FC), obtained at the water tension of 10 kPa, by the humidity value at the permanent withering point (PWP), obtained at the tension of 1500 kPa, according to Moniz (1972). From these limits, it was possible to determine the water storage capacity available in the soil (Bergamaschi *et al*., 1992).

In the laboratory, the samples with preserved structure were initially saturated by capillarity for 24 hours with a water layer of approximately 3 cm and then weighed. The samples were subsequently submitted to a sand column to obtain the humidity at the equilibrium tensions of 1, 6 and 10 kPa (Reinert and Reichert, 2006) and at the tensions of 33, 100, 500, 1000 and 1500 kPa in Richards pressure chambers (Klute, 1986). The volumetric humidity of the samples was obtained by the relation between the amount of retained water at a determined tension and the volume of the cylinder. At the end the samples were taken to the drying oven for about two days, where the soil dry weight was obtained, also estimating the density of the soil (Blake and Hartge, 1986). During the elaboration of the soil water retention curve with the addition of hydrogel, the variation of the sample volume was not considered as the humidity varied according to the expansion of the hydrogel.

With the humidity values at the tensions of 1, 6, 10, 33, 100, 500, 1000 and 1500 kPa, the soil water retention curve was adjusted, using the Van Genuchten (1980) model through the SWRC (*Soil Water Retention Curve*) software.

2.2. Physiological analysis

Until the 47th day after planting, daily evaluations of water stress symptoms, chlorophyll contents and greenhouse temperature verification were performed. For visual symptomatology, analysis criterion of Navroski *et al.* (2014) was adopted, noting the number of days the plant remained in each condition: SEM- days without symptoms (turgid plant, visually vigorous, with no evidence of water deficit); SLM- days with mild withering symptoms; SMM- days with moderated symptoms (plant in permanent withered state, with curved and darkened apex); SSM- days with severe wilting symptoms (dry leafs/in abscission). For survival, the code PPV was adopted – the number of days the plant remained alive.

Chlorophyll content was obtained using the portable chlorophyll meter SPAD-502 (Minolta Camera Co. Ltd.), measured in two physiologically mature leaves on opposite sides, in the middle portion of the plant top, the leaves were marked for further measures. From the

four readings, the average was calculated using the portable chlorophyll meter itself. The measurements were performed in ten repetitions, in all treatments, always in the pre-irrigation period.

Gas exchange evaluations were performed only in treatments with presence and absence of hydrogel, with a frequency of three days of irrigation, using five repetitions, and it was not possible to evaluate the other treatments, due to mortality that gradually occurred during the experiment. The evaluations were performed using an Infrared Gas Analyzer portable photosynthesis meter (Li-6400xt), determining the assimilation values of CO_2 (*A*), stomatal conductance (g_s), transpiration (*E*), relation between intercellular and atmospheric concentration CO_2 (*Ci/Ca*) and water-use efficiency (*WUE*). The water-use efficiency (*WUE*) was obtained by dividing *A* by *E*. Inside the greenhouse, the plants received, during the measurements, an irradiance of approximately 900 mmol of photons m^{-2} s^{-1}. Gas exchange evaluation was performed immediately before and 24 hours after irrigation, and 43 days after planting and the start of intermittent irrigation. Assessments on both days began around 8:00 am.

2.3. Dry biomass

At the end of the experiment (48 days), by a destructive method, the aerial and root biomass of the plants was manually collected. Collection was performed only in treatments that received intermittent irrigation, in the frequencies of three and six days, regardless of the absence or presence of the hydrogel. These were separated and stored in paper bags, kept in a forced air circulation oven (65 ± 3°C) until they reached constant mass, after weighing to determine the dry matter content by using a precision balance (0.01 g).

2.4. Statistical Analysis

After confirming the normality and homogeneity of the data of the analyzed variables, a parametric variance analysis was performed at a 5% error probability level. When the value of "F" was significant, the qualitative treatments had their averages compared by the Scott-Knott test or t-test, at 5% probability of error. The SISVAR software was used to perform the data analysis and for graphics the Origin Pro 9 and the Graphed 8 softwares were used.

3. RESULTS AND DISCUSSION

3.1. Available water retention curve

By determining the soil's water retention curve, it was found that the use of hydrogel incorporated in the soil caused a significant in the amount of water retention when compared to the same soil with the absence of the polymer, although this increase was more evident at tensions below 10 kPa (Figure 1). In any case, the addition of hydro-retaining polymers positively influenced the soil water-storage capacity.

The field capacity (FC) was higher (0.427 m^3 m^{-3}) in the presence of hydrogel than in the absence (0.349 m^3 m^{-3}). Regarding the permanent wilting point (PWP), hydrogel presence promoted inferior results when soil received the polymer (0.189 m^3 m^{-3}) compared to when it did not (0.206 m^3 m^{-3}). As a result, the available soil water storage capacity (WSC) was higher in the presence of the polymer (0.238 m^3 m^{-3}) compared to the absence (0.143 m^3 m^{-3}), resulting in a relative increase of 66%. The available water storage capacity increased due to the hydro-retaining polymer having a greater activity in ion exchange capacity with the soil solution, as it has a larger diffuse double layer than the sandy particles that are predominant in the soil used in the experiment. Therefore, it is expected that this effect will be more pronounced in sandy-textured soils, as in the present study, than in clayey-textured soils.

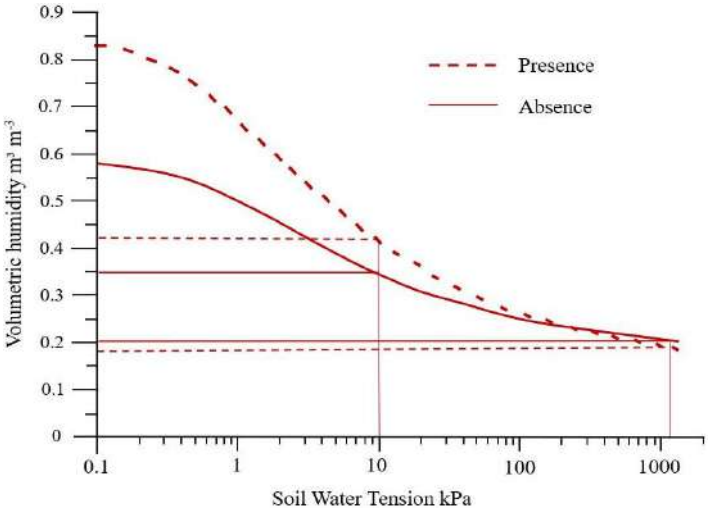


Figure 1. Soil water retention curve in Aluminium Humic Cambisol, with the absence (0 g L^{-1}) and presence of hydrogel (3 g L^{-1}), subjected to tensions of 1, 6, 10, 33 and 100, 500, 1000 and 1500 kPa.

This result can be explained by the high water absorption capacity of the hydrogel. According to Prevedello and Loyola (2007), the hydrogel has the capacity to absorb 150 to 400 times its dry mass and can be used to increase water storage capacity, minimizing problems associated with irregular or deficient water availability of water in the soil, when it may negatively affect plant development.

3.2. Photosynthetic analysis

Analyzing the hydrogel efficiency before irrigation and 24 hours afterwards, with the absence or presence of this polymer in the cultivation soil, the direct effect on gas exchange of this species can be observed (Table 1). Overall, there was no significant difference in the results of photosynthetic analyses collected prior to irrigation using the hydrogel. However, there was a difference ($p<0.05$) when analyses occurred 24 hours after irrigation. It was observed that the treatments without hydrogel presented the best values for all analyzed variables.

This fact can be explained by the drought-tolerance mechanism that the plant develops at the cellular level where the production or accumulation of osmotically active solutes occurs, a process known as "osmotic adjustment," in order to maintain turgor and water potential balance in the cell (Nunes, 2007). For Serraj and Sinclair (2002), this mechanism allows the survival of the water-deficient plant, but does not maintain plant growth and productivity.

Table 1. Liquid CO_2 assimilation (*A*), stomatal conductance (g_s), transpiration (*E*), relation between intercellular and atmospheric CO_2 concentration (*Ci/Ca*), and water use efficiency (*WUE*) in *Eucalyptus dunnii* before, and 24 hours after irrigation and with or without hydrogel in the soil.

Period	Hydrogel	*A*	g_s	*E*	*Ci/Ca*	*WUE*
		µmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	mol mol^{-1}	µmol $mmol^{-1}$
Before	With	2.44 Aa*	0.016 Aa	0.247 Aa	0.337 Aa	9.82 Ba
Before	Without	2.89 Aa	0.021 Aa	0.335 Aa	0.429 Aa	8.06 Aa
After	With	5.38 Ab	0.043 Aa	0.949 Ab	0.422 Aa	5.79 Ab
After	Without	8.18 Bb	0.092 Bb	1.563 Bb	0.557 Bb	5.59 Ab
CV (%)		21.5	19.2	20.4	26.1	16.8

*Averages followed by the same letter, uppercase for hydrogel and lowercase for period do not differ between each other by t-test (0.05).

Irrespective of the presence or not of irrigation, the plants with the absence of the hydro-retaining polymer presented the best physiological performance, presenting the better stomatal control and consequently higher values of *A*, *E* and *Ci/Ca*.

With the addition of the polymer, *A*, *E* and g_s recovery was visible 24 hours after irrigation, demonstrating that *A* limitation probably occurred due to stomatal limitation, given the rapid recovery. These results show that all physiological processes of the cell are directly or indirectly affected by water supply. However, Agaba *et al*. (2011), state that the hydrogel's ability to hold and make water available more slowly may favor the ability of seedlings to return to normal after water stress has occurred.

As for the *WUE* values, they maintained their higher values in the presence of hydrogel, both before irrigation and 24 hours after it. This fact may be justified due to the strategy that the plant develops to tolerate the water deficit. The control of plant water loss by stomatal closure keeps the water potential and relative leaf water content high (Souza *et al*., 2004). As a consequence, there are restrictions (or reductions) in stomatal conductance and gas exchange, which reduces the rate of transpiration and assimilation of CO_2 as observed.

The first and most sensitive response to water deficit is the decrease in turgidity, through which stomata closure, photosynthesis reduction and cell elongation decrease, especially in extension (Larcher, 2006). This stress caused by water deficiency is produced by both soil water limitation and excessive transpiration loss in relation to root absorption, and these processes are influenced by environmental factors and plant characteristics such as species or genotype (Sant'Anna, 2009).

3.3. Chlorophyll

The chlorophyll level of *Eucalyptus dunnii* leaves in both treatments (presence and absence) presented statistically higher averages (t-test) regarding the use of hydrogel in the three irrigation frequencies, and the highest rates were observed in the treatments with the presence of this polymer (Figure 2).

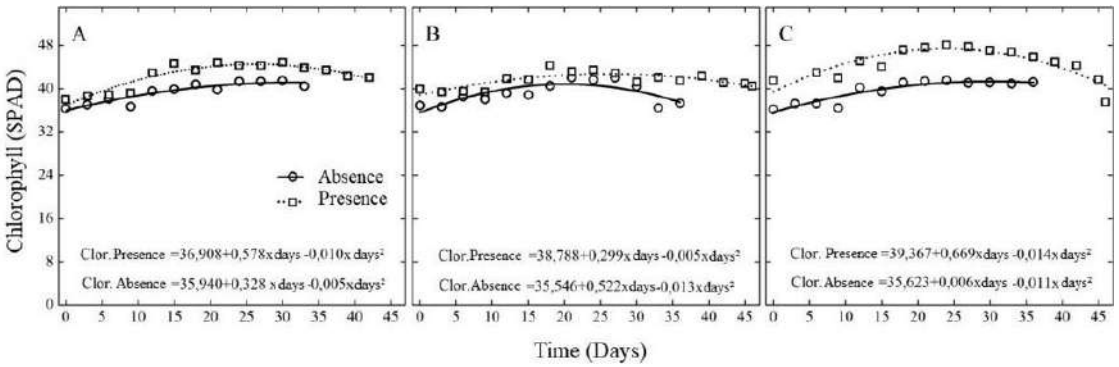


Figure 2. Chlorophyll content in plants as a function of the presence or absence of hydrogel and different irrigation frequencies. A-irrigation only at planting; B-irrigation every 3 days; C-irrigation every 6 days.

In the analysis of chlorophyll between irrigation frequencies, it was not possible to perceive behavior change between treatments. The chlorophyll content over time showed a quadratic behavior, with values close to 35 at the beginning of the experiment, reaching 45-48 around 25 days and decreasing again to close to 40 at the end of evaluation (47 days). And at all periods and over time, the values were higher for hydrogel treatment, as already discussed.

With these results, it is believed that the addition of this polymer possibly decreases nutrient loss by leaching (Navroski *et al*., 2015b), providing higher photosynthetic rate due to higher nitrogen contents, where this nutrient is directly linked to higher photosynthetic rate of the plant. In this sense, for the soil with higher water availability the mineralization rate of

organic matter increases in relation to the soil with lower humidity, thus making a larger amount of nitrogen available to the plant.

Tohidi-Moghadam *et al*. (2009), studying the response of six canola genotypes to water stress and hydrogel application, observed that water deficiency also reduced the chlorophyll content and that under the field conditions the hydrogel increased the performance of physiological characters, water deficiency and absence of hydrogel led to a decrease in all parameters evaluated. According to these authors, these results may be related to the reduction of photosynthesis and also chlorophyll content.

3.4. Symptomatology

Analyzing the irrigation frequencies for the classification days without symptoms (WOT), the obtained data showed there was interaction (p<0,05) between the factors of the evaluated variables. There was no effect of the presence or absence of hydrogel on irrigation use only at planting. In the presence of hydrogel, the frequencies of 3 and 6 days showed a relatively high gain without the appearance of symptoms, compared to the absence of this polymer, which increased the time for the emergence of stress compared to irrigation only at planting, demonstrating once again that the use of hydrogel responds positively to water scarcity in the soil.

No interaction was observed between hydrogel use and irrigation frequency in the evaluation of plants with mild withering symptoms (MWS), independent of irrigation frequency. With the presence of hydrogel, *Eucalyptus dunnii* seedlings remained more days without the onset of water stress, delaying the appearance of symptoms at all levels, compared to the absence of the polymer, where symptoms appeared much earlier (Table 2).

Regarding irrigation in the presence of the polymer, the frequency of 6 days showed a gain in days without the onset of these symptoms. Not using it, even with irrigation, there was no accelerated occurrence of these symptoms. It is noteworthy that at this stress level the seedlings still have a total physiological condition to recover in the occurrence of rain. The use of irrigation had a positive effect, as expected, and at the frequency of 3 days there was the greatest delay in the onset of symptoms.

In the appearance of the moderate withering symptoms class (MWS), where the plant was close to the permanent wilting point, the analyzed data showed that there was interaction between the factors of the variables. In this class, there was also a delay in stress symptoms when hydrogel was present.

In the class of severe withering symptoms (SWS) where, according to Navroski *et al*. (2014), at this level of stress a recovery of the plant with new irrigations or rainfall is already difficult, the hydrogel still continued to be efficient, and there was a delay of more than five days in the onset of these symptoms when using irrigation only in the planting, in relation to not using it.

These results corroborate the findings of Navroski *et al*. (2014) in the study of the influence of the hydro retainer polymer on the survival of *Eucalyptus dunnii* seedlings under different water-management regimens, where its use mitigated water scarcity in the soil, allowing the delay of water deficit symptoms, with its influence being greater when irrigation was performed less frequently.

It's important to highlight that, in the implantation of *Eucalyptus* forests, it is usually observed that the most critical period for the loss of seedlings due to lack of water is in the first 20 days, when the plant is being established and its root system is still in formation (Fernández *et al*., 2010). In the field planting, it can be assumed that in cases where irrigation is frequent, or where rainfall is higher, the use of the polymer could be discarded (Navroski *et al*., 2015a). However, under less frequent rain or more-spaced irrigation, the use of hydrogel may be recommended.

Table 2. Averages results of duration of symptoms of water stress, in days, evaluated from planting until the 47th day after planting *Eucalyptus dunnii* in pots, due to the presence or absence of hydrogel and different irrigation frequencies, where: WOT – no withering symptoms; MLS – mild withering symptoms; MWS – moderate withering symptoms; SWS – severe withering symptoms and PRA – plants remained alive.

Variable	Hydrogel	Appearance of stress symptoms (days)			Averages
		Irrigation frequency			
		Planting	6 days	3 days	
WOT**	Absence	23.10 aB	24.80 bB	31.70 bA	26.54
	Presence	24.10 aC	34.12 aB	44.90 aA	24.37
	Average	23.60	29.21	38.30	
MLS	Absence	27.10	32.70	44.00	34.60
	Presence	31.00	34.33	46.00	37.20
	Average	29.05B	33.47 B	45.00 A	
MWS	Absence	32.00 bC	36.50 bB	44.50 aA	37.66
	Presence	36.50 aB	45.56 aA	46.00 aA	42.58
	Average	34.25	40.78	45.25	
SWS	Absence	35.40 bC	40.80 bB	44.70 aA	40.30
	Presence	41.70 aB	46.00 aA	46.00 aA	44.52
	Average	38.55	43.26	45.35	
PRA	Absence	35.40 bC	40.80 bB	44.70 aA	40.30
	Presence	41.70 aB	45.55 aA	46.00 aA	44.37
	Average	38.55	43.18	45.35	

* Averages followed by the same letter, uppercase letter in the line (Scott-Knott test), and lowercase in the column (t-test) do not differ from each other at 5% probability of error.

In the evaluation of the number of days the plants remained alive (PRA), it was noticed that the use of hydrogel presented a difference when irrigation occurred every 6 days and only at planting. In this case, with the presence of the hydrogel in the soil, an increase of approximately 5 to 6 days in which they remained alive was obtained, thus justifying the expenses with the use of the polymer.

In the use of post-planting irrigations, frequently no significant effect of hydrogel was observed. This may indicate that the use of hydrogel is beneficial in delaying seedling mortality when frequent or infrequent irrigation is used only at planting, allowing a gain of a few days waiting for rain to occur.

3.5. Dry biomass

The results showed that for the root dry mass variable, the hydrogel interaction and irrigation frequency time was not significant ($p>0.05$), there was only difference for the isolated factors. In the presence of hydrogel, there was a significant difference, where the root dry mass was higher, presenting 2.17 g and in the absence only 1.12 g. As expected, the addition of the hydrogel contributed to the development of *Eucalyptus dunnii* seedlings, presenting a significant increase in root dry mass. Eloy *et al*. (2013) state that this variable is of great importance in the development of plants, since when there is a good rooting, they have a greater potential for growth and field survival.

In the evaluation of the presence of hydrogel, within the frequencies of 3 and 6 days, there was no significant difference. These results indicate that regardless of irrigation time, the use of hydrogel favored the increase of root dry mass of seedlings. In the absence of the hydrogel, there was a significant difference between the frequencies, where the frequency of 3 days had 1.97 g of root dry mass and for the frequency of irrigation of 6 days the root dry mass was lower, with 1.33 grams.

For the variable dry mass of the aerial parts, the hydrogel interaction and irrigation frequency were not significant ($p>0.05$). In the presence of hydrogel there was significant difference, and the highest values were found for the treatment with hydrogel, in which the dry mass of the aerial parts was higher (4.35 g), compared to the absence (2.34 g).

For the frequency of irrigation, there was a significant difference, and in the frequency of 3 days the dry mass of the aerial parts was higher (3.99 g) compared to the frequency of 6 days (2.7 g). The results indicate that the use of hydrogel incorporated into the soil and associated with different irrigation frequencies positively influenced the development of aerial parts of *Eucalyptus dunnii* seedlings.

4. CONCLUSION

- The use of hydrogel promotes a significant increase in water retention in the soil, allowing the delay of water deficit symptoms, although the effect of retention was greater at lower tensions (less than 10 kPa).

- The presence of the hydro retainer polymer in the soil presents higher averages for morphological characteristics, and in general decreased symptoms of water deficit in plants.

- There is a direct effect on gas exchange, with recovery of the variables analyzed 24 hours after irrigation. However, plants grown in the absence of the hydrogel exhibit the best physiological performance, so further studies should be conducted (preferably in the field) to understand physiological processes.

- In general, the hydrogel allows the reduction of the frequency of irrigation, showing a better result when the plants are under water restriction.

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Lovatel et al. *New Zealand Journal of Forestry Science* (2021) 51:11
https://doi.org/10.33494/nzjfs512021x84x
E-ISSN: 1179-5395
published on-line: 04/09/2021

RESEARCH ARTICLE Open Access

New Zealand Journal of Forestry Science

Impacts of genetic selection on *Sequoia sempervirens* mini-cutting rooting and initial growth in the field

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(Received for publication 26 November 2019; accepted in revised form 24 August 2021)

Abstract

Background: Vegetative propagation from superior individuals allows multiple copies of plants that are genetically identical to the parent plant to be obtained. However, vegetative propagation success varies among individual genotypes, with some clones having more difficulty forming roots than others. The aim of this study was to evaluate the genetic gain in *Sequoia sempervirens* (D.Don) Endl. clones using parameters describing vegetative propagation success and initial growth in field.

Methods: Vegetative propagation success was quantified for 16 clones in a completely randomised design consisting of 10 replications, each containing 10 mini-cuttings. At 90 days, rooting (RT), survival (SV) and the number of new shoots (NS) were evaluated. Performance after planting in the field was assessed using 13 clones from the previous experiment, arranged in linear parcels of 10 plants with 8 replicates. After 18 months, survival (SV), stem diameter (SD), height (H) and dominance breakdown (DB) were assessed. Estimates of variance components, heritability and genetic correlations were obtained using the Selegen-REML/BLUP software.

Results: The mini cuttings of the 16 clones had a coefficient of genetic variation ($CV_{gi}\%$) of 32.32% for RT, 5.44% for SV and 5.35% for NS. The heritability of the total genetic effects ($H^2 g$) for RT was 0.68. The clones with the best predicted genotypic classifications for the characteristics evaluated in the field were A116, A140 and A138 for SV, A126, A140 and A138 for SD, A138, A140 and A117 for H and A138, A228 and A116 for DB.

Conclusions: In general, it was possible to obtain high genetic gain for rooting and medium gain for dendrometric variables in the field.

Keywords: vegetative propagation; clonal test; Selegen-REML/BLUP; selection gain.

Introduction

The species *Sequoia sempervirens* (D.Don) Endl. known worldwide as sequoia or redwood, is native to North America, mainly from the central and northern California coast, a region with moderate to high rainfall in winter and fog in summer (Olson et al. 1990; Cown and McKinley 2008). Sequoia wood is widely used for the construction of decks, fences, windows, doors, shutters and interior applications where appearance and stability are important requirements (Cown and McKinley 2008). Sequoia is a species that mainly propagates through seed production, however, the seed germination rate is generally less than 10% and seedling viability is very low (Ozudogru et al. 2011). In addition, young plants have lower seed viability, with higher values being obtained from 250-year-old trees (Olson et al. 1990). Alternatively, in natural stands the species can reproduce by vegetative propagation. When a tree is cut or burned, sprouts appear from the cut trunk or already established roots and grow more vigorously than other understory plants. Thus, an individual's genetic information is the same as that of the trees that inhabited the site thousands of years ago (Luna 2008).

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For commercial forestry, vegetative propagation from superior individuals allows the multiplication of the best genotypes and plants that are genetically identical to the parent plant to be obtained. It also allows the multiplication of selected trees that contain favourable genetic combinations and the production of genetically homogeneous material that can develop in a predictable and uniform manner (Wendling et al. 2014).

Mini cuttings (cuttings taken from mini hedges not previously rejuvenated by in vitro techniques) is one of the most widely used vegetative propagation techniques. It allows the selection of those individuals with the best characteristics in tree breeding programmes and can reduce the length of the breeding cycle (Freitas et al. 2016). Compared with traditional cutting methods, which suffer from "physiological ageing" problems with the donor plants, mini-cuttings offer advantages in the small size of clonal mini-hedges, high productivity, lower cost and better rooting indices (Majada et al, 2011).

Plant responses to vegetative propagation and their consequences have received little attention in the quantitative genetics literature. By contrast, studies in horticulture and silviculture have examined some aspects of this problem. Genetic variation in rooting ability has been observed in several economically important species and environmental effects on rooting have been widely studied to find methods to optimise rooting of cuttings for commercial production (Schwaegerle 2005). Although rooting is an important factor in the selection of clones in forestry, few studies address the use of this characteristic (rooting) in the early selection of superior genotypes. Our hypothesis is that characteristics such as the rooting of cuttings and initial growth in the field following out-planting can be used as selection criteria for sequoia clones. Therefore, the objective of this work was to evaluate the genetic gain in *Sequoia sempervirens* clones using parameters related to vegetative propagation success and initial growth in the field.

Methods

Vegetative propagation and rooting success of mini-cuttings

The experiment compared the propagation success and rooting of mini-cuttings from sixteen different clones (A100, A113, A115, A116, A117, A126, A127, A130, A131, A133, A135, A136, A138, A140, A227 and A228). Material for the experiment was sourced from stumps of selected 40-year-old sequoia parent trees. Conventional cuttings were collected from the matrix trees 120 days after partial or complete annealing, as described by Pereira et al. (2017). This material was transported to the forest nursery in Lages, Santa Catarina state, Brazil. From this material, 10 cm cuttings were made, each containing a pair of acicular leaves with the total leaf area reduced by 50%. These cuttings were treated with 6,000 mg L^{-1} of indole-3-butyric acid (IBA), set in tubes (180 cm^3) containing medium-sized (2–4 mm) vermiculite and commercial substrate (1:1 v/v) and placed in a mini-tunnel for rooting to occur.

Five months after these cuttings were set, rooted plants were acclimated in a shade house for 30 days and then transferred to a greenhouse to complete acclimatisation for another 30 days. Subsequently, plants approximately 20 cm high were transferred to 5-L pots filled with a commercial substrate consisting of peat and decomposed pine bark (50%) and medium-sized (2–4 mm) vermiculite (50%). Fifteen days after installation (the time needed to adapt the plants to the system) the apex of the plant's main sprout was pruned to a height of 10 cm (± 2 cm), thus forming the mini-stumps for the establishment of the clonal mini-garden. Pruning of the mini-stumps was performed each month over a four-month period.

The nutrient solution used in the clonal mini-garden was based on commercial fertiliser comprising 10% N (water-soluble); 42% P_2O_5 (water soluble); 10% K_2O (water soluble); 0.6% Mg; 0.1% Fe and 0.02% Br. Fertigation was carried out twice a week, with each mini-strain receiving 50 mL of the solution composed by diluting 1.5 g of fertiliser in 1L of water. After the formation of mini-stumps, shoots were collected for the experiments.

From each clone, 8 to 10 cm long mini-cuttings were prepared, with the basal portion bevel cut and the upper portion incised transversely, and with a pair of acicular leaves cut in half. Once prepared, the cuttings were placed to root in 180 cm^3 polypropylene tubes containing an average size particle (2–4 mm) of vermiculite and commercial substrate (1:1 v/v) with the addition of 6 g L^{-1} of controlled release fertiliser, in which the insertion of approximately 2 cm from the base of the mini-cutting into the substrate was made. The commercial plant substrate, according to the manufacturer, is composed of peat, expanded vermiculite, pine bark and charcoal. The chemical and physical characteristics after substrate analysis are as follows: pH = 6.6; electrical conductivity = 0.13 mS cm^{-1}; wet density = 450.8 kg m^{-3}; dry density = 302.7 kg m^{-3}; current humidity = 32.8 (%); total porosity = 72.2 (%); aeration space = 17.0 (%); easily available water = 12.2 (%); buffering water = 2.4 (%); remaining water = 40.5 (%); WHC10 – water holding capacity at 10 cm = 55.1; WHC50 = 42.9 and WHC100 = 40.5.

The trays containing the tubes with the mini-cuttings were placed in a mini-tunnel – plastic covered greenhouse structure (measuring 8.0 m long x 1.20 m wide x 0.9 m high). The temperature inside the greenhouse usually varied between 20–32°C and the relative humidity remained above 80%, being irrigated by micro-sprinkling for 5 minutes, five times a day. The mini-cuttings remained in this condition for 90 days, at which time the evaluation of the experiment was performed. The experiment followed a completely randomised design with 10 replications of 10 mini-cuttings from each clone.

Survival percentage (SV), percentage of rooted cuttings (RT) and the number of new shoots (NS) were evaluated. Mini-cuttings with live wood, old leaves or young shoots, rooted or not, were considered survivors. The rooting percentage was considered over the total, not only the surviving mini-cuttings. The number of

roots was not evaluated because the plants from the experiment were destined for field planting. Counting the number of roots implies substrate disruption, causing serious damage to the seedling roots.

Initial growth in the field

The clonal mini-cuttings test was installed in Curitibanos – Santa Cristina (Brazil) in October 2017. The region, according to Koppen, has the mesothermal humid subtropical climate (Cfb) (Alvares et al. 2013). Prior to planting, subsoiling and a rotary hoe were used to prepare the site. Plants from the rooting experiment were used for this study. At the time of planting, they were nine months old. Of the 16 clones used in the rooting experiment, four of them did not have sufficient rooted material for initial growth in the field (A113, A115, A127, A13). Clone A110 was added to field planting experiment but was not used in the rooting experiment.

At the time of planting, the mini-cuttings were approximately 30 cm tall and had a root-collar diameter greater than 4 mm. Fifteen days before planting, they underwent an acclimatisation period, with reduced irrigation and maintenance in full sun. Fifteen days after planting, 150 g of NPK (5-20-20) fertiliser was applied to each plant. The growth study was undertaken using 13 clones (A100, A110, A116, A117, A126, A130, A131, A135, A136, A138, A140, A227 and A228) in lines of 10 plants per clone with 8 replicates for a total of 80 plants tested per clone. The planting spacing used was 3.0 x 3.0 m. All clonal stock was in the form of mini-cuttings produced from the clonal mini-garden.

Eighteen months after out-planting in the field, the following variables were measured: survival (SV), stem diameter (SD), height (H) and dominance breakdown (DB) in relation to the early testing of sequoia clones in the field.

Statistical analysis

Estimates of variance components, heritability and genetic correlations were obtained from mixed models fitted using the Selegen-REML/BLUP software (Resende 2016). An alternative to be used in the construction of indexes that can lead to a more accurate selection process is the use of restricted maximum likelihood (REML) and best linear unbiased prediction (BLUP). This method is based on the assumptions that the smaller the standard deviation of genotypic behaviour between two sites, the greater the harmonic mean of their genotypic values between two sites (Silva et al. 2011; Rosado et al. 2012). The REML/BLUP procedure has several advantages as it considers genotypic effects as random, can deal with imbalance, non-orthogonality and heterogeneity of variances, outliers, correlated errors within locations, provides breeding values after discounting environmental effects, and can be applied to any number of environments (Resende 2007).

Vegetative propagation and rooting success were evaluated assuming a completely randomised design, with testing of unrelated clones and more than one plant per parcel. The following statistical model was used for determining the genetic variance components:

$$y = Xu + Zg + e \quad [1]$$

where: y = data vector; u = fixed effect vector of the general average; g = vector of genotypic effects assumed to be random; e = error vector or random residue.

The variance components analysed for vegetative propagation and rooting were: σ^2_g = genotypic variance, σ^2_e = residual variance, σ_{fi} = individual phenotypic variance, h^2_g = heritability of individual parcels in the broad sense (i.e., effects of total genotypes), CV_{gi} (%) = genotypic variation coefficient and CV_e (%) = residual variation coefficient and overall mean of the experiment. Analysis of the different parameters related to the initial growth in the field was undertaken assuming a randomised block experimental design with several plants per parcel and testing of unrelated clones. The following statistical model was used for determining the genetic variance components:

$$y = Xr + Zg + Wp + e \quad [2]$$

where y = data vector; r = repeating effects vector (assumed to be random) plus the overall mean; g = genotypic effects vector assumed to be random; p = parcel effects vector; e = error vector or random residual. The variance components analysed in the field clone test were: σ^2_g = genotypic variance, σ^2_{parc} = environmental variance between parcels, σ^2_e = residual variance, σ^2_{fi} = individual phenotypic variance, h^2_g = heritability of individual parcels in the broad sense (i.e., the effects of total genotypes), h^2_{aj} = individual heritability in the broad sense (adjusted for parcel purposes), C^2_{parc} = coefficients for determining the effects of parcels, h^2_{mc} = heritability of the mean genotype, Ac_{clon}(%) = the genotype selection accuracy, CV_{gi}(%) = coefficient of genotypic variation, CV_e(%) = residual coefficient of variation, CV_r = coefficient of relative variation, PEV = prediction error variance of the genotypic values, SEP = standard deviation of predicted genotypic value and overall mean of the experiment.

Results

Vegetative propagation and rooting of mini-cuttings

Results from the experiments with sequoia mini-cuttings showed that there was considerable potential to improve vegetative propagation success through genetic selection. The coefficient of genetic variation (CV_{gi}%) for RT was 32.32% (Table 1), which was high when compared with the same parameter obtained for SV (5.44) and NS (5.35). Heritability estimates for the SV and NS variables were low, being 0.16 and 0.04, respectively. With this wide variation, genotypic means for RT ranged from 23.73% for clone A138 to 98.77% for clone A113 (Table 2). Mean SV values were above 85% for all clones and NS percentage was above 76%. The best results for RT were obtained for clones A113 (97.7%), A127 (97.8%), A136 (96.9%) and A115 (94.5%). These same clones exhibited good performance across the other traits that were assessed.

The heritability of the total genetic effects (h^2_g) for the rooting variable was considered high (0.68), which has

TABLE 1: Variance components for the survival (SV), rooting (RT) and number of new shoots (NS) for 16 *Sequoia sempervirens* clones measured 90 days after setting.

Component	SV (%)	RT (%)	NS
σ^2_{ga}	26.70	580.00	19.45
σ^2_{e}	131.31	262.49	401.17
σ^2_{fi}	158.02	842.50	420.62
h^2_{a}	0.1689 ± (0.0841)	0.6884 ± (0.1698)	0.0462 ± (0.044)
CV_{gi} *(%)*	5.44	32.32	5.35
CV_{e} *(%)*	12.06	21.74	24.30
General average	94.98	74.49	82.41

important implications for the genetic selection of clones as this is the most limiting feature for the propagation of clones via mini-cuttings.

Initial growth in the field

The clones with the highest survival when planted in the field were A116, A140 and A138; for stem diameter the best performing clones were A126, A140 and A138; for height: the best performing clones were A138, A140 and A117 and for dominance breakdown the best performing clones were A138, A228 and A116 (Table 3). One of the best performing clones in the field (A138) had the worst rooting score in the greenhouse experiment, and by contrast clone A136, which showed poorer field performance, had the third best rooting score (Table 2). Genotypic mean values (*u*+*g*) ranged from 0.33 to 0.81 (%) for SV; 12.02 to 27.25 (mm) for SD; 58.60 to 118.53 (cm) for H and 0.42 to 0.70 (%) for DB. The worst performing clones in terms of survival, stem diameter, height and dominance breakdown were A117, A228, A228 and A136, respectively. High values of genotypic coefficients of variation (20.06 to 34.48%) were found, indicating that these traits, especially survival, have good potential to be selected for (Table 4). The heritability of

TABLE 2: Genotype classification by predicted genotypic effect (*g*) and genotypic mean (*u*+*g*) for rooting (RT), survival (SV) and number of new shoots (NS) of *Sequoia sempervirens* clones evaluated at 90 days after setting.

Clone	RT (%)			SV (%)			NS		
	RK[1]	*g*	*u + g*	RK	*g*	*u + g*	RK	*g*	*u + g*
A100	15	-35.8052	38.6894	4	3.2440	98.2274	11	-0.902	81.5105
A113	1	24.2842	98.7788	7	2.0225	97.0060	7	0.786	83.1988
A115	4	20.0443	94.5389	6	2.3987	97.3822	13	-1.314	81.0987
A116	8	9.4153	83.9099	11	1.0166	96.0000	10	-0.687	81.7257
A117	13	-25.0068	49.4878	13	-4.7069	90.2765	3	2.621	85.0745
A126	14	-33.6137	40.8809	16	-9.7276	85.2559	14	-2.504	79.9084
A127	2	23.3836	97.8782	5	2.6909	97.6744	2	3.671	86.1796
A130	11	2.8962	77.3908	9	1.5486	96.5320	15	-3.831	78.5816
A131	12	-6.8018	67.6928	8	1.9007	96.8842	12	-0.942	81.4709
A133	9	9.3564	83.8510	1	3.7779	98.7614	1	4.094	86.5065
A135	10	4.8486	79.3433	12	0.1708	95.1543	9	-0.196	82.2168
A136	3	22.4858	96.9804	3	3.2440	98.2274	4	1.732	84.1442
A138	16	-50.7630	23.7316	2	3.7779	98.7614	16	-5.761	76.6514
A140	7	11.1684	85.6630	14	-6.4140	88.5695	5	1.707	84.1199
A227	5	12.5510	87.0457	10	1.5201	96.5035	8	0.185	82.5976
A228	6	11.5567	86.0513	15	-6.4642	88.5193	6	1.202	83.6145

[1] Genotype ranking

TABLE 3: Genotype classification by predicted genotypic effect (*g*) and genotypic mean (*u*+*g*) for the survival (SV), stem diameter (SD), height (H) and dominance break (DB) for *Sequoia sempervirens* clones evaluated at 18 months after out-planting in the field.

Clone	SV (%)			SD (mm)			H (cm)			DB		
	RK[1]	*g*	*u + g*	RK	*g*	*u + g*	RK	*g*	*u + g*	RK	*g*	*u + g*
A100	4	0.1446	0.6680	9	-1.3003	19.8897	7	-0.4797	88.3223	7	0.0569	0.6393
A110	10	-0.1230	0.4005	12	-1.8618	19.3283	8	-4.1648	84.6372	4	0.1026	0.6850
A116	1	0.2866	0.8100	5	0.8082	21.9982	4	6.7229	95.5248	3	0.1031	0.6855
A117	13	-0.1947	0.3288	8	-0.7677	20.4224	3	7.5452	96.3472	6	0.0619	0.6443
A126	5	0.0568	0.5802	1	6.0628	27.2528	5	2.4033	91.2052	12	-0.1270	0.4554
A130	12	-0.1738	0.3496	7	-0.6574	20.5327	10	-5.3187	83.4832	10	-0.1183	0.4641
A131	11	-0.1428	0.3807	10	-1.5317	19.6584	6	1.1233	89.9253	8	0.0070	0.5894
A135	8	-0.0845	0.4389	4	1.9368	23.1268	9	-4.2276	84.5743	5	0.0792	0.6616
A136	9	-0.1031	0.4203	11	-1.5331	19.6570	12	-10.0620	78.7400	13	-0.1601	0.4223
A138	3	0.1700	0.6934	3	3.8569	25.0469	1	29.7256	118.5276	1	0.1226	0.7050
A140	2	0.1802	0.7037	2	4.4807	25.6707	2	14.7124	103.5143	9	-0.1142	0.4681
A227	7	-0.0485	0.4749	6	-0.3222	20.8678	11	-7.7769	81.0251	11	-0.1226	0.4597
A228	6	0.0325	0.5560	13	-9.1713	12.0187	13	-30.2030	58.5989	2	0.1089	0.6913

[1] RK – Genotype ranking

parcels in relation to genotypes (0.093 to 0.157) was not as high as that for rooting (0.688), but values for the SD and H traits were close to those found for quantitative selection traits in other species (0.157 and 0.148 respectively). Individual heritability values for parcel effects were very similar compared to the effects of total genotypes (0.103 to 0.157).

Discussion

The coefficients of genetic variation, both at an individual level (Cv_{gi}) and progeny level (Cv_{gp}) are accepted as essential indicators of existing variation, allowing the estimation of genetic gains in provenance and progeny tests (Sebbenn et al. 2009; Rosado et al. 2012). The genotypic coefficient of variation values estimated for

TABLE 4: Variance components for survival (SV), stem diameter (SD), height (H) and dominance breakdown (DB) of *Sequoia sempervirens* clones evaluated at 18 months after out-planting in the field.

Component	SV (%)	SD (mm)	H (cm)	DB
σ^2_g	0.03	22.83	317.30	0.02
σ^2_{parc}	0.00	0.23	92.15	0.03
σ^2_e	0.21	122.13	1722.83	0.20
σ^2_{fi}	0.25	145.20	2132.28	0.25
h^2_g	0.1320 ± 0.0557	0.1572 ± 0.0799	0.1488 ± 0.0777	0.0929 ± 0.0614
h^2_{aj}	0.1321	0.1575	0.1555	0.1033
C^2_{parc}	0.00	0.00	0.04	0.10
h^2_{mc}	0.6628	0.7035	0.5854	0.3645
Ac_{clon} *(%)*	0.81	0.84	0.77	0.60
CV_{gi}*(%)*	34.48	22.55	20.06	26.19
CV_e*(%)*	24.59	14.64	16.88	34.58
CV_r*(%)*	1.40	1.54	1.19	0.76
PEV (%)	0.01	6.77	131.54	0.01
SEP (%)	0.10	2.60	11.47	0.12
General average	0.52	21.19	88.80	0.58

the RT trait in mini-cuttings and for SV, SD, H and DB traits in field trials were all above 20%. Coefficients of genetic variation (Cv_g) above 10%, as found in the present study, indicate that there is considerable genetic variability to be explored and that there are genetically superior clones in the experiment (Villacorta et al. 2015; Stovall et al. 2011). This value demonstrates elevated experimental precision, meaning that the model was able to capture most of the variation in the test and, as such, the estimates can be trusted. These values are similar to those found by Westbrook et al. (2015) and Sykes et al. (2006) for loblolly pine (*Pinus taeda*).

Based on Resende (2007), the accuracy observed was high for survival (SV), stem diameter (SD) and height (H), and was similar to the accuracy levels reported in other studies on forestry species (Gapare et al. 2015). The observed value of Ac_{clon} indicates that selection based on SV, SD and H can be considered accurate, as the true values (which are unknown) and predicted values are very similar.

Estimates of genetic parameters are important for directing breeding programmes, as they aid the selection process and serve as a theoretical framework to support the recommendations of commercial materials (Maia et al. 2009). However, it should be noted that this variation cannot be related only to the effects of heritability in the broad sense, i.e., the proportion of variability caused by the effects of genes (Jung et al. 2008). This variation may also be linked to the clonal C-effects (Pereira et al. 2018). C-effects can result in an artificial increase in clonal variation, which may increase the estimates of genetic gains in clonal selection (Frampton & Foster 1993). According to the same authors, this discrepancy may be due to the problems associated with vegetative propagation and the degree of environmental variation in the place where the clonal test is performed.

Overall, the best rooting clones were the ones with the poorest field performances, as was the case with clones A138 and A140, which had good rankings for the field variables, while in rooting their positions were intermediate to poor (16 and 7 respectively). By contrast, clone A136 which ranked third best for the RT trait demonstrated poorer field results for all four characteristics evaluated. Differences in rooting may be associated with differences in subsequent growth. These differences may be related to carryover effects, or C-effects, which are intergenerational environmental effects that occur when the performance of vegetative propagules is influenced by the environment in which the propagules developed. C-effects can also bias estimates of genetic and environmental parameters in plant growth studies by inflating variation among genotypes. C-effects arise from environmental differences among plants used as the source for cuttings: plants of the previous clonal generation (Schwaegerle 2005).

Although good levels of rooting were generally achieved, plants produced by sequoia mini-cuttings can be inefficient in the acclimatisation process and this can lead to high mortality, which requires careful consideration of environmental variables, particularly high temperatures (Luna 2008). Clones A113, A115 and A127, which were in the top four in the percentage of RT, were not field tested due to high levels of nursery mortality and the resulting lack of material for planting.

The heritability of individual parcels of total genotypic effects found for rooting was high (0.68); values above 0.5 are considered high (Resende 2002), indicating the possibility for satisfactory gains with selection for this propagation trait for sequoia mini-cuttings. However, vegetative propagation traits, such as rooting, must have a secondary and auxiliary character, the most important parameters for selection being those related to the survival, shape and productivity of trees in the field.

For field evaluations, heritability was only found to be close to the normal range for quantitative traits (0.15– 0.5) for SD and H. Polygenic traits such as diameter and height have complex gene interactions. They are highly influenced by the environment, making it difficult to identify superior genotypes based on the phenotype (Chinelato et al. 2014). Heritability cannot be fully assumed as part of the additive genetic variance, which is relative to the variance due to the additive effects of the genes and should only serve as an indication of the possibility of gain for future generations. One of the most important functions of this parameter in genetic breeding studies is its predictive role. Heritability is the main indicator of success in the selection process, with higher values indicating greater genetic gains (Braga et al. 2020).

One of the reasons that the heritability values for SD (0.158) and H (0.149) are not higher in the present study may be due to the early evaluation period, since these traits have greater variation over time. Variation in plant size can arise from genetic and environmental responses to vegetative propagation, in addition to variation caused by direct genetic and environmental effects on plant growth (Schwaegerle 2005).

The low heritability for DB (0.09) was undesirable, as this characteristic is fundamental for stem quality, which is directly related to the final value of the product. Therefore, the presence of apical dominance may be a limiting factor in the selection. In another field planting study reported by Pereira (2018), sequoia plants lost their apical dominance due to attack by a grasshopper (*Chromacris speciosa*), something that may have happened in this field test, where the presence of the insect was observed.

Although it did not cause high mortality in the current study, the occurrence of frost may cause the breakdown of apical dominance. Very intense frosts can cause mortality, as observed in the study by Pereira (2018). Frosts were also responsible for the loss of trees planted in colder areas of New Zealand (Dean 2007). For each plant species there is a temperature at which protoplasm freezing will occur and may occur before the air temperature reaches 0°C. Some species may have a higher resistance to freezing, remaining alive even after the phenomenon has occurred, but mortality may occur in younger tissues, such as the apex (Perissato et al. 2013). Kreyling et al. (2015) observed a cold tolerance of up to -9.2°C for *Sequoia sempervirens*. In the same study, the authors also observed that the thermal amplitude

was one of the main factors responsible for high levels of damage to the plants.

Overall, considering genetic heritability, RT selection is easier. In addition to being a qualitative trait (influenced by fewer genes than quantitative traits), which generally has a higher heritability, it is an important variable determining the success of vegetative propagation of plants on a large scale for supplying commercial plantations. Among the various genetic parameters estimated in progeny tests, one of the most important is the heritability coefficient, which measures the genetic control existing in a trait, and therefore the breeder's potential to improve a trait through genetic selection. However, the phenotypic characteristics observed in the field, such as stem shape, growth and adaptation are the most important for the selection of superior genetic materials.

Therefore, consideration should be given to the development of clones in the field over time, in accordance with the desirable characteristics of the final product (e.g., volume, height, diameter, density, etc.) and thus to define other selection parameters. In general, the results from this study allow for different scenarios for the selection of genetic material (clones). Good results for selection were obtained for rooting, but as already stated, this trait must be complementary to phenotypic traits in the field which are the most important for selection, particularly for species grown in commercial plantations in Brazil. However, further studies and new introductions of genetic materials should be carried out, given that the origin of the seminal material that generated the rescued and propagated sequoia mother trees is unknown. There may be genetic proximity between the clones used in this study if the seeds were collected at the same site.

Conclusions

Genetic selection for rooting success in sequoia clones proved to be effective, with this trait showing a high heritability. All traits evaluated in field yielded satisfactory results for selection, indicating that there is potential for genetic gain through clonal selection.

Authors' contributions

QCL and GTR evaluated the experiment, conducted statistical and genetic analysis, wrote the manuscript, and provided critical revisions of the manuscript. ACSS, BCL, ELT and RARD evaluated the experiment and provided critical revisions of the manuscript. MOP and MCN designed the experiment and supervised the entire research. All authors read and approved the final manuscript.

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Ciência Rural, Santa Maria, v.51:10, e20200549, 2021
http://doi.org/10.1590/0103-8478cr20200549
ISSNe 1678-4596
FORESTRY SCIENCE

Liming and macronutrient on early growth of *Eucalyptus benthamii*

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ABSTRACT: *Macronutrient suppression is one way to identify which chemical elements is the most important in the early development of seedlings of forest species. This study evaluated the initial growth of Eucalyptus benthamii seedlings through morphological and physiological variables, submitted to doses of N, P and K, and liming. The experimental design to install the experiment was completely randomized, with a 2x9 factorial scheme, in which levels of factor "A" referred to absence and presence of liming and levels of factor "B", to the different treatments of NPK. Morphological variables of SD (stem diameter) and H (height) and the physiological variables of A (photosynthesis), E (transpiration), gs (stomatal conductance), Ci/Ca (relationship between intercellular and atmospheric CO_2 concentration) and WUE (water use efficiency) were measured. For morphological variables, the absence of N directly affected the means and there was a direct relationship between increase of the dose and increase of SD and H. There was a positive relationship of P mainly in relation to shoot dry matter content (SDMC) and root dry matter content (RDMC). For the physiological variables, there was no direct response with the doses of N or P, with K being the element that most influenced the variables, especially when limestone was applied, resulting in higher averages for photosynthesis, transpiration and stomatal conductance. With the purpose of meeting the morphological and physiological demands, the recommendation for cultivation of Eucalyptus benthamii under the conditions of this study is the doses 150-200-150, without liming application.*
Key words: *omission of chemical element, initial development of seedlings, photosynthesis, stomatal conductance.*

Calcário e macronutrientes no crescimento inicial de *Eucalyptus benthamii*

RESUMO: *A supressão dos macronutrientes é uma das maneiras de identificar qual ou quais dos elementos químicos é o mais importante no desenvolvimento inicial das mudas de espécies florestais. O estudo objetivou avaliar o crescimento inicial de mudas de Eucalyptus benthamii por meio de variáveis morfológicas e fisiológicas, submetidas a doses de N, P e K, e calagem. O delineamento experimental para instalação do experimento foi em delineamento inteiramente casualizado, com esquema fatorial 2x9, em que os níveis do fator "A" se referiram a ausência e a presença de calagem e os níveis do fator "B" aos diferentes tratamentos de NPK. Variáveis morfológicas de DAC (diâmetro a altura do colo) e H (altura) e as variáveis fisiológicas de A (fotossíntese), E (transpiração), gs (condutância estomática), Ci/Ca (relação entre a concentração intercelular e atmosférica de CO_2) e WUE (eficiência no uso da água) foram determinadas. Para as variáveis morfológicas, a ausência do N afetou diretamente as médias, havendo uma relação direta entre o aumento da dose com o aumento das variáveis. Há uma relação positiva do P principalmente em relação a MSPA e MSPR. Para as variáveis fisiológicas, não houve resposta direta com as doses de N ou P, sendo o K o elemento que mais influenciou as variáveis, principalmente quando o calcário foi aplicado, resultando em maiores médias para fotossíntese, transpiração e condutância estomática. Com o objetivo de atender às demandas morfológicas e fisiológicas, a recomendação para o cultivo de Eucalyptus benthamii nas condições deste estudo é a dose 150-200-150, sem aplicação de calagem.*
Palavras-chave: *omissão de elementos químicos, desenvolvimento inicial de mudas, fotossíntese, condutância estomática.*

INTRODUCTION

Eucalyptus ranks among the most economically important trees globally (BAYLE et al., 2019). They exhibit traits, such as fast growth (LORENTZ & MINOGUE, 2015) and high water and light use efficiency (MAIER et al., 2017), that contribute to their candidacy as an excellent biofuel crop (GONZALEZ et al., 2018). Most eucalypt species are native to Australia and Indonesia and are adapted to tropical and subtropical environments; however, some species have developed frost tolerance through advancements in biotechnology or adaptation (STANTURF et al., 2013). *Eucalyptus benthamii*

Received 06.09.20 Approved 02.11.21 Returned by the author 04.14.21
CR-2020-0549.R2

has been shown to exhibit competitive frost tolerance when compared to members of the same genus (ARNOLD et al., 2015), making this species the most important currently in southern Brazil.

For the species to manifest its productive potential, it is necessary that the essential elements are available in the soil for absorption, given that the species originally occurs in fertile soils. In this sense, considering the low fertility of most Brazilian soils, liming and fertilization practices are essential to provide nutrients that are not in satisfactory concentrations to meet the demands of plants in the reforestation areas (SILVA et al., 2012).

Due to the fact that Brazilian soils do not have high fertility, there is a requirement for nutritional corrections, in which the fertilization method can be used. Thus, because it is a species with desirable characteristics, such as rapid growth, resistance to frost, good shape of the stem and rusticity, there is an increase in planting areas generating an increasing need for in-depth studies related to the relevant aspects for the species and its production (CUNHA et al., 2019).

When the nutritional requirements of the species are known, it is possible to identify and correct their needs in the soil, as nutrients have essential and specific functions in the metabolism of plants. Thus, when one of the essential elements are not present in satisfactory quantities or in conditions that make it poorly available, its deficiency promotes changes in the plant's metabolism. The symptoms of mineral deficiencies are more or less characteristic for each nutrient, species/cultivar and environmental factors (PRADO et al., 2010).

The lack of essential nutrients for plants such as nitrogen, phosphorus and potassium can represent a drastic decrease in growth and productivity. Nitrogen is one of the mineral elements most demanded by plants, and plants grown under inadequate amounts of N do not fully express their genetic potential, with a series of morphophysiological changes, negatively affecting the development of crops (MATOS et al., 2011). Likewise, plants with a deficiency in phosphorus and potassium have retarded growth, as they are affected by processes such as protein synthesis, nucleic acids, and reduction of high-energy storage compounds, such as ATP (Adenosine Triphosphate). It is through the use of this energy that the plant performs photosynthesis, actively absorbs nutrients from the soil and synthesizes various organic compounds (VIÉGAS et al., 2011).

One of the methods to identify the most needed nutrients for a given species and the symptoms it may show in the case of a nutrient deficiency is the missing element technique. Given this assumption, the general objective of the study was to evaluate the effect of doses of N, P and K, as well as liming on the initial growth of *Eucalyptus benthamii* seedlings through morphological and physiological variables.

MATERIALS AND METHODS

The study was carried out in forest nursery of the State University of Santa Catarina, located in Lagos, southern Brazil (coordinates 27°47'33"S and 50°18'4"W), with an altitude of approximately 900 meters. The experiment was conducted in a greenhouse with a 150 micron plastic cover, with an average temperature of 25-30 ºC and humidity between 65 and 90%. According to the Köeppen classification, the climate of Lages/SC is humid mesothermal with mild summer (temperate Cfb). The average annual temperature is 15 °C and the average annual rainfall is 1300 to 1500 mm (ALVARES et al., 2013). The implementation of the experiment occurred in early January 2018.

The soil used to fill the pots consisted of soil taken from a depth from 0 to 50 cm from a Humic Cambisol (World Reference Base - WRB/FAO systems). The physical and chemical soil analysis are: pH=4.7 H_2O^2; SMP Index=4.90; $Ca^{(2)}$=2.2 $cmol_c$ dm^{-3}; $Mg^{(2)}$ =1.2 $cmol_c$ dm^{-3}; $Al^{(2)}$=4.9 $cmol_c$ dm^{-3}; P Mehlich$^{(2)}$=3.7 mg dm^{-3}; $K^{(2)}$=48 mg dm^{-3}; organic matter – OM=2.9%; Effective CEC=6.46 $cmol_c$ dm^{-3}; CEC pH 7.0=13.1 $cmol_c$ dm^{-3}; Base saturation =26.8%; Al saturation=58.2%; clay= 47.6%, silt=24.4% and sand=28%.

The seedlings of seminal origin of *Eucalyptus benthamii* were obtained from the forest nursery of the region, with approximately 120 days. They presented an adequate development for field planting, that is, a height close to 25 cm, between 4-5 pairs of leaves and 2 mm of root collar diameter, as recommended by NAVROSKI et al. (2015).

The experiment was conducted in a completely randomized design in a 2x9 factorial scheme. The factor "A" consists of presence or absence of limestone (0.865 g kg^{-1} PRNT 70%) and the factor "B", combinations of nutrients (mg kg^{-1}), N (nitrogen), P (phosphorus) and K (potassium): T1: 0-0-0; T2: 0-200-150; T3: 150-0-150; T4: 150-200-0; T5: 150-200-150; T6: 150-200-300; T7: 150-400-150; T8: 300-200-150; and; T9: 300-400-300. The determination of the liming treatments and doses of NPK followed the recommendation of MAEDA & BONOLA (2012) and ROS et al. (2016),

assuming 150-200-150 as the recommended dosage according to the nutrients used in the present study. The treatments (NPK dosages) were then assembled in which each chemical element was omitted, the recommended dose and twice the recommended dose.

To compose the treatments, the nutrient formulations used were: N: 45% urea; P: 42% P_2O_5 TSP (triple superphosphate); and K: 60% KCl (potassium chloride). Each treatment consisted of 10 repetitions (pots) of a plant, given that each pot had a capacity of 5 L. The pots had five holes in the base for draining irrigation water. The nutrients were weighed in analytical balance and separated in single doses for K and P. N was applied in six installments, every 15 days. The respective doses of N were diluted in distilled water, by mixing for 30 minutes and after being applied to the plants.

Initially the soil was spread on a plastic tarp inside the greenhouse and subjected to constant revolving for nutrient incorporation. Subsequently, the pots were filled with 5 kg of soil and placed in a location with the same intensity of irrigation and luminosity (solar energy). After filling the pots, the seedlings were transplanted, and a manual hole of approximately 300 cm³ was opened in the center of each pot for planting. The irrigation in the greenhouse was carried out with micro-sprinklers, with four daily irrigations (9:00 am; 12:00 pm; 3:00 pm; 6:00 pm) of approximately 8 daily mm of water. The water used for irrigation of the experiment is the same as that used in the nursery, being originally from an artesian well at the university.

Morphological variables

Height and diameter biometric assessments were performed every 30 days for 4 months, and an initial assessment was performed at the time of the experiment implementation. The height of the seedlings (cm) was determined with the aid of a millimeter ruler or tape measure, measuring from the base of the plant to the insertion of the highest leaf. The stem diameter (ST) was measured in mm and determined with the aid of a digital caliper (Digimess®) with 0,01mm of resolution. After the data collection period, the manual collection of the total biomass of the plants was held by a destructive method. The samples were separated into root dry matter content (RDMC) and shoot dry matter content (SDMC). They were stored in paper bags kept in a forced air circulation oven (65±3 °C) and weighed every 8h until constant mass was obtained. After 36h, when the constant mass (g) was reached, the dry matter contents of the components were determined in precision balance (0.01 g) and the Dickson quality indexes were calculated. The Dickson quality index - DQI (DICKSON et al., 1960) is a tool to evaluate seedling quality as a function of total dry matter, shoot height, stem diameter, shoot dry matter – sum of stem base dry matter and leaf dry matter – and root dry matter.

Gas exchange

Gas exchange evaluations were performed with the aid of a portable photosynthesis system - Infra-Red Gas Analyzer – IRGA (Licor, model Li-6400XT, United State) in 4 repetitions, where the values for photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*), relationship between intercellular and atmospheric CO_2 concentration (*Ci/Ca*) and water use efficiency (*WUE*) determined by the ratio of *A* by *E* were determined. The photosynthetically active radiation used during measurements was 800 µmol photons m^2s^{-1}. This value was determined by the light saturation curve measured for eucalyptus, ranging between 800 and 1000 µmol of photons $m^{-2}s^{-1}$ (SILVA et al., 2000). Gas exchange evaluations for *E. benthamii* species of seminal origin were performed at 4 months after the last collection of growth data. For this evaluation, the same plant and the same leaf of each repetition was used, and the treatments were evaluated sequentially so that all treatments were covered in the same period of light of the day. The evaluations were always carried out between 7:00 and 9:30 am, being that the readings were stable in 2-3 minutes, when recorded by the equipment.

Statistical analyses

The treatment variances were tested for homogeneity by the Bartlett test. When there was homogeneity of variances, the data was subjected to analysis of variance and when there was a significant difference by the F test, the Scott-Knott's at the 5% probability level. When necessary, the data were transformed with a boxcox test. The analyzes were performed with the Sisvar statistical software (FERREIRA, 2019).

RESULTS

Morphological variables

For the SD variable, the results comparing the factors with and without liming had a statistical difference in the dose 300-200-150 (N-P-K), presenting a mean for the factor without liming 26% higher when compared to the factor with liming (Table 1). Another difference was reported in the 150-200-300 (N-P-K), where the factor without

Table 1 - Means of the variables SD (stem diameter – mm), H (height – cm), HD (relationship between SD and H), shoot dry matter content (SDMC), root dry matter content (RDMC) and Dickson Quality Index (DQI) for *Eucalyptus benthamii* at 120 days.

Treatment N-P-K (mg kg^{-1})	----------SD (mm)----------		----------H (cm)----------		----------HD----------	
	----------Liming----------					
	without	with	without	with	without	with
0-0-0	7.1 Ab*	6.3 Ac	87.0 Ac	72.8 Ab	12.4 Aa	11.8 Aa
0-200-150	7.8 Ab	8.4 Ab	103.8 BC	78.4 Bb	13.5 Aa	9.4 Ba
150-0-150	7.2 Ab	8.2 Ab	96.2 Ac	85.4 Bb	14.0 Aa	10.9 Ba
150-200-0	9.5 Aa	8.9 Ab	125.0 Ab	111.6 Aa	13.2 Aa	12.7 Aa
150-200-150	9.5 Aa	10.1 Aa	139.8 Aa	91.6 Bb	15.0 Aa	9.1 Ba
150-200-300	8.7 Bb	11.1 Aa	103.8 Bc	122.4 Aa	12.2 Aa	11.1 Aa
150-400-150	10.6 Aa	9.1 Ab	124.8 Ab	87.2 Bb	11.9 Aa	9.7 Aa
300-200-150	11.7 Aa	8.6 Bb	148.4 Aa	87.0 Bb	12.8 Aa	10.2 Ba
300-400-300	9.4 Aa	9.8 Aa	108.8 Ac	92.2 Ab	11.7 Aa	9.9 Aa
Treatment N-P-K (mg kg^{-1})	----------SDMC (g)----------		----------RDMC (g)----------		----------DQI----------	
	----------Liming----------					
	without	with	without	with	without	with
0-0-0	10.8 Ad*	9.6 Ab	7.2 Ab	4.4 Ab	1.38 Ab	1.04 Ab
0-200-150	14.4 Ad	15.2 Ab	4.8 Ab	10.4 Ab	1.22 Ab	2.34 Ab
150-0-150	16.8 Ad	13.2 Ab	6.8 Ab	5.2 Ab	1.55 Ab	1.47 Ab
150-200-0	27.6 Ac	25.4 Aa	12.4 Ab	10.0 Ab	2.62 Ab	2.44 Ab
150-200-150	28.8 Ac	32.4 Aa	13.6 Ab	16.0 Aa	2.60 Bb	4.35 Aa
150-200-300	22.0 Bc	34.4 Aa	10.0 Bb	20.0 Aa	2.30 Bb	4.28 Aa
150-400-150	35.2 Ab	26.8 Aa	17.6 Aa	10.4 Ab	3.78 Aa	3.10 Aa
300-200-150	52.4 Aa	19.6 Bb	23.6 Aa	10.4 Bb	5.15 Aa	2.46 Bb
300-400-300	28.4 Ac	25.6 Aa	10.8 Ab	13.2 Aa	2.83 Ab	3.79 Aa

*Means followed by the same letter, lowercase letters in the column and uppercase letters in the row for each variable do not differ from each other by the Scott-Knott's test at 5% probability.

liming obtained a mean 22% higher when compared to the one with liming. Considering the factor without liming, when comparing the NPK doses, the highest mean was obtained by 300-200-150 and was not statistically different from 150-200-150; 150-400-150; 150-200-0; and 300-400-300. Considering the factor with liming, the treatment with highest mean was 150-200-300 and was not statistically different from 150-200-150 and 300-400-300. The treatment with the lowest mean was the dose 0-0-0.

The highest average height was observed for 300-200-150, in the factor without liming, 41% greater than the average factor with liming for the same treatment. Unfolding the NPK dosages within the factor without liming, the highest mean height was observed for 300-200-150 which was not statistically different from 150-200-150, but 41% greater than the lowest average (0-0-0). In the liming factor, the highest average was 150-200-300, which did not differ from 150-200-0, and was 40% higher than the lowest average (0-0-0). Only the dosage 150-200-300 showed a lower average for the lack of liming when compared to the liming treatment. The other treatments showed no difference with the application of lime or showed higher averages without liming.

Plants grown in doses 150-200-150; 0-200-150; 300-200-150; and 150-0-150 obtained higher values of the HD ratio for the factor without liming when compared to those with liming. The highest mean was observed for 150-200-150 treatment without liming, differing by 39% when compared to the same treatment with liming. Among the factors, no statistical differences were obtained for the means, without or with liming.

The higher mean to SDM was observed when comparing the liming factor for the dosage 300-200-150 without liming, which was 63% higher than the same treatment with liming (Table 1). The treatment 150-200-300 with liming was superior to the one without liming by 36%. When comparing

the results within the factor without liming, the 300-200-150 treatment obtained the highest mean, being 79% higher than the 0-0-0 treatment that obtained the lowest mean. Considering the liming factor, the highest mean was observed for the 150-200-300 treatment.

Similar results were observed for SDMC and RDMC when comparing the factors with liming. There was statistical difference for the dosage 300-200-150, and in the factor without liming the mean was 56% higher than in the factor with liming. In the unfolding of NPK dosage within the liming factor, the highest mean without liming was obtained for 300-200-150, 71% higher than the mean of 150-0-150 (lowest mean). In the NPK dosage with liming the highest mean was 150-200-300. The lowest mean for the factor with liming was in the dosage 0-0-0, which was 78% lower when compared to the highest mean observed.

For Dickson Quality Index (DQI), the NPK dosage 300-200-150 without liming presented a higher mean than the same treatment with liming, being 52% higher. The dosage 150-200-150 and 150-200-300 without liming were 40 and 46% lower, respectively, when compared to their respective with liming. Within the factor without liming, the dosage 300-200-150 obtained highest mean, whereas with liming the highest mean was obtained for 150-200-150. The lowest mean with liming was observed in the dosage 0-0-0, which was 76% lower than the highest mean NPK dosage.

Physiological variables

For variable *A*, the only NPK dosage that had no statistical difference between the liming factor was the 150-200-150; there were differences among the other treatments (Table 2). For the liming factor, the treatment of highest mean was the dosage 150-200-0. Considering the factor without liming, the highest mean was for 0-200-150, which was higher in 46% than the dosage with lowest mean (300-400-300).

The NPK dosage 0-200-150; 150-0-150; and 150-200-300 without liming had higher means – 29%, 36 and 28%, respectively – when compared to the same treatments with liming to the transpiration (*E*). The mean of dosage 0-0-0 and 300-400-300 with liming were higher and statistically differed from the same treatments without liming. In the unfolding of NPK dosage within the factor without liming, the highest mean was presented by 0-200-150 and was 64% higher than the dosage of lowest mean (300-400-300). In the liming factor, the highest mean was for 150-200-0, higher in 54% of the dosage of lowest means (300-200-150 and 150-0-150).

For the variable stomatal condutance (*gs*), the NPK dosage 0-0-0; 300-200-150; 150-400-150; 150-200-0; 150-200-300 and 300-400-300 with liming had higher means and differed statistically from the same treatments without liming. Within the factor without liming the highest mean obtained was for 0-200-150, 77% higher than the lowest mean (300-400-300). In the factor with liming the highest mean was obtained for 150-200-0 which differed statistically from the other treatments. The lowest mean was observed in 300-400-300, which was not statistically different from 300-200-150 and 150-0-150 and was 52% lower compared to the dosage of highest mean.

In general, the factor with liming obtained the highest means of *Ci/Ca*. The greatest difference occurred in dosage 300-400-300, which presented a 34% higher value compared to treatment without liming. There was no statistical difference between the factors without and with liming for dosage 0-200-150; 150-0-150 and 150-400-150 to *Ci/Ca*. For the presence of liming, the dosage 0-0-0 showed the highest average of *Ci/Ca*. The lowest averages were obtained in treatments with higher N (300-200-150 and 300-400-300). In the unfolding of treatments within the factor without liming the highest mean was observed for 0-200-150, 39% higher compared to the dosage of lowest mean (300-400-300). The lowest mean was observed in dosage 300-200-150 which did not differ statistically from 300-400-300 and was 25% lower than the treatment of highest mean.

The NPK dosage 0-0-0 and 300-400-300 without liming, were superior to those with liming to water use efficiency (*WUE*). In the unfolding of treatments within the factor without liming the highest mean was observed in dosage 300-400-300, 36% higher than the lowest average (0-200-150). For the liming factor, the highest mean was in the dosage 300-200-150, the lowest mean was observed for 0-0-0 which differed from the other treatments and was 44% lower compared to the treatment of highest mean.

DISCUSSION

Morphological variables

For morphological variables, the results of treatments with N or P omission are the most affected, showing reduction in SD, H, SDMC and RDMC when compared with treatments with these nutrients. The decrease in growth in plants with low nitrogen, as observed in our study with *E. benthamii* is a function of the element's participation in the structure of numerous molecules, being the main limitation of growth, as it is part of proteins, nucleic acids and

Table 2 - Means of variables *A* (photosynthesis – µmol m^{-2} s^{-1}), *E* (transpiration – mmol of H_2O m^{-2} s^{-1}), *gs* (stomatal condutance – mmol of H_2O m^{-2} s^{-1}) *ci/ca* (ratio between intercellular and atmospheric CO_2 concentration – µmol µmol^{-1}), and *WUE* (water use efficiency) for *Eucalyptus benthamii* at 120 days.

Treatment N-P-K (mg kg^{-1})	----------*A* (µmol m^{-2} s^{-1})----------		---------*E* (mmol of H_2O m^{-2} s^{-1})-------		------*gs* (mmol of H_2O m^{-2} s^{-1})------	
	----------Liming----------					
	without	with	without	with	without	with
0-0-0	7.47 Ac*	5.97 Be	1.88 Bc	2.40 Ab	0.13 Bd	0.19 Ab
0-200-150	10.86 Aa	9.61 Bc	3.37 Aa	2.39 Bb	0.22 Aa	0.20 Ab
150-0-150	8.60 Ab	7.56 Bd	2.46 Ab	1.57 Bc	0.12 Ad	0.12 Ad
150-200-0	8.93 Bb	12.52 Aa	2.45 Bb	3.39 Aa	0.12 Bd	0.25 Aa
150-200-150	9.60 Ab	9.44 Ac	2.11 Ac	2.05 Ac	0.15 Ac	0.17 Ab
150-200-300	10.14 Aa	7.64 Bd	3.35 Aa	2.41 Bb	0.18 Ab	0.16 Ac
150-400-150	8.90 Bb	10.43 Ab	2.90 Ab	2.55 Ab	0.15 Bc	0.20 Ab
300-200-150	6.77 Bc	9.00 Ac	1.51 Ad	1.57 Ac	0.10 Be	0.13 Ad
300-400-300	5.82 Bd	8.86 Ac	1.21 Bd	1.93 Ac	0.05 Bf	0.12 Ad

Treatment N-P-K (mg kg^{-1})	----------*Ci/Ca* (*µ*mol µmol^{-1})----------		----------*WUE* ----------	
	----------Liming----------			
	without	with	Without	with
0-0-0	0.67 Bb*	0.83 Aa	4.10 Ab	3.21 Bd
0-200-150	0.75 Aa	0.75 Ab	3.40 Bc	4.20 Ac
150-0-150	0.65 Ab	0.68 Ac	4.01 Bb	4.90 Ab
150-200-0	0.63 Bc	0.74 Ab	3.95 Ab	4.17 Ac
150-200-150	0.61 Bc	0.71 Ac	4.90 Aa	5.35 Aa
150-200-300	0.67 Bb	0.72 Ac	3.49 Bc	4.11 Ac
150-400-150	0.69 Ab	0.71 Ac	3.53 Bc	4.47 Ac
300-200-150	0.57 Bd	0.62 Ad	4.95 Ba	5.76 Aa
300-400-300	0.46 Be	0.65 Ad	5.30 Aa	4.80 Bb

*Means followed by the same letter, lowercase letters in the column and uppercase letters in the row for each variable do not differ from each other by the Scott-Knott's test at 5% probability.

many other cellular constituents, such as membranes and hormones (SOUZA & FERNANDES, 2006). Phosphorus is also crucial in plant metabolism, since that acts in the transfer of energy in the cell, in the respiratory rate, photosynthesis, composes structures of nucleic acids, genes, chromosomes as well as several coenzymes (MENEGHETTE et al., 2017).

According to VIEIRA et al. (2016), in a similar investigation with macronutrient suppression, with the omission of N there was a significant reduction in plant growth, especially in relation to shoot and root dry matter contents. According to the authors, this reduction in growth in omission to N is expected because, in general, this is the most required element in the early stage of development. According to SMETHURST et al. (2004), the greatest need for N is in the first two years of the cycle. ROS et al. (2016), when studying initial growth of *E. grandis* with macronutrient suppression, observed that there were significant reductions in height and diameter at breast height with N suppression.

As noted in the present study, *E. benthamii* is highly responsive to phosphate fertilization. This same behavior was observed by DIAS et al. (2014) in which the highest dose of P (300 kg ha^{-1}) caused an increase of 2.6 times in the diameter of the neck, 1.1 times in the height of the plants and 9.9 times in the volume of wood per hectare, in relation to the control. The responses to phosphate fertilization of *E. benthamii* were superior to *E. dunnii* in the experiment, which was evaluated 11 months after planting in the field.

For all variables, the treatment that presents the highest mean in the absence of liming is the one that hase twice the value of the dose recommended for N (300-200-150). Conversely, in the treatments with liming, the highest means are presented in the treatments with twice the dose of K and the recommended for N and P (150-200-300). Even so, there is a significant reduction in the mean of treatments with absence of N and P. In treatments with suppression of K only, both for presence and absence of liming, the result for the morphological variables is not affected. There seems to be a greater dependence on N to liming, and less effect on P and K. Positive effect of liming on decreasing N application was observed by WYNGAARD et al. (2012), when liming was not performed, there was a need for an increase in N. According to our results, when liming was not performed, in the absence of N and P, there is also a significant reduction in the means of the morphological variables, also showing an effect on P. Possibly the omission of only K did not present a harmful effect because the soil used (in the region) presented values within what is considered ideal for the genus *Eucalyptus* (MALAVOLTA et al., 1997).

The absence of P significantly affected the results for both treatments, without and with liming. STAHL et al. (2013) stated that the addition of P to soil increases the production of shoot and root dry matter contents of *E. benthamii* in pots. The production of shoot dry matter content (SDMC) is significantly influenced by the application of P doses in soil (MAEDA & BONOLA, 2012). There is usually a reduction in plant growth with P suppression, as it is the most limiting nutrient, due to the naturally low levels and the high P adsorption capacity of most soils in tropical regions (NOVAIS et al., 2007). In addition, the reduction in plant growth due to P suppression occurs with greater intensity in young stands (GAVA, 1997). Very high doses of NPK, as in the treatment (300-400-300) also did not show a favorable response, suggesting that using large amounts of nutrients is unfeasible. In addition to being economically unviable, it can also cause contamination of the environment. This result may also be related to the cultivation environment being pots, and not the field environment. In the pots, there is a higher root/soil ratio compared to plants grown in the field. In pots, the root system explores the small volume of existing soil more intensely, as there is an overlap of the depletion zones, especially of P around the roots, which hardly happens in the field (STAHL et al., 2013).

There were no superior responses in treatments with liming. In most treatments the means of the morphological variables obtained with the presence of liming were lower when compared to the treatments without liming. Only in the treatments with the highest K dosage, the means obtained were higher than in the absence of liming, indicating that for the soil and species conditions of this study, liming did not affect the growth of morphological variables. Probably this response is due to the soil having the necessary Ca and mainly Mg reserves (main nutrients required) to supply the beginning of seedling development. It is important to highlight that the liming was carried out at the same time as the fertilization, and in many cultures the recommendation is to do it beforehand. For *Eucalyptus* cultivation, mainly in the reform area (area with old planting), liming when applied is applied just before the time of planting the seedlings. Liming before phosphate fertilization can also decrease the capacity for adsorption or fixation of P by the soil (RAIJ, 1992).

Physiological variables

For physiological variables the result showed a more homogeneous behavior for treatment with the recommended dosages for N, P and K (150-200-150) in the presence or absence of liming. In general, it was not possible to find a relationship between the growth of eucalyptus trees and photosynthetic rates. OTTO et al. (2013) highlighted that this absence of relationship can occur, at least in part, because the evaluations of the variables happen in distinct moments, that is, the growth is the result of a continuous process that results in the accumulation of biomass, while the photosynthesis evaluation is carried out on a specific occasion during the growth of the plant.

No significant influence was observed for the suppression of N and P elements in the treatments. To N, this result can be explained by the OM content in the soil; which although not ideal, meets the basic requirements of the genus (PERDOMO et al., 2007). Nitrogen is one of the macronutrients that most influences photosynthesis in forest species. In some species, fertilization with N caused a decrease in photosynthesis as in *E. grandis* (CLEARWATER & MEINZER, 2001), *Pinus sylvestris* (SMOLANDER & OKER-BLOM, 1989), *P. taeda* (GREEN & MITCHELL, 1992), but did not increase photosynthesis in *E. nitens* (FORRESTER et al., 2012) and *E. globulus* (TURNBULL et al., 2007).

For treatment with twice the recommended dose for N (300-200-150) with liming there was also significant response when compared to treatment without liming. The addition of N promotes formation of active photosynthetic pigments and participates in

processes such as ionic absorption, photosynthesis, respiration, multiplication and cell differentiation (TEIXEIRA FILHO et al., 2011). The highest dosages of NPK the response was also positive with the presence of liming. But this in practice, as previously discussed, would not be applicable, due to the increase in costs and environmental problems.

Treatment with K suppression in the absence of liming was directly influenced in the decrease in physiological variables. According to DECHEN & NACHTIGALL (2007), in the absence of K, the plant presents reduced photosynthesis and increased respiration. These two conditions (reduction in photosynthesis and increase in plant respiration), when there is a K deficiency, reduce the accumulation of carbohydrates, resulting in reduced plant growth. The lower photosynthetic performance in plants with K deficiency is related to stomatal opening not happening on a regular basis, reducing the entry of CO_2 (PRADO, 2008).

According to VIEIRA et al. (2016), one of the most required nutrients in the production of shoot dry mass is K. Due to the authors' assumption, the decrease in physiological activities may be related to the lack of K in one of the treatments (150-200-0) with greater influence on the decrease of physiological activities. Potassium can confer an adequate dynamic of stomatal conductance to plants, besides being an extremely important element in the activation of Rubisco's carboxylase function, which contributes to greater photosynthetic activity (PRADO, 2008).

In general the treatment that did not change with the presence or absence of liming for physiological responses was the treatment with the recommended dose (150-200-150), assuming that in relation to the physiological activity of the species under the conditions of this study, this is the best treatment. This treatment also showed good responses in the morphological variables, such as SD and H, and this treatment can be recommended as the most appropriate in the initial growth of *E. benthamii* when balancing physiological and morphological variables.

CONCLUSION

The absence of N negatively affected the means of the morphological variables and there was a direct relationship between increasing the dose and increasing the means. The increase in P also increased some variables such as SDMC and RDMC. K did not promote changes in morphological variables.

For physiological variables, the absence of fertilization or very high doses caused a decrease in photosynthesis. Higher doses of K caused a decrease in photosynthesis and stomatal conductance. Except for the treatment without K, in which liming increased the averages of physiological variables, the other doses of NPK showed no difference with the addition of liming. In general, the dosage 150-200-150 showed the best result for the physiological variables.

Based on the recommendations for each situation exposed in the study, to meet the morphological and physiological demands, the recommendation for cultivation of *E. benthamii* under the conditions of this study is the doses 150-200-150, without liming application.

ACKNOWLEDGEMENTS

The authors thank the Universidade do Estado de Santa Catarina (UDESC), for the institutional support for this research. To FAPESC for funding the research group Management of Planted and Natural Forests (PAP2019031000055) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for partial finance support, Brasil - Finance code 001.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript. All authors critically revised the manuscript and approved of the final version.

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Influence of shadowing in *Sequoia sempervirens* (D. Don) Endl. mini-stumps and mini-cuttings

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10.1590/0034-737X202269040008

ABSTRACT

The aim of the work was to analyze the influence of variables such as shading, temperature and humidity in the production and rooting of mini-cuttings of *Sequoia sempervirens* (D. Dom) Endl. in a clonal mini-garden. The experiment was conducted at the Forest Nursery at the University of Santa Catarina State - CAV/UDESC, Lages - SC. The minicuttings were obtained by vegetative rescue of a single clone (A228). The original *S. sempervirens* individuals studied are located in the National Forest of São Francisco de Paula -RS (FLONA). Sprouts were collected 90 days after the application of girdling rescue techniques. Subsequently, mini-stumps were obtained, which were submitted to three different treatments: T1 - Mini-stumps without shading protection, T2 - Mini-stumps with single layer of shading and T3 - Mini-stumps with double layer of shading. There was no significant difference for humidity and temperature in relation to the production of mini-cuttings by the mini-stumps, while for shading the treatments T2 and T3 showed greater efficiency. It is possible that the shading treatments on *S. sempervirens* mini-stumps allows the vegetative tissue to be more juvenile, obtaining excellent results in cutting.

Keywords: clonal forestry; mini-cutting; illuminance; rooting.

INTRODUCTION

Sequoia sempervirens (D. Dom) Endl. belongs to the Cupressaceae family botany and is native to the United States of America (U.S.A), where it grows along the fog belt from the Pacific coast of southwest Oregon to central California (Cown, 2008). Sequoia wood has the following characteristics: good machinability and good adhesion for paints and varnishes, in addition to not having resins, typical of coniferous wood (Spichinger, 2004). Such qualities make Sequoia wood attractive also for panel production and use in the furniture industry.

However, the species has some characteristics that prevent its sexual reproduction for commercial production purposes, such as, for example, delay in the appearance of seeds, loss of viability in a short period, low seed germination rate (average of 10%) and seedling viability is also considerably low (Ozudogru *et al.*, 2011). In addition, water, temperature, edaphic conditions and especially light are environmental factors that interfere in the development of plants (Pereira *et al.*, 2020).

Alternatively, vegetative propagation via cuttings or mini-cuttings may be an option. These techniques consist of harnessing the juvenile potential of propagules to induce rooting (Gibson *et al*., 2021). However, according to Sukumar *et al.* (2013), the species presents difficulties in rooting and low production of fine roots, in addition to slow rooting, requiring the use of growth regulators framed in the group of auxins that provide as a response a higher percentage of root formation, better quality of the same and uniformity in rooting (Oliveira *et al.*, 2012).

In general, among the main advantages of the vegetative propagation of forest species can be mentioned the formation of clonal plantations of high productivity and uniformity. Because of consecutive selection, there is the improvement of the quality of the wood and its products, the multiplication of genetics materials resistant to diseases

Submitted on October 27th, 2020 and accepted on May 24th, 2022.

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and pests and adapted specific sites. This can happen from generation to generation, of additive and non-additive genetic components, which results in greater gains within the same generation of selection (Stuepp *et al.*, 2015).

Therefore, based on the information that luminosity has a direct action on the degradation of photolabile compounds such as auxin, light control can be an easy-to-use alternative, leveraging the rooting potential of the species and reducing or eliminating the use of synthetic hormones. Therefore, the aim of the present study was to analyze the influence of light on the production of mini-cuttings in a clonal mini-garden and the rooting of *S. sempervirens*.

MATERIAL AND METHODS

The rescue area of *S. sempervirens* vegetal material occurred in the National Forest of São Francisco de Paula (RS), Brazil. According to the climatic classification of Köeppen, its climate is of the *Cfb* type, mesothermal, super humid, with mild summer and cold winter. The area is part of a planting carried out between 1974 and 1975 using seeds from California, U.S.A. Through observation of the area and considering the spacing used, it is estimated that approximately 50 seedlings were planted, of which 24 trees remained, used for the plant material rescue experiment.

We measured the total height (h) in all trees using a Vertex IV hypsometer and the diameter at breast height (DBH) with a tape-measure. The individuals used for the vegetative rescue had an average height of 26.4 m (11.7-37.3 m) and DBH of 52.3 cm (11.6-95.6 cm), with approximately 40 years of age at the time of vegetative rescue. The vegetative rescue was carried out during the spring, and the methods for vegetative rescue were both partial and complete girdling at the base of the trunk, for promoting the epicormics sprouting. The evaluations started 90 days after the application of the girdling techniques, counting the percentage of sprouted trees (%).

We transported the sprouts in polystyrene boxes within ice and water to avoid excessive evapotranspiration of the vegetal material. After transportation, we cut the sprouts into cuttings and placed them in tubes of 110 or 180 cm^3, with commercial substrate composed on pine bark and vermiculite (1:1) with an addition of 6 g L^{-1} of controlled release fertilizer. Five months after cutting, we acclimatized the rooted cuttings in a shade house for 30 days, taking them to acclimatization in a greenhouse for another 30 days afterwards. After this period, we transferred the seedlings with approximately 20 cm high to pots with a capacity of 5 L, filled with substrate based on pine bark (50%) and medium vermiculite (50%). After fifteen days of adaptation of the seedlings to the system, we performed the pruning of the apex of the main sprout at a height of 10 cm (± 2 cm), constituting the mini-stumps for the constitution of the clonal mini-garden. Monthly, for four months, we performed the pruning of the mini-stumps aiming the production of lateral sprouting.

The nutrient solution worn in the clonal mini-garden was the base of the commercial fertilizer. It was composed of 10% N; 10% K_2O (water soluble); 42% P_2O_5 (water soluble); 0.1% Fe; 0.6% Mg and 0.02 Br. We conducted the fertigation once a week, were each mini-stump got 50 ml of the solution composed by diluting 1.5 grams of the fertilizer per liter of water. After the formation of the clonal mini-garden, consisting of approximately 3 months, mini-cuttings were collected for the experiment.

For evaluation, in this study, we used only one clone (A228) with DBH of 68.2 cm and height of 33.9 m. Only one clone was used in order not to show genetic variation between clones, and all the variation obtained was attributed to the treatments tested. We arranged the pots containing the mini-stumps in a completely randomized design composed by 15 replicates per treatment. The three procedures contained in different levels of shadowing: T1 - Mini-stumps without shadow protection; T2 - Mini-stumps with single layer of shading, and; T3 - Mini-stumps with double layer of shading.

We achieved the different levels of irradiance with the use of Sombrite® polyethylene (PE) with Anti-UV stabilizing mesh corresponding to a shading blade in T2 and two blades in T3. We positioned the coverings on metal gratings in order to provide full coverage of the underlying gratings. For the control treatment, we only exposed the mini-stumps to the natural light of the greenhouse. Daily, for 15 weeks, we evaluated the illuminance, temperature and humidity using a Luxmeter with a range of 20000 - reading x 10 (lux) and a data logger measuring the temperature in Celsius degrees (Cº) and the humidity in percentage (%).

After 15 weeks, we performed the pruning of the sprouts by repetition in each treatment, cut those into mini-cuttings containing up to 8±2 cm in length, with one to three pairs of leaves cut in half, counted and put them to root inside a mini-tunnel system. Weekly, after 60 days of conditioning in the greenhouse for up to 95 days, we evaluated the survival of mini-cuttings (%), rooting (%), callus formation (%), number of roots emitted and root origin. We considered alive cuttings those with live wood, old leaves or young sprouts, rooted or not. We considered the percentage of rooted mini-cuttings on the total, not based only on the surviving ones. Was considered rooted mini-cuttings to be those with roots longer than 2 mm.

For root origin, we classified it after callus formation, after woody tissue (which may be from the cortex, cambium, vascular radius or phloem parenchyma) and from both places (Figure 1).

After checking the homogeneity by the Bartlett test and normality of the data by means of the Kolmogorov-

Smirnov we performed the analysis of variance (ANOVA). When necessary, the data was transformed by the function $(x + 0.5)^{0.5}$ and compared the means by the Tukey test at 5% probability. The R package "laercio" was used to compute the statistics (Silva, 2010).

RESULTS AND DISCUSSION

Mini-cuttings production

In the production of *S. sempervirens* mini-cuttings, the different light levels did not show statistical difference, varying between 7.5 mini-cuttings for T2 and 6.4 for T3. The control treatment (T1) presented similar average (7.2).

However, although the results do not show significance regarding the amount of mini-cuttings according to the treatments, during the evaluation it was possible to observe visual differences in qualitative variables such as the degree of lignification of the sprouts. The sprouts from T2 and T3 presented a lower degree of lignification and according to Lowe *et al.* (2021) this may favor rooting. Lima *et al*. (2011), studying the rooting capacity of *Maytenus muelleri* Schwacke cuttings, reported that the use of less lignified material results from pruning, can promote rooting rates above 50%.

For relative air humidity (UR%) and temperature (ºC), there was no great variation between treatments (Figure 2). This similarity is probably due to the stability provided by the greenhouse, and the microclimate created by the shading is not significant for these variables. As for the light rate, the different layers of shading in T2 and T3, proved efficient in blocking solar radiation, reducing the luminous flux to values less than 300 lux.

Although there are still no detailed studies on the influence of environmental conditions on the production of propagating material (cuttings or mini-cuttings) of the species under study, knowledge of the ideal conditions of light rate, relative humidity and temperature for the development and growth of plants is extremely important for an efficient propagation of the species worked. Cell division is favored by the increase in temperature and, consequently, aids the formation of roots and the production of sprouts (Hartmann *et al.*, 2011). The majority of studies with conifer rooting achieve better results with lower temperatures during the night (15-18 ºC) and higher during the day (25-27 ºC). Working with the rooting in the seasons of the year in *Sequoia sempervirens*, Pereira *et al*. (2018) got rooting higher than 85% in all seasons, with humidity greater than 80% and average temperatures ranging from 15 to 26 ºC.

Mini-cuttings rooting according to shading levels

After 95 days in the greenhouse, the mini-cuttings obtained a 100% survival rate for all treatments (Table 1). Navroski *et al.* (2015) state that the high survival of the cuttings may be the result of good weather conditions during the experiment, as well as the good ability of the *Sequoia sempervirens* to survive after making into mini-cuttings. According to Cunha *et al.* (2009), the high survival characterizes an adequacy of the cultural treatments used, such as good nutritional, water levels and handling of mini-stumps, which indicates the possibility of using these clonal mini-garden systems to produce sprouts (mini-cuttings) for this species.

Another factor that may have guaranteed the high survival rate was the high presence at the base of the mini-cuttings of calluses (Figure 3). According to Hartmann *et al.* (2011), the presence of calluses at the base of the cuttings, for some time, can perform the function of the roots, that is, absorption of water and mineral salts.

The T2 and T3 treatment presented lower callus formation rates since the start of the evaluations, confirming the fact that a balance in the auxin concentrations supposedly provided by the simple shading condition reduces the formation of calluses at the base of

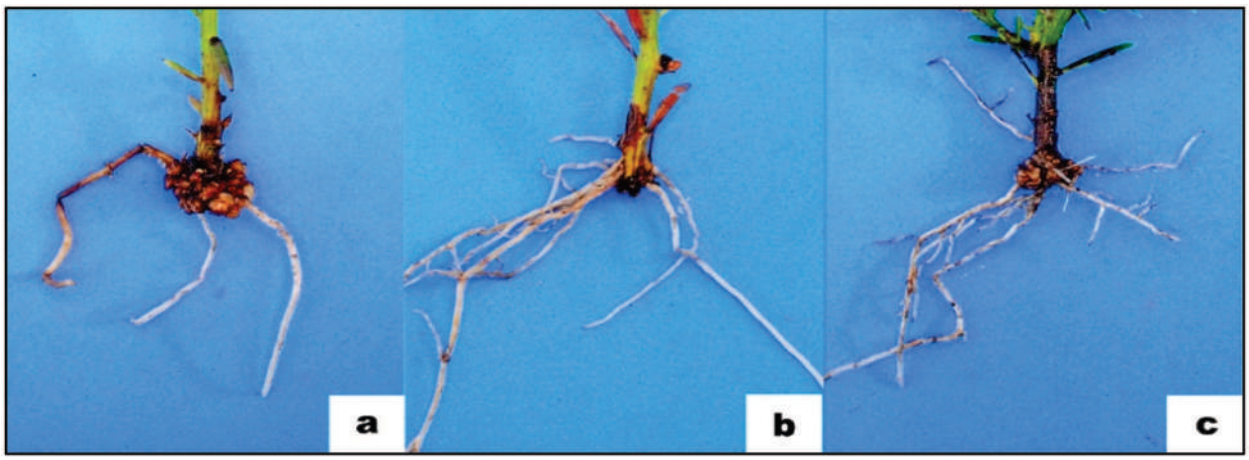


Figure 1: Different origins of roots for mini-cuttings of *S. sempervirens* a: callus; b: woody tissue; c: both places.

the *S. sempervirens* mini-cuttings. The fact that T1 presented greater callus formation at the base of the cuttings may be related to the concentration of auxin. Rasmussen *et al.* (2009) reports that the formation of root callus is favored by the degradation of auxin in the basal region of the propagule and according to Guerra & Nodari (2016), high concentrations of auxin, may increase the formation of calluses.

In this way, it is possible to conclude that simple shading balances the auxin rate in mini-cuttings, thus reducing the formation of callus. The calluses consist of an irregular mass of parenchymal cells in various lignification states, usually developing at the base of the cuttings when placed in favorable environmental conditions for rooting. With reports that the first roots appear frequently through the callus, this leads to the hypothesis that callus formation is essential for rooting in some species, as in *Sequoia sempervirens* (Pereira *et al.*, 2019).

For rooting, there was also a difference between treatments, in which, T3 presented a higher percentage (Figure 4). These results agree with Kim & Guak (2014) in all three varieties of blueberries, were the percentage of rooting and growth of the area and roots increased in the treatment with 30% of shading in relation to the treatment without shading (control). However, levels greater than 50% of shading were harmful to rooting hardwood cuttings of highbush blueberries.

This result surpassed the one found by Navroski *et al.* (2015), with 160 days after *S. sempervirens* mini-cuttings put to root with the application of exogenous Indoleacetic

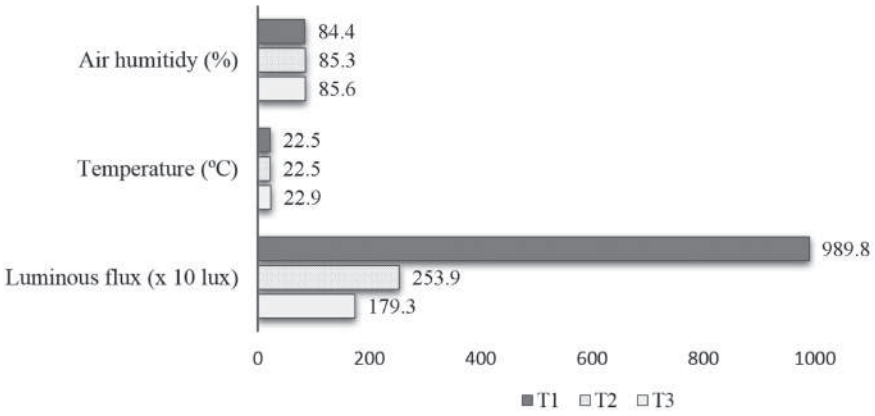


Figure 2: Variation between light (x 10 lux), temperature (ºC) and air humidity (%) averages between the three treatments of shading in mini-stumps of *S. sempervirens*.

Table 1: Survival (%), callus formation (%), rooting (%) and number of roots of *S. sempervirens* mini-cuttings 95 after put to root according to different shading levels of the mini-stumps

Shading levels	Variable			
	Survival (%)	Callus (%)	Rooting (%)	Nº of roots
T1	100.0 a *	100.0 a	77.7 b	5.7 a
T2	100.0 a	71.4 b	80.9 b	3.9 a
T3	100.0 a	78.2 b	93.7 a	5.5 a

*Treatments followed by the same letter do not differ from the average Tukey test at 5% probability.

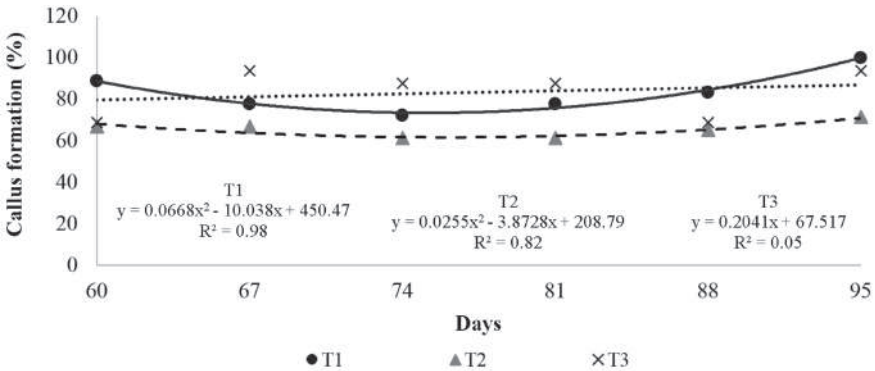


Figure 3: Percentage of callus formation in *S. sempervirens* minicuttings over the period of 60 to 95 days according to shading treatments in the clonal mini-garden.

acid (IAA), with a rooting of 80.9%. It is important to highlight the increasing and linear behavior of T3, surpassing T2 and T1 at the end of the experiment. This indicates that the concentration of endogenous auxin present in this experiment has not yet reached the inhibitory level of root production caused by the phytotoxic effect of auxin (Meneguzzi, 2017).

In addition to rooting, the number of roots on cuttings or mini-cuttings is one of the most relevant variables in plants production through vegetative (Kouakou *et al*., 2016). Regarding the number of roots, it is clear that T2 had a certain similarity during the evaluation days, whereas T1 and T3 presented an increase in quantity over the days (Figure 5). This fact can be associated with the presence of calluses in the base of the mini-cuttings of both treatments.

These variables are very important in this species cutting process, as according to Luna (2008) even with rooting, *S. sempervirens* cuttings can present high mortality in the acclimatization process. Therefore, it is necessary to be careful with environmental conditions, mainly regarding to high temperatures.

When analyzing the origin of the roots, it was possible to notice that those derived from calluses the T1 presented higher averages in relation to T2 and T3. This event is probably due to the elongation provided in T2 and T3, with reduced lignification, and consequently easier rooting. Corroborating with the results of the present study, Kim & Guak (2014) states that the use of shading layers reduces the formation of callus during the rooting process.

Thus, from the results of the origin of the roots in the mini cuttings of *S. sempervirens*, it is possible to observe that T2 and T3 presented a higher percentage of roots from the woody tissue (Table 2). It is worth noting that these roots were also visually more elongated and better distributed at the base of the cuttings, which should favor the development of the seedling. The number of roots that originated from both sites (callus or woody tissue) was also measured, however, it did not show significant variation for the three treatments studied. Grossnickle (2012) states that seedlings that have a more adequate root system tend to survive better than those that have an inferior root system, especially in the first weeks, when adverse conditions can compromise their survival.

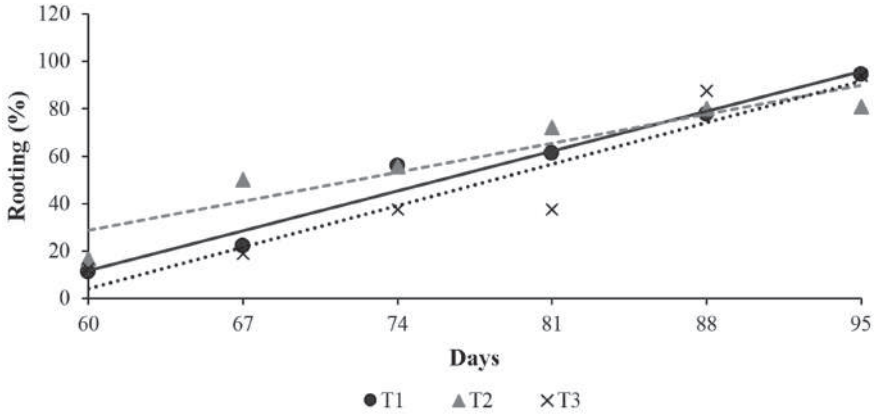


Figure 4: Rooting percentage of *S. sempervirens* mini-cuttings over the period of 60 to 95 days as a function of shading treatments in a clonal mini-garden.

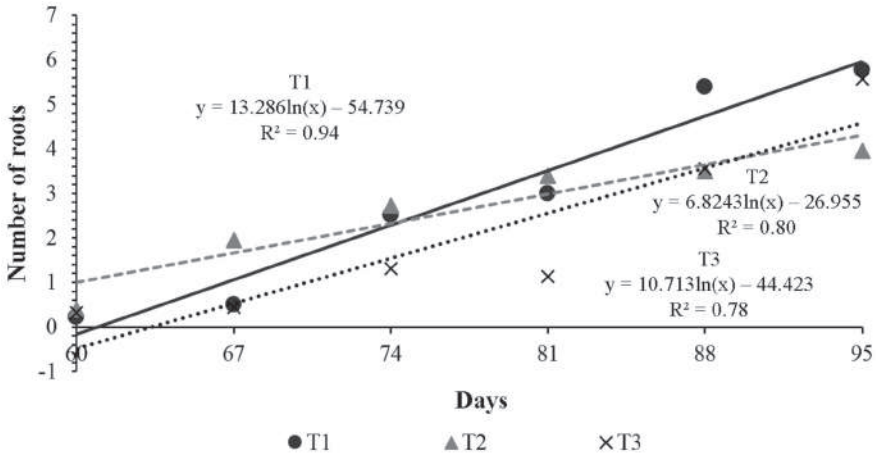


Figure 5: Number of roots in mini-cuttings of *S. sempervirens* over the period of 60 to 95 days as a function of shading treatments in a clonal mini-garden.

Table 2: Roots origin – % (callus, woody tissue or both places) in *S. sempervirens* mini-cuttings during rooting process

Shading levels	Root origin (%)		
	Callus	Woody tissue	Callus/Woody tissue
T1	63.3 a *	26.5 b	10.2 a
T2	29.4 b	61.8 a	8.8 a
T3	39.1 b	45.6 a	15.2 a

*Treatments followed by the same letter do not differ from the average Tukey test at 5% probability.

CONCLUSIONS

In the production of *S. sempervirens* mini-cuttings, the different levels of shading had no influence.

For the rooting of the mini-cuttings, a difference was found between the treatments, in which two blades of Sombrite® with 95 days after cutting presented a higher percentage of rooting. As for the origin, the roots derived from calluses and no shadowing presented higher averages in relation to any shadowing levels.

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Australian Journal of Crop Science AJCS

AJCS 16(07):949-954 (2022) ISSN:1835-2707
doi: 10.21475/ajcs.22.16.07.p3625

Photosynthetic light response curves in *Eucalyptus benthamii* and *Eucalyptus dunnii* clones

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Abstract

The photosynthetic light-response curve reflects the instantaneous response of the net photosynthetic rate to different gradients of photosynthetically active radiation. Genetic materials can respond differently to light and consequently alter productivity. Thus, this work aimed to compare clones of *Eucalyptus benthamii* and *Eucalyptus dunnii* by checking the CO_2 assimilation rate due to the increase in photosynthetically active radiation. The evaluations were carried out in mini-stumps implanted in a clonal mini-garden system. The CO_2 assimilation rate curves were determined with the aid of a portable photosynthesis meter. The curves of CO_2 assimilation rate (*A*) in response to the increase in photosynthetically active radiation (*PAR*) were evaluated at values of 1500, 1000, 700, 450, 250, 120, 50 and 0 µmol m^{-2} s^{-1} for clones of *E. benthamii* and *E. dunnii*. The three *Eucalyptus* clones evaluated showed a similar behavior of the *A/PAR* curve, showing a high demand for photosynthetically active radiation. In general, the three clones were very similar in terms of CO_2 assimilation rate due to the increase in photosynthetically active radiation.

Keywords: photosynthetically active radiation, quantum efficiency of photosynthesis, respiration.

Introduction

Eucalyptus is ranked globally among the most economically important trees (Bayle et al., 2019). They exhibit traits such as fast growth (Lorentz and Minogue, 2015) and high water and light use efficiency (Maier et al., 2017) that contribute to their candidacy as an excellent biofuel crop (Gonzalez et al., 2011). *Eucalyptus* plants are classified as C3 – carbon dioxide fixation and follow a C3 pentose phosphate cycle (Machado et al., 2011). Most eucalypt species are native to Australia and Indonesia and are adapted to tropical and subtropical environments. However, some species have developed frost tolerance through advancements in biotechnology or adaptation (Stanturf et al., 2013). Two species, in particular *Eucalyptus benthamii* and *Eucalyptus dunnii*, has been shown to exhibit competitive frost tolerance when compared to members of the same genus (Arnold et al., 2015).

In Brazil, these two species are well adapted to southern Brazil. However, in many places in this region it is common to have many cloudy days (Aguiar et al., 2018), which can significantly decrease the growth of species, whether in the field or in a nursery. Light absorption capacity is thought to be a major factor limiting growth at tree scale across a broad range of environmental contexts and management (Binkley et al., 2013). However, the positive effect of light absorption on gross primary productivity and biomass production can be obscured by other limiting factors (Will et al., 2001). The effect of light absorption on forest productivity is expected to vary among genotypes, soil, climate and management conditions (Forrester et al., 2018).

In order to better understand and predict the productivity of different genetic materials, studies involving photosynthetic capabilities can facilitate this process. Photosynthesis is the process used by plants to transform less than 5% of the solar radiation into the energy needed to drive carbon dioxide fixation and to form the organic matter of plant tissues, and to promote plant growth (Solarte et al., 2010). Photosynthesis is critical for support of life systems on the Earth. Each plant species has its own optimal light intensity range, soil conditions, and other environmental needs for photosynthesis (Liao et al., 2020).

The radiation used in photosynthesis ranges between 400 to 700 nm of the solar radiation spectrum and is known as photosynthetically active radiation (*PAR*) (Azcón-Bieto et al., 2008). The *PAR* intercepted and absorbed by the leaves, and the efficiency of the conversion of carbohydrates into chemical energy are key factors to understanding plant growth (Woittiez et al., 2017). One way to study these factors is with light saturation curves, which represent the response of the net photosynthetic rate to the *PAR*.

The photosynthetic light-response curve reflects the instantaneous response of the net photosynthetic rate (*Pn*) to different gradients of photosynthetically active radiation (*PAR*). It can provide measures of many photosynthetic indicators, such as the maximum *Pn* (*Pmax*), dark respiration

rate (*Rd*), apparent quantum yield (*AQY*), light compensation point (*LCP*) and light saturated point (*LSP*), for analysing plant photosynthetic activity (Coble et al., 2016). Therefore, our work aimed to compare clones of *Eucalyptus benthamii* and *Eucalyptus dunnii* by checking the CO_2 assimilation rate due to the increase in photosynthetically active radiation.

Results and Discussion

In average, there was no difference between the evaluated clones (Figure 1b) in photosynthetically active radiation (*PAR*), whereas the general average obtained was 13.22 µmol CO_2 m^{-2} s^{-1}.

The three *Eucalyptus* clones showed very similar *A/PAR* curves (Figure 2), showing a pattern of higher increase in CO_2 *A* assimilation rate (µmol CO_2 m^{-2} s^{-1}) up to approximately 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation (*PAR*). For all clones there was a high increase in *A* assimilation rate mainly up to 450 µmol m^{-2} s^{-1} as we raised *PAR*.

Dark respiration (*Rd*) values ranged from 2.15 µmol CO_2 m^{-2} s^{-1} for clone C (*E. dunnii*) to 2.76 µmol CO_2 m^{-2} s^{-1} for clone A (*E. benthamii*), representing an increase of 22%. Clone B had a *Rd* value closer to clone A (also from *E. bethamii*). For maximum photosynthesis (A_{max}), *E. benthamii* clones (clones A and B) showed higher values compared to clone C, with a difference of less than 5%.

The quantum efficiency of photosynthesis (*AQY*) ranged between 0.0719 and 0.0810 µmol CO_2 µmol of photons^{-1}. The highest value obtained for clone A, with a difference of 12% higher in relation to the other clone of *E. benthamii* (B). Regarding the light compensation point (*φ*), the *E. benthamii* clones obtained the highest values, close to 35 µmol of photons m^{-2} s^{-1}, while the *E. dunnii* clone C presented a value lower than 30 µmol of photons m^{-2} s^{-1}.

The three *Eucalyptus* clones showed a similar behavior of the *A/PAR* curve, showing a high demand for photosynthetically active radiation (greater than 800 µmol m^{-2} s^{-1}). This result demonstrates that in the presence of light clones highly respond to photosynthetic production. Many species also exhibit such as behavior. Berry and Goldsmith (2020) reported that *Heliocarpus americanus* L., *Cecropia polyphlebia* Donn. Sm. and *Ficus* spp require at least 800 µmol m^{-2} s^{-1} to reach to the maximum photosynthetic capacity. Other species such as *Meliosma vernicosa* Liebm. and *Conostegia rufescens* Naudin reached the maximum photosynthetic capacity with only 400 µmol m^{-2} s^{-1}.

Working with *Eucalyptus grandis* (W. Hill ex Maiden) and the hybrid between *E. grandis x Eucalyptus camaldulensis* (Dehnh.), Shem et al. (2009) obtained a limitation of 800 µmol m^{-2} s^{-1} for the species, and values lower than this were assumed to limit assimilation. In this same study, the maximum photosynthesis obtained for *E. grandis* was 19.2 µmol m^{-2} s^{-1} and for the hybrid *E. grandis x Eucalyptus camaldulensis*, 24.6 µmol m^{-2} s^{-1}. It is important to highlight that Shem et al. (2009) studied 3-year-old field plantations, while our results are from younger plants planted in a mini-clonal garden for minicutting production, periodically pruned with continuous fertilization. It is noteworthy that there are no studies on the need for light in a nursery environment for the production of mini-cuttings. The authors consider this variation between species due to differences between them in the light absorption properties of leaves. The same is applied to our study, however with less accentuated differences between the two species (*E. benthamii* and *E. dunnii*).

In general, the three clones presented very similar pattern in *A/PAR* curve. However numerically, clone A of *E. benthamii* presented a higher A_{max}, about 4.3% higher than clone C, demonstrating a possible greater potential for mass accumulation. However, the *Rd* value of about 28.5% higher in clone A compared to C can neutralize this higher A_{max}, or even, in the final carbon balance fixed in a 24h cycle, it can be negative. The higher *Rd* can also mean higher capacity/potential to relocate the starch accumulated by photosynthesis in the leaves. The maximum net photosynthetic rate (A_{max}) is an important parameter of plant growth rate, describing the maximum net light plant potential, and reflecting the maximum potential of plants can turn the CO_2 from the atmosphere into organic matter in the unit area (Xiong et al., 2012).

Dark respiration reflects a condition, in which with no absorption of light radiation by leaves, the amount of CO_2 released by respiration exceeds that of CO_2 fixed in photosynthesis (Larcher, 2004). Differences in dark respiration rate have been used as criteria for discriminating varieties with low respiration rates and high biomass accumulation capacity (Wilson and Jones, 1982). Working with *Eucalyptus pauciflora* (Sieb. ex Spreng), Atkin et al. (2000) found values like the present study for *Rd*, obtaining 2.48 µmol CO_2 m^{-2} s^{-1} at a temperature of 30 ºC. However, this value was reduced with temperature, reaching 0.64 µmol CO_2 m^{-2} s^{-1} at 15 ºC. Our study was carried out in a greenhouse where daytime temperatures are approximately between 25 and 30 ºC. Average values of dark respiration for the plants of the three provenances of *Eucalyptus cloeziana* F. Muell. ranged from 0.61 to 1.86 µmol m^{-2} s^{-1} (Ngugi et al., 2003).

Numerically, clone A had the greatest potential for radiation response (0.081). This shows that for each mole of photon absorbed by the clone leaves, 0.081 moles of CO_2 are fixed via photosynthesis. Compared to clone C, it would be 9.46% more efficient. The decline of *AQY* implies that the conversion efficiency of low-light energy was decreased (Liang et al., 2016). Our values are higher compared to an 18-month-old *Eucalyptus cloeziana* plantation, whose apparent quantum efficiency of photosynthesis (*AQY*) was 0.04 to 0.06 µmol of CO_2 µmol photons^{-1} (Ngugi et al., 2003). In addition to the effect of species observed in this study compared to *Eucalyptus cloeziana*, the cultivation environment must also be mentioned, in which the mini-stumps are conducted aiming at the maximum production of mini-cuttings, with temperature, humidity and nutrition control, with greater response to radiation.

The compensation point reflects the number of moles of photons that are needed for the carbon balance in the leaves to be equal to zero, which is photosynthesis equal to respiration. The clone C was numerically smaller, about 22.28%, compared to clone A. This means that clone C (*E. dunnii*) presents a more characteristic photosynthetic apparatus adapted to shading conditions, which may reflect in the lower A*max* and *Rd*, since the saturation of photosynthesis by light occurs more quickly, and usually shade plants present lower Rd. Plants with lower light compensation point have stronger adaptability to environment light (Xiong et al., 2012).

There are several factors that can affect the photosynthetic rate, such as nutritional management, genetic effect, climate,

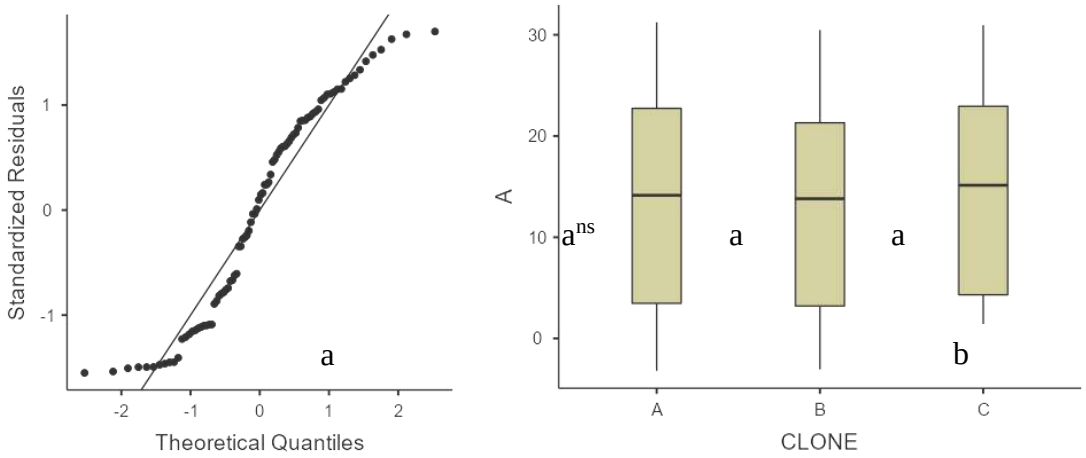
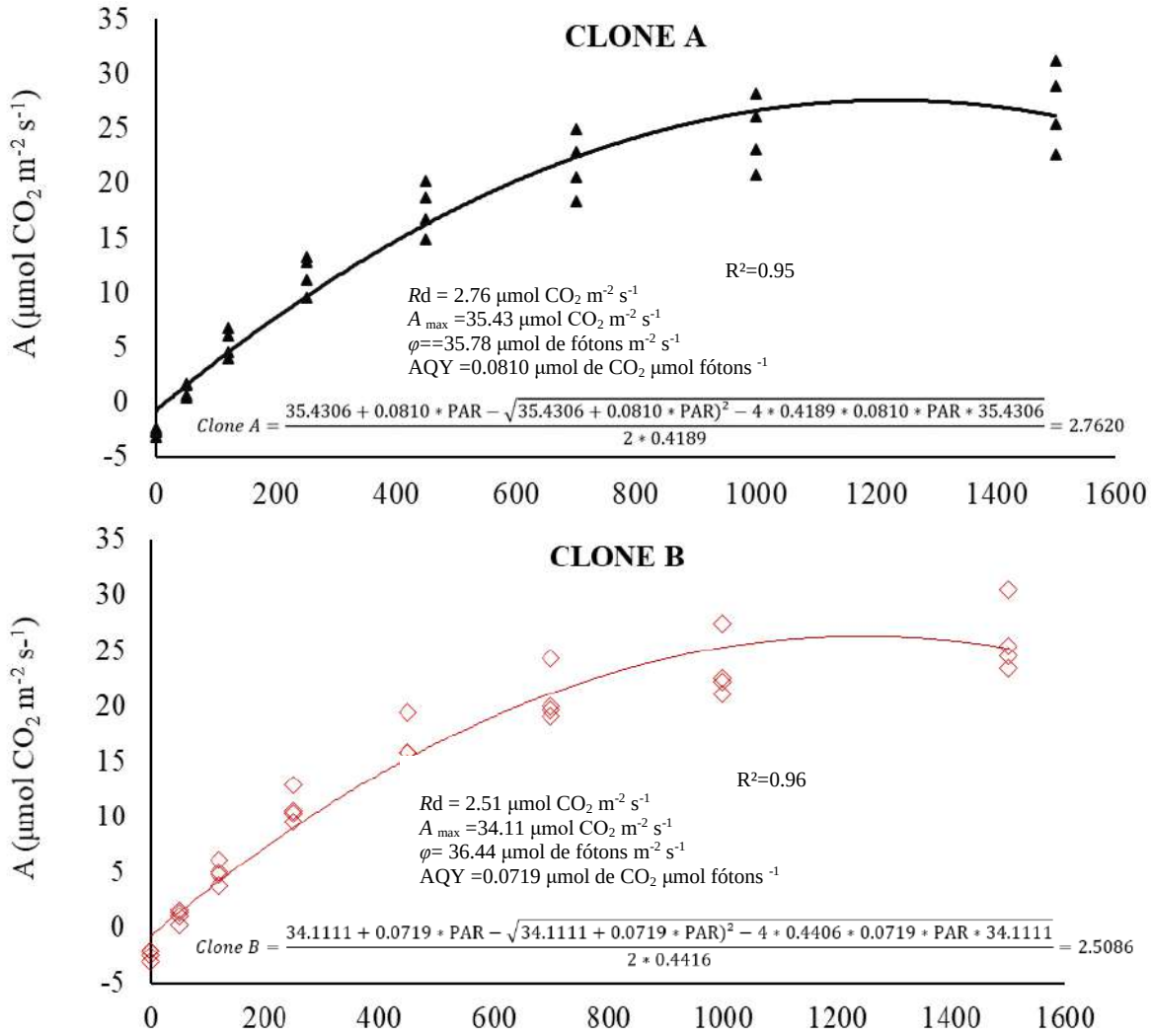


Figure 1. Standardized residuals (a) and (b) *A* assimilation rate (µmol CO_2 m^{-2} s^{-1}) em different *Eucalyptus* clones. ^{ns} - not significant at 5% error by Tukey's test.



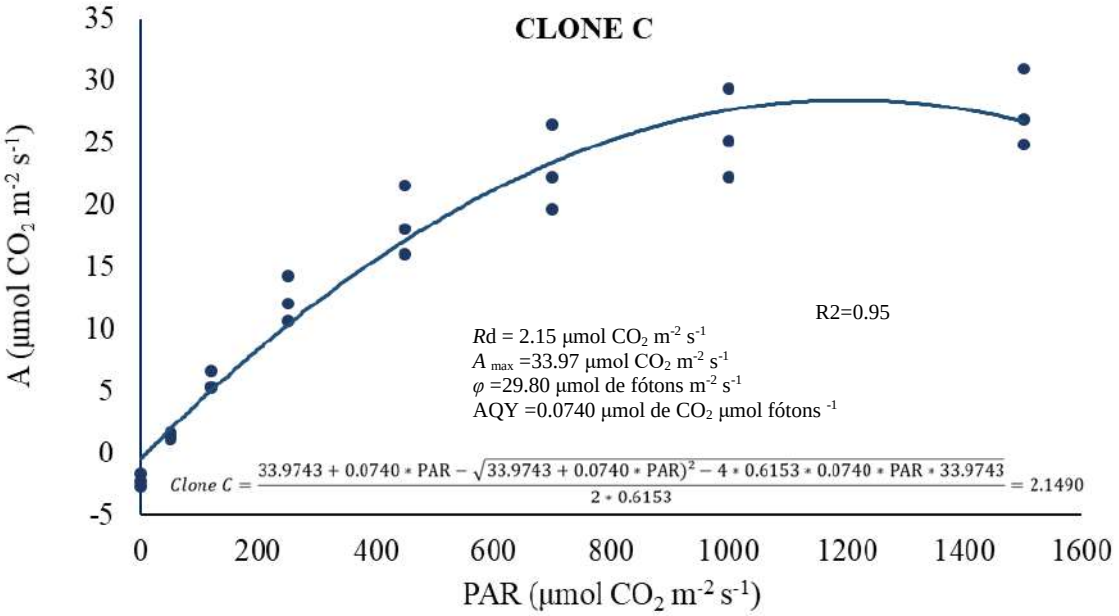


Figure 2. Liquid CO_2 assimilation curves (*A*) in response to the increase in photosynthetically active radiation (*PAR*) ranging between 0 and 1.500 µmol m^{-2} s^{-1} in three *Eucalyptus* clones (clones A and B - *Eucalyptus benthamii* and clone C - *Eucalyptus dunnii*). Where: *AQY* = apparent quantum yield (µmol de CO_2 µmol fótons $^{-1}$); *φ*= light compensation point (µmol de fótons m^{-2} s^{-1}); A_{max}= maximum net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}); *Rd*= dark breath rate (µmol CO_2 m^{-2} s^{-1}).

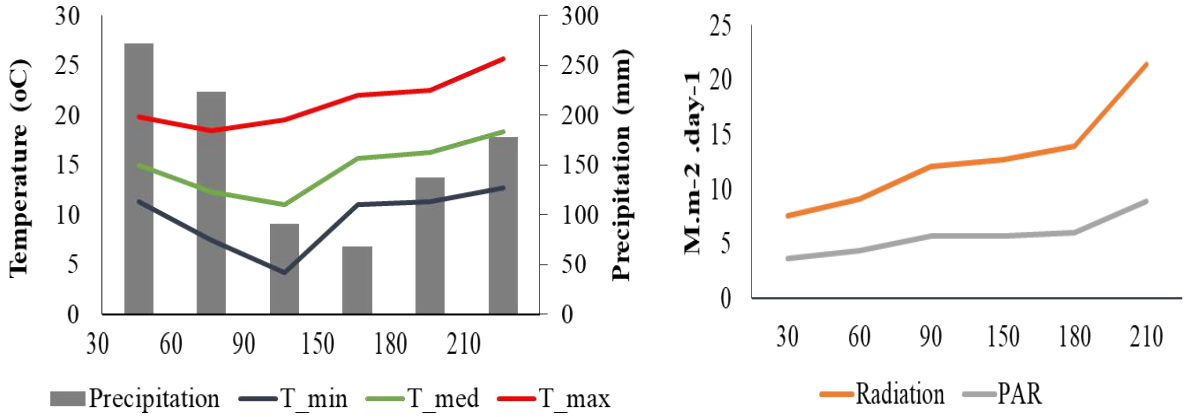


Figure 3. Summary of meteorological variables of the month in that was evaluated the experiment.



Figure 4. Partial view of the experimental greenhouse, showing an uncovered (a) and covered (b) plastic incubator.

among others, modifying the productive potential of the species (Woittiez et al., 2017). Studies have shown that there is a relationship between photosynthesis and productivity (Romero et al., 2007). Consequently, conditions that limit the components of photosynthesis (water, sunlight, CO_2, nutrients, chlorophyll and leaf area) negatively affect this process, resulting in less growth and less resistance to pests and diseases (Cayón, 1999).

In general, the three clones are very similar in terms of CO_2 assimilation rate due to the increase in photosynthetically active radiation, with little superiority for the *E. benthamii* clones. Thus, acclimatization and development studies with field monitoring are necessary to achieve a better understanding of the light needs of each genetic material.

Materials and methods

Conduction of study

This study was conducted in the greenhouse of a Forestry company located at Otacílio Costa, Santa Catarina state, Brazil. The area has a temperate climate of type Cfb (Alvares et al., 2013). In this area, cloudy days prevail throughout the year, especially in fall and winter, reducing the availability of light for the mini-stumps, meaning that a highly controlled environment is required for optimizing the performance of mini-stumps and the production of shoots for mini-cuttings (Aguiar et al., 2018).

Meteorological data

Meteorological variables (Figure 3) were recorded daily from an automatic station available at Forestry company. The meteorological variables were correlated with the amount of mini-cuttings produced and with the photosynthetic variables. The following variables were used: minimum, mean and maximum temperature (°C); relative humidity (%); precipitation (mm); radiation (M m^{-2} day^{-1}); and photosynthetic active radiation (*PAR*) (M m^{-2} day^{-1}).

Plant material and experimental design

The experiment was installed in March 2017 and conducted in a plastic incubator with mini-stumps of two clones of *E. benthamii* (referred as A and B) and one clone of *E. dunnii* (clone C). The incubator (Figure 4) had 16.6 in length, 0.8 m of width and a height of 0.8 m, located inside a greenhouse with plastic cover. The plastic of the greenhouse and incubator was transparent with a thickness of 150 microns, diffusing light transmission and protection against ultraviolet rays. The incubator was open during the day and closed at night. Humidity inside the incubator was generally greater than 80% at night (closed) and variable during the day (open). Photoperiod in this region varied from approximately 10 h in winter to 14 h in summer.

Mini-stumps were grown in a substrate consisting of a layer of sand covering a narrow layer of crushed stone, making up a total depth of 30 cm. The mini-stumps were originated from cuttings of each of the clones evaluated. Mini-stumps were planted in consecutive lines spaced 10x10 cm apart. Each clone was represented by eight lines, each one having eight mini-stumps planted in sand. Four plots per clones were planted in the incubator. Mini-stumps were adapted to the incubators, receiving daily irrigation with a balanced nutrient solution for continuous production of mini-cuttings. The nutrient solution was prepared with the same concentrations described in our previous work (Aguiar et al., 2018): 165 mg L^{-1} N, 32.7 mg L^{-1} P, 255 mg L^{-1} K, 200 mg L^{-1} Ca, 40 mg L^{-1} Mg, 52 mg L^{-1} S, 0.4 mg L^{-1} B, 0.05 mg L^{-1} Zn, 0.06 mg L^{-1} Cu, 1.6 mg L^{-1} Fe, 1.04 mg L^{-1} Mn, and 0.02 mg L^{-1} Mo. In winter, two events of fertigation were conducted per day, while in summer the frequency was increased to three or four events. Each fertigation lasted between 2 and 4 minutes.

The incubator was also equipped with LED lamps with red/rose light or a mixture of red and blue light (red/blue). The specifications of each type of lamps were: (i) red lamp - 36", 1W, AC85 – 265 V, red/rose = 660:730= 1:1, LED quantity: 36 pcs; (ii) red/blue - 36", 1W, AC85 – 265 V, red/blue = 660:450 = 2:1, LED quantity: 36 pcs. The lamps were placed at the top of the incubator, at a height of 0.8 m. The mini-stumps were exposed to the artificial light for 14 h (between 6 a.m. and 10 p.m.) on a daily basis. Eight replicates of each clone were not exposed to artificial light (control treatment). To separate light-exposure treatments, eight more lines of mini-stumps (borders) were planted between lamps (a space with no direct exposure to any lamp). For the present experiment (*A/PAR* curves) only the control treatment was used.

Evaluated variables and statistical analysis

One year after the installation of the light experiment in the clonal minigarden (in February 2018) with the aid of a portable photosynthesis meter, the CO_2 assimilation rate curves were determined. The portable meter is model 6400XT (Li-Cor, Biosciences), equipped with a light source type LI-6400-02B and operating in an open system. The CO_2 assimilation rate curves (A, µmol CO_2 m^{-2} s^{-1}) were evaluated at values of 1500, 1000, 700, 450, 250, 120, 50 and 0 µmol m^{-2} s^{-1}, for clones A and B (*E. benthamii*) and clone C (*E. dunnii*) in response to the increase in photosynthetically active radiation (PAR).

The evaluations were carried out between the hours of 8 and 12h, on the third pair of leaves, starting from the apex, using 4 repetitions. From the data, the response curve of A was adjusted as a function of *PAR*, adjusting the non-rectangular hyperbolic function, given by the equation:

$$A = \{[A_{max} + (\alpha * PAR) - [(A_{max} + (\alpha * PAR))^2 - (4 * \alpha * \theta * PAR * A_{max})]^{0.5}] / (2\vartheta)\} - Rd + \varepsilon,$$ where:

- A is the assimilation of photosynthesis - µmol CO_2 m^{-2} s^{-1}
- A_{max} is maximum photosynthesis - µmol CO_2 m^{-2} s^{-1}
- Rd is respiration in the dark - µmol CO_2 m^{-2} s^{-1}
- α is apparent quantum yield of A;
- θ is one dimensional convexity;
- and ε is the standard error.

Statistical analyses were performed using the statistical package "Jamovi" for Windows Version 1.6.23. The least significant difference between mean values was calculated using Tukey's multiple range test at the 5% significance level.

Conclusion

The three *Eucalyptus* clones evaluated showed a similar behavior of the *A/PAR* curve, showing a high demand for photosynthetically active radiation. Regarding the light compensation point (φ), *E. benthamii* clones obtained the highest values (close to 35 µmol of photons m^{-2} s^{-1}).

The clones are very similar in terms of CO_2 assimilation rate due to the increase in photosynthetically active radiation little superiority for *E. benthamii* clones.

Acknowledgements

The authors thank the Universidad do Estado de Santa Catarina (UDESC), for the institutional support for this research. To FAPESC for funding the research group Management of Planted and Natural Forests (PAP2019031000055). We also thank the National Council for Scientific and Technological Development (CNPq) to the Research Productivity Scholarship.

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BOSQUE 43(2): 135-147, 2022 DOI: 10.4067/S0717-92002022000200135

Vegetative rescue and *in vitro* propagation of *Persea willdenovii*

Rescate vegetativo y propagación *in vitro* de *Persea willdenovii*

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SUMMARY

Persea willdenovii is a native species popularly known for its high potential for medicinal use. Due to problems in low seed production and germination, vegetative propagation appears promising. Thus, we seek with this work to evaluate techniques of vegetative rescue and the potential use of shoots obtained in tissue culture. Adult trees of the species were used to apply total and partial girdling treatments and obtain pruned branches as vegetative rescue techniques. Shoot production was monitored over time (90 to 240 days). Sprouts were used for *in vitro* propagation, and disinfestation treatments were carried out using biocides such as NaClO and Plant Preservative Mixture™ (PPM™), changing the time and concentrations of the products. Multiplication was also tested using doses of 6-benzylaminopurine (BAP) and media and the potential for callogenesis under combinations of BAP and Naphthalene Acetic Acid (ANA). The vegetative rescue by complete or semi-girdling has the potential to produce shoots, though pruned branches proved to be one of the best techniques for rescue. In the micropropagation, the use of biocides such NaOCl for a long period of time (2 % for 20 min) with PPM™ added to the culture medium shows a potential for disinfestation in the *in vitro* establishment. For *in vitro* multiplication, the usage of WPM and BAP (2 a 4 g L^{-1}) promotes higher shoot length, shoot number and leaf number. For indirect organogenesis by leaf segments, the cultivation with BAP and ANA was not responsive to the induction of callogenesis.

Keywords: vegetative propagation, girdling, pruned branches, micropropagation.

RESUMEN

Persea willdenovii es una especie nativa popularmente conocida por su uso medicinal. Debido a problemas en la baja producción y germinación de semillas, la propagación vegetativa parece promisoria. Consecuentemente, en este trabajo se evaluaron técnicas de rescate vegetativo y el uso potencial de brotes obtenidos en cultivo de tejidos. Se usaron árboles adultos de la especie para realizar tratamientos de anillo total y parcial, y obtener ramas podadas como técnicas de rescate vegetativo. La producción de brotes se controló a lo largo del tiempo (90 a 240 días). Se utilizaron brotes para la propagación *in vitro*, y los tratamientos de desinfestación se llevaron a cabo utilizando biocidas como NaClO y Mezcla Conservante de Plantas (PPM™), cambiando el tiempo y las concentraciones de los productos. La multiplicación también se probó utilizando dosis de BAP y medios y el potencial de callogénesis en combinaciones de 6-Bencilaminopurina (BAP) y ácido naftalenacético (ANA). El rescate vegetativo mediante anillo completo o parcial tiene el potencial de producir brotes, pero las ramas podadas demostraron ser una de las mejores técnicas. En la micropropagación, el uso de biocidas como el NaOCl durante un período prolongado (2 % durante 20 min) con PPM™ añadido al medio de cultivo muestra un potencial de desinfestación en el establecimiento *in vitro*. Para la multiplicación *in vitro*, el uso de WPM y BAP (2 a 4 g L^{-1}) promueve una mayor longitud y número de brotes, y número de hojas. Para la organogénesis indirecta por segmentos de hojas, el cultivo con BAP y ANA no respondieron a la inducción de callogénesis.

Palabras clave: propagación vegetativa, anillo, ramas podadas, micropropagación.

INTRODUCTION

Persea willdenovii Kosterm, popularly known as bush avocado, maçaranduba, pink cinnamon or pau de Andrade belongs to the Lauraceae family. The species is known for its diversity of uses in urban forestry, civil building, and medical properties (Batista *et al*. 2010). The local population uses the bark of this species to make tea as treatment of gastric ulcers and wound healing. These claims were supported by pharmaceutical and phytochemical studies due to the presence of mucilaginous structures that are related to protective action on inflamed mucous (Rosa *et al*. 2017).

Despite its high potential, *Persea willdenovii* is present in several endangered species lists and is considered critically endangered in the Red List of Rio Grande do Sul state's flora (CNCFlora 2020), mainly due to the exploratory use in popular medicine through removal of the bark

with the consequential death of individuals in the forests. In addition to this problem, the species has an irregular fruit maturation which can compromise the quality of seeds and storage potential, making the species more vulnerable to human actions and therefore more susceptible to the extinction process. The vegetative propagation of species that present low seed germination and / or recalcitrant seeds, such as *Persea willdenovii,* can ensure the preservation of the species in addition to multiplying individuals with desirable traits for commercial purposes (Fior *et al.* 2007).

Studies conducted on aspects of propagation of *Persea willdenovii* by Fior *et al*. (2007) presented limitations, mainly related to restriction in the morphogenic potential, presence of endogenous contamination and high tissue oxidation. There are many factors influencing the success of the propagation such as genotype, maturation, type of explant, age and size of the explant, and extrinsic factors such as plant regulators and culture medium (Lee and Pijut 2017, Silva *et al*. 2020).

Few species are easily propagated using mature (old material) propagules. In the case of tree species, this difficulty is directly linked to the process of maturation, because the older the plant, the more reduced the cell division rates and regenerative capacity (Nascimento *et al*. 2018), necessary to rejuvenate the mother plant before starting any propagation work. In addition, there is an increase of inhibitors (anatomical barriers, lignification, gibberellins, phenols) and consequently decrease of rooting as the age of the plant increases (Bisognin *et al*. 2015). In woody forest species the gradient of youth grows towards the base of the tree. The basal region of the tree is younger in the ontogenetic age and this is due to the later formation of the apical meristems (terminal regions) in relation to the basal meristems in a tree. It is important to know this ontogenetic behavior of the different parts of the tree, since, depending on the location of the removal of vegetative material (part of the plant), it will certainly interfere in the rooting potential of the propagule and consequently in the production of seedlings (Stuepp *et al*. 2018).

The *in vitro* culture may be an important strategy in solving problems not only in propagation but also in genetic improving and plant biotechnology, especially in perennial woody species (Warakagoda and Subasinghe 2013). The *in vitro* culture of *Persea willdenovii* is still not well explored and has potential among other techniques of asexual propagation, however, there are hindrances related to low morphogenic potential and explant contamination (Fior *et al*. 2007). According to Oliveira *et al*. (2013), high contamination of native tree species is common, since they have superior lignification, endogenous microorganisms, often adverse climatic conditions, and difficulty in carrying out preventive treatment.

Various parts of the mother plant can be used as source for the explant. The choice of material can be influenced by factors such as: availability of the material, contamination level, juvenility of the tissue, among others. The nutritional requirements can also vary, as well as the type and efficiency of the phytoregulators (Cid and Teixeira 2010). However, it is important to highlight that the success of the micropropagation, regardless of the explant used, is subject to the effect of the genotype from the mother plant in response to the stimulus *in vitro* (Stein *et al.* 2009).

To identify and characterize relevant aspects in the production of plants, we conducted a series of experiments with vegetative rescue and *in vitro* propagation. We aimed at verifying if the species has the possibility of vegetative rescue by girdling or pruned branches and at assessing the potential for establishment and cultivation *in vitro*. We are looking for a new propagation alternative for the species that has problems with seed germination. Our hypothesis is that it is possible to use rescue techniques to produce juvenile shoots that will be used in *in vitro* propagation, and that in this environment the species has the possibility of establishment and multiplication, thus allowing to produce clonal plants with sanitary quality, and still seek new studies exploring the medicinal characteristics of the species.

Thus, the objectives of the study are to determine the best method for vegetative rescue in adult plants and evaluate various protocols of *in vitro* propagation of the rescue material of *Persea willdenovii*, a species with great potential popular interest, mainly in medicinal functions.

METHODS

Vegetative rescue. To conduct the study, adult plants (reproductive age) of *Persea willdenovii* located in a native area in the municipality of Urupema, Santa Catarina state, Brazil, were studied. The study area is located in the Catarinense mountain range at approximately 1,400 m a.s.l., at the coordinates of 28° 17’ 38’’ S and 49° 55’ 54’’ W.

This region has a humid temperate climate (Cfb), with average annual precipitation around 1,800 mm, well distributed throughout the year. The average annual temperature is 13 °C, with a very distinct winter and summer. The average temperature for these two seasons is 8 °C and 18 °C, respectively. As characteristics of the region, there may be snow and minimum temperatures of up to -14 °C (EMBRAPA, 1998).The region belongs to the altomontana mixed ombrophilous forest, characterized by the presence of the species *Araucaria angustifolia* (Bertol) Kutze (from 1,000 m a.s.l.), forming groupis in association with other species.

The vegetative rescue was conducted in August 2015. The treatments were: girdling (100 %), semi-girdling at 75 % of the trunk circumference, semi-girdling at 50 % of the trunk and the induction of shoots from pruned branches. The trunk girdling and semi-girdling consisted in the removal of an approximately 2 cm wide bark ring (figure 1A) at a height of approximately 30 cm from the soil, made by sectioning two transverse rows with a machete, to break the bark without damaging the wood. A total of se-

ven trees were used in each treatment (girdling and semi-girdling). The experiment was conducted in a completely randomized system.

For the epicormic sprouts induction treatment from pruned branches, branches were removed, from five mother trees, in the lowest position of the canopy to minimize the effects of maturation and ontogenetic age. Ten branches per mother trees were cut to an approximate size of 1-meter-long and had their ends protected with plastic bags to avoid water loss (figure 1B). The material was placed in a mini-tunnel under a greenhouse (33 % of light blocking), with constant relative humidity (80 %) and an average temperature of 25 °C, in the forest nursery of the State University of Santa Catarina (city of Lages, Santa Catalina state, Brazil).

The evaluations and harvesting of the sprouts were held from the first signal of shooting onwards, at 90, 120, 150, 180, 210 and 240 days (December 2015 to May 2016) after application of vegetative rescue treatments. After each harvest, the number and size (length) of these sprouts were registered, and afterwards the percentage of sprouting per harvest was calculated. For the pruned branches technique, evaluations were made in relation to the length of the branches (sprout number m^{-1}) as done by Nascimento *et al*. (2018).

In the field, diameter at breast height (cm), height (m) and the amount of incident light (lux) in the trunk base of each tree were measured (table 1). An amount of light was measured with a portable digital light meter (Minipa MLM-1011), with three measures interspersed between trees. The assessment was always carried out with a clear sky, without the sun being covered by any cloud. This reading (incident light) was repeated in all harvests (90, 120, 150, 180, 210 and 240 days).

In vitro propagation. For the *in vitro* propagation of *Persea willdenovii*, the sprouts originated from the vegetative rescue (pruned branches or girdling). Figure 2 shows a summary of the micropropagation process carried out, summarizing the experiments in each phase. During the production and harvest of sprouts, the calcium oxychloride based fungicide, bactericide and sporicide Frexus® was

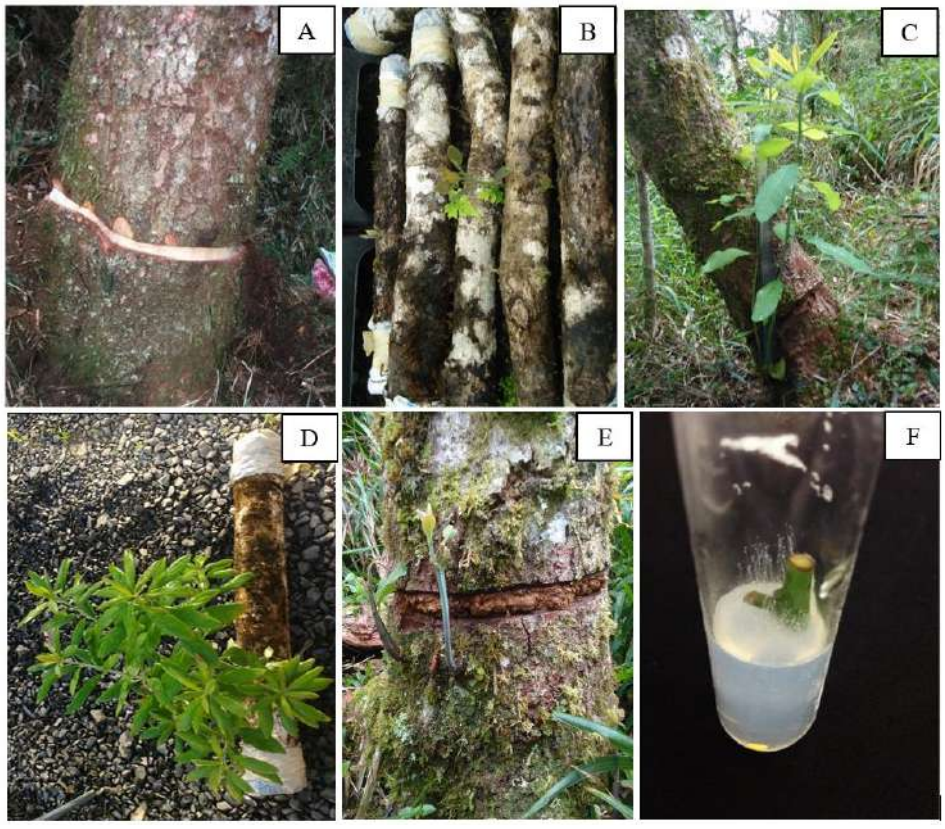


Figure 1. Vegetative rescue and *in vitro* propagation of *Persea willdenovii*. A) girdling in an adult tree in the field, B) pruned branches starting to sprout, C) sprouts in field trees submitted to girdling, D) sprouts on pruned branches, E) healing in the girdling region and F) explant *in vitro* showing contamination.

Rescate vegetativo y propagación *in vitro* de *Persea willdenovii*. A) anillo en un árbol adulto en el campo; B) ramas podadas que comienzan a brotar; C) brotes en árboles de campo sometidos a anillo; D) brotes en ramas podadas; E) curación en la región de anillo; y F) explante *in vitro* que muestra contaminación.

Table 1. List of *Persea willdenovii* trees submitted to vegetative rescue treatments with their respective values: percentage of girdling applied to the circumference of the trunk - treatment (%), tree height (m), diameter at breast height (DBH) (cm) and incident light (lux).

Árboles de *Persea willdenovii* sometidos a tratamientos de rescate vegetativo con sus respectivos valores: porcentaje de anillos aplicadas a la circunferencia del tronco - tratamiento (%); altura del árbol (m); diámetro a la altura del pecho (cm) y luz incidente (lux).

Mother plant	Treatment - % of girdling	Height (m)	DBH (cm)	Average of incident light (lux)
1	100	7.9	24.8	230 (40)*
2	50	12.3	40.7	190 (30)
3	75	13.1	32.8	166 (30)
4	100	6.7	16.5	300 (50)
5	50	6.6	20.5	250 (40)
6	100	4.5	5.7	8,500 (1,200)
7	50	4.7	18.0	250 (30)
8	75	7.8	18.5	300 (30)
9	100	6.4	20.0	360 (50)
10	100	9.3	28.5	2,800 (300)
11	75	9.5	23.7	1,900 (240)
12	50	7.5	24.5	100 (20)
13	75	12.0	29.0	300 (40)
14	50	11.5	42.0	810 (90)
15	75	13.2	31.4	900 (110)
16	100	6.8	20.5	7,030 (900)
17	50	7.1	39.0	200 (30)
18	75	8.4	28.5	245 (40)
19	50	11.6	40.0	1,700 (200)
20	75	8.8	26.6	160 (30)
21	100	10.4	18.2	542 (60)
Average	-	8.6	25.8	1,379.5

* Value in parentheses refers to the standard deviation of the evaluations performed at 90, 120, 150, 180, 210 and 240 days.

applied in the vegetative material, at 0.2 g L^{-1} concentration. The transportation of sprouts originated from pruned branches or girdling was made inside a Styrofoam box, with ice at the bottom and covered by paper sheets moisturized with water. The transport to the material processing site did not last more than 10 minutes (pruned branches) and 40 minutes (girdling). The *in vitro* propagation experiments took place in the vegetal micropropagation laboratory at the Agroveterinary Science Center of the State University of Santa Catarina during 2016.

First, the sprouts were superficially washed using neutral detergent and immersed in running water for 15 minutes. Later the material was segmented in smaller sizes (explants) with ± 3 cm (only nodal segments) and taken to a laminar flow cabinet where the explants were disinfected. The culture medium used was the MS (Murashige and Koog 1962) with 50 % of the concentration of the original; 30 g L^{-1} of sucrose, 0.1 g L^{-1} of myo-inositol, 6 g L^{-1} of agar and 1.5 g L^{-1} of activated charcoal were also added to the medium. The recipients were tubes (150 x 25 mm) containing 15 mL of the medium. The pH of the medium was adjusted to 5.8 ± 0.1 before autoclaving at 120 ºC and 1 atm for 20 minutes.

For the disinfestation, three methods for explant were tested: experiment I – concentrations of the disinfectant sodium hypochlorite (NaClO) in six concentrations: 1, 2, 2.5, 3, 4 and 5 % of active chlorine in contact with the explants for 15 minutes. Experiment II – use of PPM™ biocide in two concentrations: a) 1 mL L^{-1} added to the medium and b) agitating the explants for four hours in contact with 5 mL L^{-1} of the product. In the treatment with the addition of the biocide in the medium, the asepsis was carried out with NaClO 2 % (v v^{-1}) for 15 minutes. And in the treatment under agitation, there was no contact with

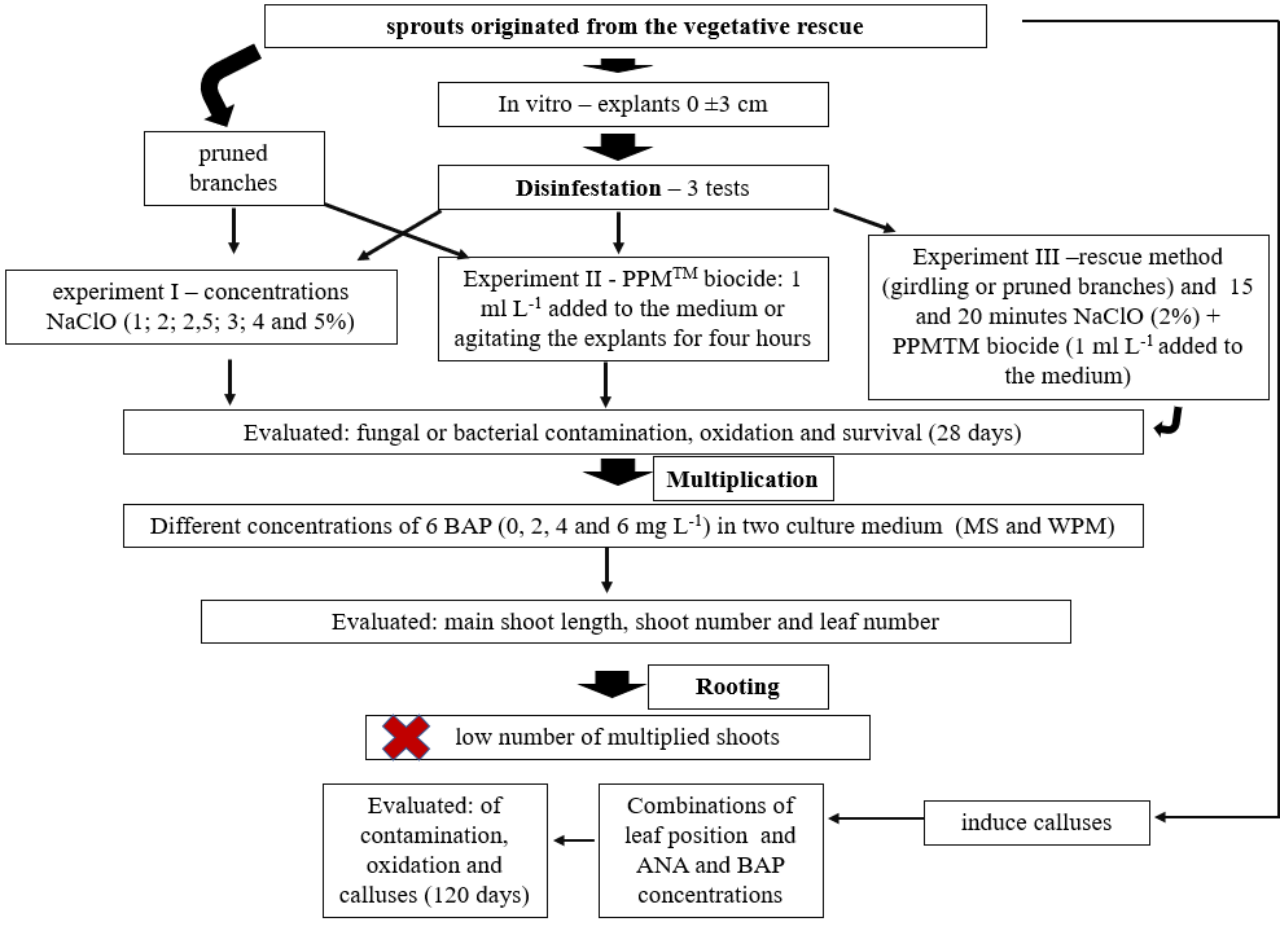


Figure 2. Scheme of the micropropagation process used in *Persea willdenovii*, showing steps from the origin of the material to the cultivation.

Esquema del proceso de micropropagación utilizado en *Persea willdenovii*, mostrando las etapas desde el origen del material hasta el cultivo.

another disinfectant; the explants were directly introduced to the medium. For experiments I and II, the vegetative material was obtained exclusively from pruned branches. Experiment III – the material was divided according to the rescue method: coming from the forest (girdling) or the mini-tunnel (pruned branches). These rescue materials were tested at different times of immersion in sodium hypochlorite (2 % active chlorine): 15 and 20 minutes of contact with the explant (%) + PPM™ biocide (1 mL L^{-1} added to the medium). All experiments used six repetitions, containing three explants in each treatment.

The low number of repetitions and non-utilization of a control treatment was established due to the limited number of nodal segments. Despite the technique that originated the material issued a considerable number of sprouts, the space between them was relatively large, resulting in a small sample of explants. After the asepsis, in all experiments, except the agitation in PPM™ biocide, the explants were washed three times in autoclaved distilled water and incubated in the culture medium. The material was kept in the dark for seven days, and after that period cultivated in the culture room under temperature of 25 ± 3 ºC, in a 16-hour photoperiod and 20 µmol m^{-2} s^{-1} photon flux density, provided by cold white fluorescent lamps. The evaluations of all experiments were carried out in a 28-day period, in which, the incidence of fungal or bacterial contamination, oxidation and survival rate of explants were evaluated.

Shoot segments with previously established shoots *in vitro*, 3 ± 0.5 cm in size, were used for multiplication. Different concentrations of 6-benzylaminopurine (BAP) (0, 2, 4 and 6 mg L^{-1}) were analyzed in two culture media, MS and Woody Plant Media WPM (Lloyd and Mccown 1980). All media were supplemented by 30 g L^{-1} of sucrose, 0.1 g L^{-1} of myo-inositol and 6 g L^{-1} of agar (Sigma Aldrich®). The experimental design was completely randomized in factorial scheme 4 x 2 (4 BAP concentrations and 2 culture medium), six repetitions containing six explants in each treatment. Later, the material was incubated in the culture room as previously described. After 60 days, the main shoot length (mm), shoot number and leaf number were evaluated.

Due to the low number of multiplied shoots, it was not possible to carry out tests involving rooting. However, it was decided to conduct an experiment to induce calluses. The leaves harvested from branches derived from the vegetative rescue were washed in running water and neutral detergent and later segmented in smaller portions (1 cm²). In a laminar flow chamber, the leaf segments were immersed in ethanol 70 % (v v^{-1}) for 1 minute and in sodium hypochlorite solution (NaClO) at 2 % active chloride for 15 minutes, finally rinsed three times in autoclaved distilled water.

The segments were later sectioned in explants of ±1 cm² containing the central leaf nerve and inoculated in abaxial and adaxial leaf positions in 100 mL flasks containing 30 mL MS medium. The mix was supplemented by 30 g L^{-1} of sucrose, 0.1 g L^{-1} of myo-inositol and 6 g L^{-1} of agar and the treatments were various combinations of BAP and ANA in concentrations of 0 to 12 mg L^{-1} (table 2). The pH of the mix was adjusted to 5.8 ± 0.1 before autoclaving at 120 °C and 1 atm for 20 minutes. The explants were kept in the dark for 10 days, and after this period, cultivated in the culture room. Evaluations of contamination, oxidation and calluses growth were carried out weekly until 120 days after the inoculation. The experimental design was completely randomized, in factorial scheme 2 x 10 (A x D), the factor A composed by leaf position and factor D comprised by the combinations of ANA and BAP concentrations, composed by five repetitions, with four leaf segments in each treatment.

To test the hypothesis that rescue by girdling or pruned branches are good strategies to produce rejuvenating sprouts that can be grown *in vitro*, we first checked the variables to normal distribution (Shapiro's test, $P < 0.05$) and variance homogeneity (Barttlett's test, $P < 0.05$). When necessary, the data were transformed with a boxcox test. After rechecking the data for their fit to the basic requirements, we proceeded with ANOVA (F test, $P < 0.05$), followed by Tukey's mean comparisons test ($P < 0.05$) or regression analysis. For the regression analysis, linear and second order (quadratic) models were tested. Tree height data, diameter at breast height and incident light were correlated by Pearson with the variables of vegetative rescue.

Table 2. Leaf face position and 6-benzylaminopurine (BAP) and Naphthalene Acetic Acid (ANA) concentrations tested in MS culture medium in the induction of *Persea willdenovii* callogenesis.

Posición de la cara de la hoja y concentraciones de 6-Bencilaminopurina (BAP) y ácido naftalenacético (ANA) evaluadas en el medio de cultivo MS en la inducción de la callogénesis de *Persea willdenovii.*

Treatment	Leaf face position	Phytoregulator (mg L^{-1})	
		BAP	ANA
1	Abaxial	0	0
2	Abaxial	4	4
3	Abaxial	8	8
4	Abaxial	12	12
5	Abaxial	0	4
6	Abaxial	0	8
7	Abaxial	0	12
8	Abaxial	4	0
9	Abaxial	8	0
10	Abaxial	12	0
11	Adaxial	0	0
12	Adaxial	4	4
13	Adaxial	8	8
14	Adaxial	12	12
15	Adaxial	0	4
16	Adaxial	0	8
17	Adaxial	0	12
18	Adaxial	4	0
19	Adaxial	8	0
20	Adaxial	12	0

RESULTS

Vegetative rescue. Despite the wide variation in dendrometric values (height and DAP) and light intensity in the trunk base, there was no significant Pearson correlation of data from the mother trees with variables evaluated (percent, number and height of sprouts). For all variables, there was an interaction between the vegetative rescue methods and harvests (days after application of the technique).

The percentage of sprouted trees (figure 3A) presented the major average in the pruned branches. This method was the only one to show sprouts (69 %) 90 days after the application of the treatment. There was sprouting in 100 % of branches in the harvests at 120, 150, 180 and 210 days; only at 240 days there was a decrease of sprouts (84 %).

The highest number of sprouts (12.2) was observed in the pruned branches technique, at 150 days (January) (figure 3B). This harvest date was also superior for girdling 50 % of the trunk (4.1). For the techniques of 100 % girdling and 75 % girdling the larger number of sprouts was observed in February after 180 days (3.5 and 4.6 respectively sprouts). After 180 days there is a reduction in the number of sprouts in all vegetative rescue techniques, and at 240 days there are practically no more shoots. It should be noted that it was decided to use the pruned branch technique in comparison to girdling to verify the production potential of the technique; however, the number of sprouts for pruned branches refers to the number per linear meter (sprout number m^{-1}), while the girdling ones refer to the amount per tree. Therefore, an average test was not used and only a regression analysis, showing a production behavior over time.

For the sprout length, in all rescue vegetative techniques, the largest size was obtained between 150 and 180 days after application of treatments (figure 3C). The treatments of complete girdling and pruned branches (figure 1D) presented the largest sizes of the sprouted branches with averages between 15 - 20 cm in height. Some individuals of the complete girdling generated sprouts of almost 50 cm (figure 1C). In the last evaluation, in addition to the absence or small number of sprouts in many trees, the size was very small. In this last evaluation, it was also possible to observe an intense healing in most trees in the girdling treatments (figure 1E).

In vitro propagation. For the *in vitro* establishment, experiments I (concentrations of the disinfectant sodium hypochlorite) and II (PPM™ biocide in two concentrations) did not have significant difference according to the Tukey test ($P < 0.05$). High contamination rate was observed during the four weeks following the removal of material from the dark room to the illuminated environment, between 80 to 92 % of explants had fungal or bacterial contamination (table 3). The oxidation varied between 18 and 33 % and the maximum survival was 20 %.

In experiment III, testing the origin of the rescue material and disinfestation treatments with different times of

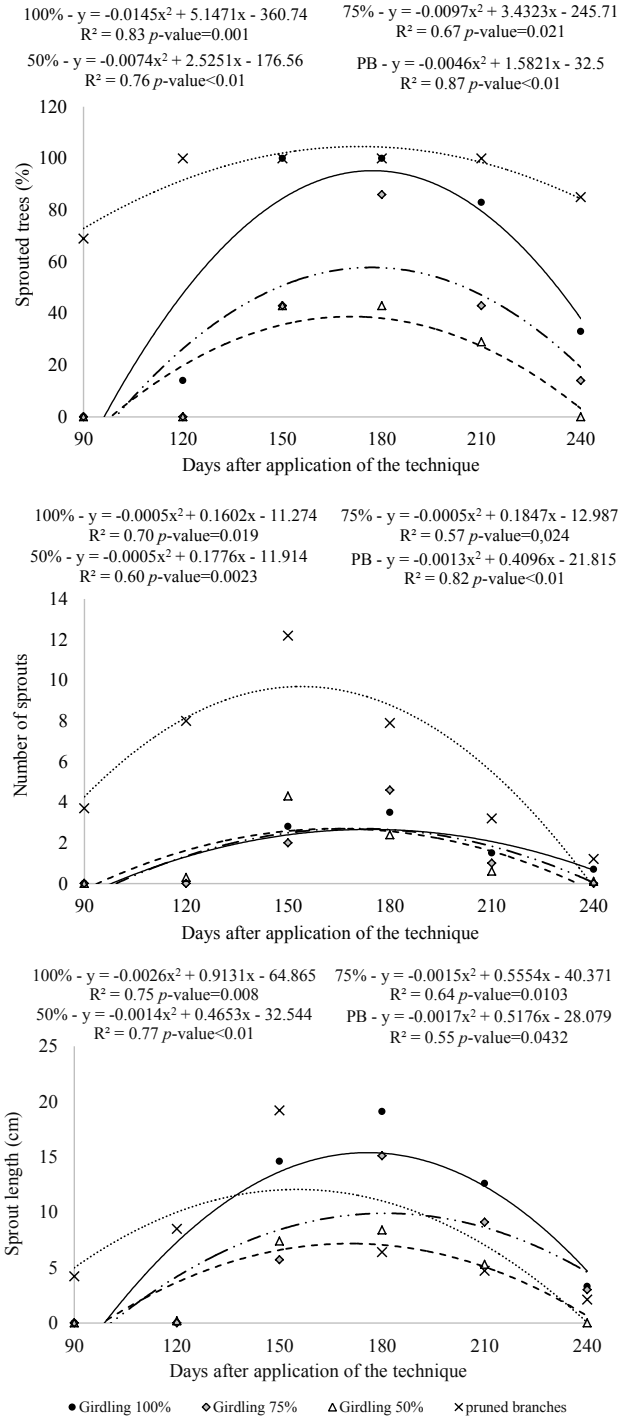


Figure 3. Percentage of sprouted trees (A), average number of sprouts (B) and length of sprouts - cm (C) in *Persea willdenovii* as a function of different vegetative rescue treatments. The days after applying the technique, at which time the assessments took place, correspond to from 90 days (December 2015) up to 240 days (May 2016).

Porcentaje de árboles brotados (A); número promedio de brotes (B) y longitud de los brotes - cm (C) en *Persea willdenovii* en función de diferentes tratamientos de rescate vegetativo. Los días posteriores a la aplicación de la técnica, momento en el que se realizaron las evaluaciones, corresponden a 90 días (diciembre 2015) hasta 240 días (mayo 2016).

NaClO supplemented with PPM™ biocide (1 mL L^{-1}), we observed a difference between treatments. The girdling material presented higher fungal/bacterial contamination (between 82 and 86 %), higher oxidation rate (between 38 and 41 %) and lower explant survival rates (39 to 45 %), regardless of the time of exposure to the NaClO (figure 4). However, the pruned branches material also showed inferior results, similar to those of girdling. The treatment with

Table 3. Fungal / bacterial contamination (%), oxidation (%) and survival (%) in *Persea willdenovii* explants grown *in vitro* after carrying out disinfestation experiments.

Contaminación por hongos / bacterias (%), oxidación (%) y supervivencia (%) en explantes de *Persea willdenovii* cultivados *in vitro* después de realizar experimentos de desinfestación.

		Disinfestation/variables		
Experiment	Treatment	Fungal/ Bacterial contamination (%)	Oxidation (%)	Survival (%)
experiment I – concentrations NaClO	1 %	92^{ns} (8)	33^{ns} (21)	10^{ns} (20)
	2 %	88 (4)	24 (14)	15 (8)
	2.5 %	85 (10)	31 (17)	15 (12)
	3 %	83 (9)	26 (19)	19 (10)
	4 %	89 (10)	30 (10)	7 (8)
	CV (%) (*p*-value)	35.3 (0.843)	19.9 (0.441)	14.4 (0.093)
Experiment II - PPM™ biocide	1 mL L^{-1} added to the medium	80^{ns} (13)	18^{ns} (11)	20^{ns} (10)
	agitating with explants	82 (15)	22 (12)	18 (12)
	CV (%) (*p*-value)	26.4 (0.923)	15.3 (0.192)	16.1 (0.324)

^{ns} - not significant. Values in parentheses represent the standard deviation of the mean.

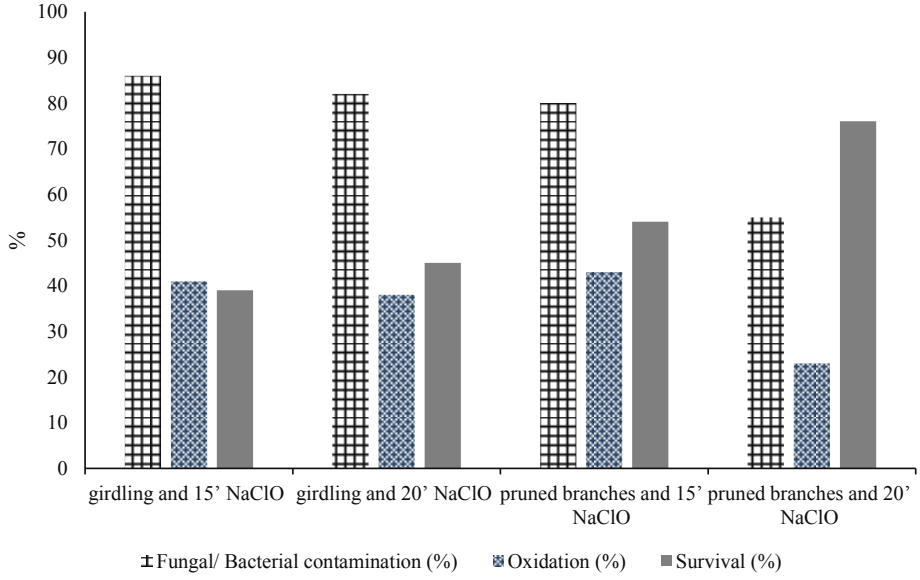


Figure 4. Percentage of fungal / bacterial contamination, oxidation and survival of *Persea willdenovii* explants depending on the rescue method (girdling or pruned branches) and 15 and 20 minutes NaClO (2 %) + PPM™ biocide (1 mL L^{-1} added to the medium) in the *in vitro* establishment. Fungal / bacterial contamination (CV % = 21.3 and *p*-value = 0.021), oxidation (CV % = 18.4 and *p*-value = 0.042) and survival (CV % = 14.2 and *p*-value = 0.0328).

Porcentaje de contaminación fúngica / bacteriana, oxidación y supervivencia de explantes de *Persea willdenovii* en función del método de rescate (anillado o ramas podadas) y 15 y 20 minutos NaClO (2 %) + biocida PPM™ (1 mL L^{-1} agregado al medio) en el establecimiento *in vitro*. Contaminación por hongos / bacterias (CV % = 21,3 y valor-*p* = 0,021), oxidación (CV % = 18,4 y valor-*p* = 0,042) y supervivencia (CV % = 14,2 y valor-*p* = 0,0328).

the best response was pruned branches with exposure for 20 minutes in NaClO. This treatment obtained less contamination (55 %), oxidation (23 %) and higher survival (76 %).

For the *in vitro* multiplication, there was no interaction between factors ($P < 0.05$) (culture medium and BAP concentrations) in the evaluated variables, nonetheless there was a difference between the isolated factors (table 4). For main shoot length (mm) there was a difference between the culture media, in which the WPM presented the highest average (7.5 mm). The addition of BAP showed no difference for this variable. For shoot number, the WPM medium also showed better response (0.51) compared to the MS medium (0.30). The addition of 4 mg L^{-1} represented a larger number of shoots, not differentiating from 2 mg L^{-1}. The absence or the highest concentration (6 mg L^{-1}) of BAP provided low number of shoots. The same behavior (higher averages) was obtained for number of leaves using the WPM medium (0.78) and the concentrations 2 and 4 mg L^{-1} of BAP (1.02 and 0.99).

There was no formation of calluses in the leaf calluses exposed to the evaluated treatments. In general, it was observed that leaves had light green coloration up to 15 days after inoculation, becoming beige after 60 days of cultivation, and after this period, the color changed to brown. After 120 days of *in vitro* cultivation, the explant tissue died, probably due to the lack of favorable conditions for development. The data are not presented because in the evaluation of 120 days, there was no formation of callogenesis or live leaf tissues, regardless of treatment.

Table 4. Main shoot length (mm), shoot number and leaf number depending on concentrations of 6-benzylaminopurine (BAP) and culture medium in *Persea willdenovii* explants multiplied *in vitro*.

Longitud de los brotes principales (mm), número de brotes y número de hojas en función de las concentraciones de 6-bencilaminopurina (BAP) y del medio de cultivo en explantes de *Persea willdenovii* multiplicados *in vitro*.

BAP (mg L^{-1})	MS	WPM	Average
	main shoot length (mm)		
0	5.3 (3.4)	7.1 (2.4)	6.2^{ns}
2	5.1 (2.9)	7.9 (2.0)	6.5
4	6.4 (3.8)	8.3 (4.2)	7.1
6	6.0 (2.5)	6.9 (3.5)	6.4
Average	5.7 B*	7.5 A	
p-value interaction = 0.0963	*p*-value BAP = 0.094 *p*-value medium = 0.023		
	shoot number		
0	0.20 (0.22)	0.40 (0.26)	0.30 b
2	0.35 (0.13)	0.55 (0.27)	0.45 ab
4	0.52 (0.26)	0.73 (0.33)	0.62 a
6	0.14 (0.22)	0.36 (0.15)	0.25 b
Average	0.30 B	0.51 A	
p-value interaction = 0.122	*p*-value BAP = 0.006 *p*-value medium = 0.0162		
	leaf number		
0	0.24 (0.12)	0.41 (0.20)	0.32 a
2	0.72 (0.23)	1.33 (0.37)	1.02 a
4	0.84 (0.31)	1.15 (0.34)	0.99 a
6	0.26 (0.12)	0.24 (0.21)	0.25 b
Average	0.44 B	0.78 A	
p-value interaction = 0.083	*p*-value BAP = 0.001 *p*-value medium = 0.0093		

* Means with different capital letters on the line (culture medium) and lower-case letters on the column (BAP) show a significant difference by the Tukey test at 5 % error. Values in parentheses represent the standard deviation of the mean. ^{ns} - not significant.

DISCUSSION

Vegetative rescue. There was no record of the death of trees subjected to girdling and semi-girdling treatments, meaning that the species supports the removal of a partial or complete girdling of the bark without damaging tree survival. The pruned branches also survived until the end of the evaluations (April 2016), showing a high environmental control of moisture and temperature inside the mini-tunnel, and possession of endogenous reserves, allowing the use of this technique to produce sprouts.

There was no correlation among number of shoots with diameter, height of trees and light intensity. In a work with *Toona ciliata* M. Roem., Pereira *et al.* (2015) did not find correlation between diameter of the mother trees and number of emitted sprouts through vegetative techniques, either. In the same study, dead trees were not observed after vegetative rescue treatments. In contrast, the work of Pinto *et al.* (2013) with *Pterogyne nitens* Tul. presented a positive correlation with the increase in diameter of the plant and increased number and strength of sprouts. There are few published works available to try to understand the influence of the degree of maturation in the growth in diameter and plant height and, consequently, in the production of sprouts. Studies point to some factors prevailing on the regrowth capacity and survival of strains in woody species, including age, level of allocated reserve substances, the genetics of the species and the effect of climate variations throughout the year (Wendling *et al*. 2013, Pereira *et al*. 2017, Nascimento *et al*. 2018).

In relation to climate variations, January and February are the months with a history of higher temperatures in the region (Santa Catarina) and without water deficit problems, which may have contributed to the development of new sprouts. Higher averages of shoots in *Ilex paraguariensis* A. St.-Hil. were also observed in the summer months by Nascimento *et al*. (2018), and this work was carried out in the same experimental area. The fifth and sixth harvests in the field, held in March and April, lowered the production of sprouts, probably the metabolic and physiological cutback of plants due to the decrease in average temperature and temperature range in these months. Another factor that may be responsible for the lower number of sprouts in these harvests was the healing of the girdling part as a response to the application of vegetative rescue (figure 2F). This healing behavior has also been reported to *Toona ciliata* in which the thickness of the bark checked in the ring line may have interfered with the emergency rate of sprouts in some trees (Pereira *et al.* 2015). According to Dias *et al*. (2012), competition for water, nutrients, light and space between sprouts over time may also be related to this reduction.

In addition to the environmental conditions the highest number of sprouts during January and February harvests may be explained by the possibility that these shoots are from gems that had remained dormant after the application of the treatments and did not generate any sprout after the first harvest or, are the product of a morphogenesis, when a cambial cell transforms over time, mainly by the action of auxins (Zahadat *et al.* 2017). According to the same authors, one effect of auxin is to make the bundles of unspecialized stem cells, called cambium cells, which are located near the vascular tissues, transform into vessels. This is especially interesting considering the fact that limited common resources (*e.g.* water) need to be distributed among different branches of a plant via their vessels.

Physiologically, the emission of basal sprouts benefits from the partial or total rupture of apical dominance increasing the cytokinin / auxin ratio (Hartmann *et al.* 2011). Another factor that may have induced the emergence of new sprouts, is stress by girdling, because it may have produced functional disturbances in the trees, which sprouted as a survival strategy. In plants, stress happens by interrupting the transport of photosynthates and other organic metabolites from the higher to the lowest parts in the plant, which is executed by elements and riddled cells, located in the phloem (Taiz and Zeiger 2013). The cut off techniques and stem girdling also managed to induce the emission of basal sprouts in many species such as *Tectona grandis* Linn F. (Badilla *et al.* 2016), *Ilex paraguariensis* (Stuepp *et al.* 2015, Stuepp *et al.* 2016, Nascimento *et al*. 2018), *Sequoia sempervirens* (Pereira *et al*. 2017) and *Toona ciliata* (Pereira *et al.* 2015).

The sprouting ability can also be influenced by factors such as light, thickness and depth of cut (Dinh *et al.* 2018). In this work, we can see that the percentage of girdling at the tree trunk also influences the formation of sprouts, being practically directly proportional in the mother trees of *Persea willdenovii*. For *Toona ciliata* the number of sprouts by annealing is four times higher than those observed in semi-girdling (Pereira *et al.* 2015).

Similarly, vegetative rescue by pruned branches proved to be an effective technique in induction of epicormic sprouts in *Ilex paraguariensis* trees kept in the mini-tunnel (Nascimento *et al.* 2018) and *Eucalyptus cloeziana* F. Muell. trees, where all the pruned branches that were in the greenhouse issued sprouts after 40 days of conditioning (Almeida *et al.* 2007). According to the authors, the induction of epicormic sprouts from pruned branches should be used carefully. Since the way the tissue samples it uses are positioned in the tree, it may mean they are physiologically mature, compromising the juvenility of the material. It is, therefore, important to collect branches of the lower portions of the plant, as these tend to give juvenile shoots. The physiological principle of the method, as well as in stem girdling, is based on shifting the balance between growth regulators (auxin / cytokinin) in support of the issuance of sprouts. Additionally, in most of the angiosperms trees, the dormant epicormic gems, which can be present since the formation of branches / trunk or be the product of morphogenesis, when a cambial cell transforms to yield the new sprouts, are present in the outer bark (Hartmann *et al.* 2011).

The environmental conditions in the mini-tunnel may have contributed to the success of the pruned branches technique. The conditioning in the greenhouse, or similar, creates a favorable microclimate for their survival. In this work, the mini-tunnel has a misting system that keeps the relative humidity at about 80 % and the temperature between 18 and 30 ºC, which may have favored the early sprouting (90 days). For the present study, the meteorological variables of temperature and humidity were not constantly checked, whereas in the study by Nascimento *et al*. (2018) conducted in the same structure, it obtained relative air humidity above 90 % and average temperature between 15 ºC (winter) and 26 ºC (summer). The data was verified with a datalogger. The plastic cover used in greenhouses significantly changes the balance of radiation, when related to the external environment due to the attenuation of incident solar radiation, resulting in a reduction of the internal radiation balance and thus affecting temperature, humidity and evapotranspiration. Vegetative growth is very intense and the growth rate of the aerial part of the plant gradually increases in accordance with increasing temperature.

With the pass of time, after 240 days, shoot emission was reduced, which may be associated with exhaustion of reserves present in the branches. The same was observed in detached branches to induct shoots in *Araucaria angustifolia* (Bertol.) Kuntze and in the experiments of Wendling *et al.* (2009) and *Ilex paraguariensis* (Nascimento *et al.* 2018). The vegetative rescue technique via pruned branches, in addition to getting the best results compared those applied in the field (girdling), has the advantages of having the harvest of material at the plant production site (nursery) and the possibility of higher phytosanitary control of these sprouts.

In vitro propagation. In our study, we found high contamination by fungi / bacteria and oxidation in the first weeks after removing the material that was in the dark at the beginning of the cultivation. Woody plant tissues are quite susceptible to *in vitro* browning, a direct result of stress and chemical reactions induced by polyphenol oxidases and other enzymes. The resulting phenol compounds react with oxygen to form quinine compounds, generally inhibitory of plant growth (Ahmad *et al.* 2013).

Between 80 and 90 % of explants showed contamination in experiment I (NaOCl concentration) and in the second study (PPM™ biocide in two concentrations). The bacterial contaminants manifested after the first week of evaluation, in the base of the explants cultivated *in vitro*, and continued to appear afterwards. In general, the vegetative material survived this bacterial contamination. However, these contaminations generated considerable limitations in the development of the explants. Fior *et al*. (2007) also reported high proportion of contamination by microorganisms of fungal and bacterial origin (74 %) and micropropagation of non-juvenile tissue of *P. willdenovii* during *in vitro* sowing. According to these authors, after various subcultures, the endogenous contamination remained in parts of the material.

In experiment III (establishment) testing the material according to the rescue method and different times of immersion in NaOCl (2 %) + PPM™ biocide (1 mL L^{-1} added to the medium), more promising results were obtained for *in vitro* establishment, mainly of plant material from pruned branches, which were kept in mini-tunnel and presented phytosanitary control with fungicide application. Control with fungicides in mother trees when possible is a good strategy to reduce contamination *in vitro* (Hiti-Bandaralage *et al.* 2017). Better contaminant control in this experiment (III) may also have been due to the addition of PPM™ in the culture medium. Similar results regarding the benefit of using PPM™ were observed in *Calophyllum brasiliense* Cambess., using nodal segments as starting material, where the biocide added to the culture medium reduced the contamination of explants (Silveira *et al.* 2016). According to the same authors, very high concentrations of biocides such as PPM™ and NaOCl can lead to oxidation and mortality.

The difficulties in the *in vitro* establishment of *P. willdenovii* due to contamination and oxidation of the explants continue despite the rejuvenation of the vegetative material; this is probably related to the genetics of the species. Other species of the *Persea genus* also found such obstacles in micropropagation. Studies about the avocado tree (*Persea americana* Mill.) published since 1987 with various cultivars report similar results to the ones found in this (Osorio *et al.* 2018).

As for *in vitro* multiplication, the WPM medium shows better results, with high results of main shoot length (mm), shoot number and leaf number. This may be explained by the difference in salt concentration in the medium. The WPM has fewer salts concentrations (especially nitrogen and potassium) when compared to the MS media, which has high salt concentration, the nitrate and ammonium ions specifically (Rathwell *et al.* 2016).

The use of 2 to 4 mg L^{-1} of BAP promoted higher multiplication, with an increase in the number of sprouts and mainly of new leaves. The absence of the regulator promoted a low rate of regeneration. The same result was obtained in *Melanoxylon brauna* Schott. where the use of between 2 and 4 mg L^{-1} of BAP provided a higher number of shoots (Silva *et al.* 2020). According to Brum *et al*. (2002), the multiplication of sprouts with the use of BAP may be related to the influence of the genetic charge from the mother plant, of the growth regulator used to help with cellular division and in the break of the dormant state of axillary shoots, until then inhibited by apical dormancy. The results obtained in the *in vitro* multiplication of *Persea willdenovii* are promising and show viability of this technique in multiplication and conservation programs of this species. However, newer work must be done aiming for the increase in number of sprouts, exploring, for example, experiments about the influence of the environment

(temperature, light, culture mix solidity, etc.) in the induction of new sprouts.

In the conditions of this work, BAP and ANA phytoregulators and their combinations, in doses of 0 to 12 mL L^{-1}, did not contribute to the callogenesis induction of *Persea willdenovii*, other types of phytoregulators and concentrations pending future testing. Some studies, such as Encina *et al.* (2014) in *Persea americana,* show success of the callogenesis; however, the avocado is a cultivated species, and that generally presents better results *in vitro*. Callus culture is an important technique to increase the number of secondary metabolites in medicinal species (Bansal *et al.* 2013), consequently it should receive further studies with *Persea willdenovii* considering the numerous medicinal properties that the species has.

The rooting test was not possible due to the low number of explants multiplied, owing to the loss of regenerative capacity. Thus, it is recommended to carry out further studies, primarily aimed at improving the multiplication capacity. However, in this experiment it was possible to notice good results regarding the use of cured material (mainly pruned branches), suggesting that these new works follow this methodology.

CONCLUSIONS

The vegetative rescue by complete or semi-girdling has the potential to produce shoots to be used in the vegetative propagation of *Persea willdenovii*. The technique of pruned branches proved to be one of the best techniques for rescue, because in addition to producing higher precocity and quantity of shoots, it enables higher phytosanitary control.

The micropropagation of the species presents numerous challenges, such as high fungal / bacterial contamination and oxidation. The use of biocides such NaOCl for a long period of time (2 % for 20 minutes) with PPM™ added to the culture medium shows a potential for disinfestation in the *in vitro* establishment. For *in vitro* multiplication, the usage of WPM and BAP (2 to 4 g L^{-1}) promotes higher shoot length, shoot number and leaf number. For indirect organogenesis by leaf segments, the cultivation with BAP and ANA was not responsive to the induction of callogenesis.

ACKNOWLEDGMENTS

The authors thank FAPESC for funding the research group Management of Planted and Natural Forests (PAP2019031000055).

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Recibido: 28.04.21
Aceptado: 16.06.22

rLAS® v.5, n.3 Especial (2023)
EIXO III – Ambiente, Sustentabilidades e Implicações na Saúde
ISSN: 2526-219X

Doses de fertilizante de liberação controlada no crescimento inicial de *Cedrela fissilis* Vellozo

Dosage of controlled release fertilizer in the initial growth of Cedrela fissilis Vellozo

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RESUMO

O objetivo do experimento foi avaliar os efeitos de diferentes dosagens de fertilizante de liberação controlada na germinação e crescimento inicial de mudas de *Cedrela fissilis*. Foi utilizado substrato comercial e nele foi adicionado o fertilizante de liberação controlada (15-09-12) nas doses de 0, 3, 6, 9 e 12 g L^{-1}, constituindo os tratamentos do experimento. O experimento foi instalado em delineamento inteiramente casualizado, sendo que cada tratamento foi constituído de cinco repetições de nove tubetes de 180 cm^3. Estes foram dispostos em bandejas, as quais ficaram sob as mesmas condições de temperatura e irrigação. Após 120 dias da semeadura avaliou-se o número de folhas, diâmetro do coleto (mm) e altura (cm). O diâmetro do coleto foi medido com auxílio de um paquímetro digital e a altura com uma régua graduada de 30cm. Recomenda-se o uso de 6 g L^{-1} de fertilizante de liberação controlada na produção de mudas de *Cedrela fissilis* em função dos melhores resultados obtidos para o crescimento e em razões econômicas.

Palavras-chave: Cedro. Qualidade de mudas. Parâmetros morfológicos.

ABSTRACT

The objective of the experiment was to evaluate the effects of different dosages of controlled release fertilizer on the germination and initial growth of *Cedrela fissilis* seedlings. A commercial substrate was used and controlled release fertilizer (15-09-12) was added to it at doses of 0, 3, 6, 9 and 12 g L^{-1}, constituting the treatments of the experiment. The experiment was set up in a completely randomized design, with each treatment consisting of five replicates of nine tubes of 180 cm^3. These were arranged in

trays, which were kept under the same conditions of temperature and irrigation. After 120 days of sowing, the number of leaves, stem diameter (mm) and height (cm) were evaluated. The collar diameter was measured with the aid of a digital caliper and the height with a 30cm graduated ruler. It is recommended the use of 6 g L^{-1} of controlled release fertilizer in the production of *Cedrela fissilis* seedlings due to better results obtained for growth and economic reasons.

Keywords: Cedar. Seedling quality. Morphological parameters.

1 INTRODUÇÃO

Cedro (*Cedrela fissilis*) é uma espécie florestal de sucessão secundária (inicial ou tardia) e ocorre em diversas fitofisionomias brasileiras, com germinação normalmente superior a 80%. Pertence à família botânica Meliaceae e sua madeira é deveras apreciada, pois é muito semelhante visualmente a madeira do mogno, além de ser leve, o que possibilita diversos usos (ANGELI, 2005). Normalmente o cedro ocorre em solos profundos de textura argilosa e bem drenados, mas é tolerante a outras condições incluindo solos contaminados por metais pesados (MARQUES *et al*., 2000); porém a produção de mudas em viveiros busca indivíduos saudáveis e produtivos, e utiliza-se de condutas de manejo para que obter os resultados esperados, e dentre os principais manejos conduzidos em viveiros está a adubação do substrato.

Levando-se em consideração que o cedro se comporta como uma espécie secundária inicial ou secundária tardia, a demanda por nutrientes para uma maior taxa de crescimento inicial é alta. A utilização da fertilização em viveiros de produção de mudas é essencial para que cresçam rapidamente com características vigorosas, resistentes, rústicas e, principalmente, bem nutridas. Somente desta maneira estas mudas resistirão às mais variadas condições após o plantio (GONÇALVES; BENEDETTI, 2005). Nas últimas décadas os fertilizantes de liberação lenta tornaram-se uma opção para a produção de mudas de alto rendimento, pois em consequência de o substrato ser inerte (não fornece nutrientes à planta) a adubação é fundamental para garantir a sobrevivência das mudas (MACEDO, 1993), por conseguinte diversos fertilizantes (químicos e orgânicos) foram introduzidos ao mercado.

A partir disto, o objetivo do experimento foi avaliar os efeitos de diferentes dosagens de fertilizante de liberação controlada na germinação e crescimento inicial de

mudas de *Cedrela fissilis*.

2 METODOLOGIA

O experimento foi conduzido no viveiro florestal da Universidade Estadual de Santa Catarina (UDESC). A duração do experimento foi de quatro meses (março a junho), ocorrendo integralmente durante o outono com temperatura média de 15,58 ºC e precipitação média de 105,25 mm. O delineamento utilizado foi o inteiramente casualizado, sendo que cada tratamento teve cinco repetições, cada uma contando com nove tubetes de 180 cm^3. Estes foram dispostos em bandejas, as quais ficaram sob as mesmas condições de temperatura e irrigação.

Os tubetes foram preenchidos com substrato comercial, que segundo o fabricante, é composto por turfa, vermiculita expandida, casca de pinus e carvão vegetal. Apresentando as seguintes características: pH = 6,0 (± 0,5); condutividade elétrica= 0,7 (± 0,3) mS cm-1; densidade= 500 kg m^{-3}; capacidade de retenção de água – CRA (p/p) = 150 % e umidade máxima (p/p) = 50 %. Ao substrato foi adicionado o fertilizante de liberação controlada (FLC) (15-09-12 com 5 a 6 meses de liberação) nas seguintes doses: 0, 3, 6, 9 e 12 g L^{-1}, constituindo os tratamentos do experimento.

Para a semeadura, cada tubete recebeu duas sementes (com características viáveis) de uma única matriz, para minimizar variação genética. Em seguida uma camada de vermiculita expandida de aproximadamente 1 mm de espessura foi aplicada sobre as sementes. A germinação foi avaliada visualmente na quarta semana após a semeadura e na sexta semana foi executada a repicagem, ficando cada tubete com apenas uma plântula. Aos 120 dias após a semeadura foram avaliadas as variáveis número de folhas, diâmetro do coleto (mm) e altura do caule até primeira ramificação (cm). O diâmetro do coleto foi medido com auxílio de um paquímetro digital e a altura com uma régua graduada de 30cm.

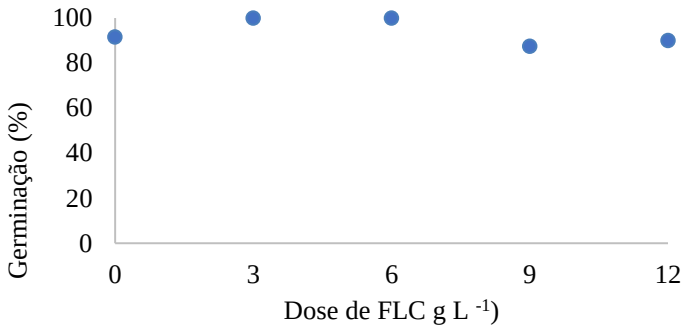
As variâncias dos tratamentos foram testadas quanto à homogeneidade pelo teste de Bartlett. Quando houve homogeneidade das variâncias os dados foram submetidos à análise de variância e quando houve diferença significativa pelo teste de F, foi realizada a regressão polinomial ao nível de 5% de probabilidade. A dose de máxima eficiência técnica (DMET) do FLC, referente a cada característica avaliada foi determinada a partir do cálculo das derivadas parciais das equações ajustadas pela análise de regressão. A

análise dos dados foi realizada no pacote estatístico SISVAR (FERREIRA, 2011).

3 RESULTADOS

Para a germinação (%) não houve diferença estatística entre os tratamentos testados. A taxa de germinação geral do experimento foi de 93,83%. As médias de cada tratamentos são demostradas na Figura 1.

Figura 1- Germinação de *Cedrela fissilis* em função dos tratamentos de fertilizante de liberação controlada.



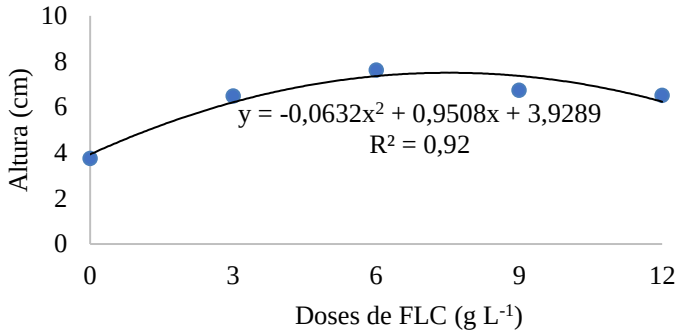
Fonte: próprios autores.

A altura das mudas de *Cedrela fissilis* apresentou variação entre os tratamentos (Figura 2). A presença do fertilizante de liberação controlada influenciou positivamente o crescimento das mudas, sendo a média de todas as dosagens superiores ao controle. A dose de 6 g L^{-1} apresentou a melhor resposta para o crescimento em altura. A dose de máxima eficiência técnica observada foi de 7,5 g L^{-1}. Doses maiores provocaram uma leve diminuição da altura, mostrando ser desnecessário doses superiores a 6 g L^{-1}.

Na relação do diâmetro do coleto (mm), houve variação entre as doses de FLC, sendo que a maior média foi obtida com 3 g L^{-1} (Figura 3). A dose 0 g L^{-1} apresentou a menor média, com diâmetro pouco superior a 2 mm. As demais doses testadas apresentaram médias semelhantes a dosagem de 3 g L^{-1}. A dose de máxima eficiência técnica obtida foi de 7,4 g L^{-1}.

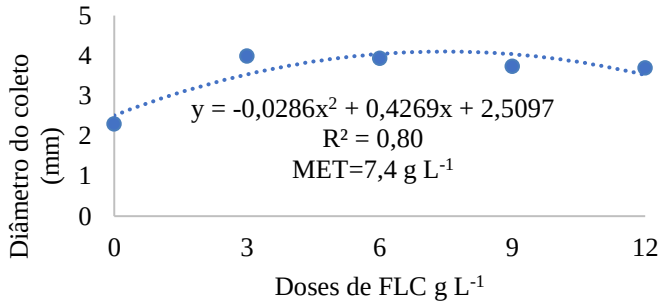
O número de folhas também apresentou variação entre as doses do fertilizante de liberação controlada. O maior número de folhas foi obtido entre as doses de 6 e 9 g L^{-1}, sendo que a máxima eficiência técnica ficou em 7,7 g L^{-1}.

Figura 2- Altura (cm) de mudas de *Cedrela fissilis* em função dos tratamentos de fertilizante de liberação controlada.



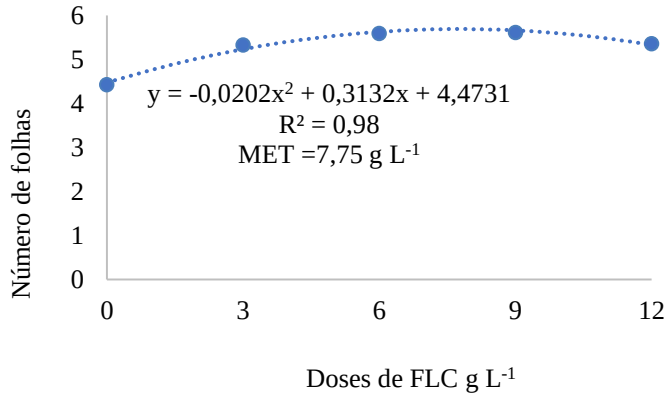
Fonte: próprios autores.

Figura 3- Diâmetro do coleto (mm) de mudas de *Cedrela fissilis* em função dos tratamentos de fertilizante de liberação controlada.



Fonte: próprios autores.

Figura 4- Número de folhas de mudas de *Cedrela fissilis* em função dos tratamentos de fertilizante de liberação controlada.



Fonte: próprios autores.

4 DISCUSSÃO

As taxas de germinação ficaram no intervalo esperado para sementes de cedro, que segundo Angeli (2005) é superior a 80%. Em geral observou-se melhores respostas de crescimento em altura, diâmetro do coleto e número de folhas para doses

intermediárias, principalmente 6 g L^{-1}. Para a maioria das variáveis a dose de máxima eficiência técnica ficou próxima a 7 g L-1, contudo, esse valor é estimado pelo ajuste da equação, podendo representar certa incoerência. Assim, analisando-se as médias e implicações econômicas, pode-se recomendar a dose de 6 g L^{-1} para produção de mudas de cedro.

Esse resultado é corroborado por outros trabalhos. Navroski *et al*. (2016) estudando a aplicação de fertilizante de liberação controlada em mudas de cedro também encontraram que doses entre 5 e 6 g L^{-1} apresentaram os melhores resultados. Estudando doses de fertilizante de liberação controlada na produção de mudas de grápia, Pias *et al*. (2013), observou que doses próximas a 6 g L^{-1} também apresentaram melhor crescimento das mudas.

A adubação de base com o uso de adubos de liberação controlada é amplamente utilizada em viveiros de produção de mudas de espécies florestais. Brondani *et al*. (2008) estudando fertilização de liberação controlada no crescimento inicial de angico-branco obteve uma DMET de 2,7 g dm-3 de fertilizante correspondendo uma altura de 17,2 cm e um acréscimo de aproximadamente 16 % em relação a testemunha (substrato sem adição de Osmocote ®).

Para espécies com crescimento mais lento, como é o caso do cedro, muitas vezes são necessárias doses mais elevadas de adubação para o suprimento nutricional. A volatilização e a lixiviação dos nutrientes podem representar uma diminuição do crescimento das mudas com o passar do tempo, dessa forma, a utilização de doses adequadas de FLC são essenciais para geração de mudas de boa qualidade. Essa característica de liberação controlada pode garantir a manutenção de um sincronismo entre liberação de nutrientes ao longo do tempo, e as necessidades nutricionais, favorecendo um crescimento adequado para as plantas (CASARIN; STIPP, 2013).

5 CONCLUSÃO

Recomenda-se o uso de 6 g L-1 de fertilizante de liberação controlada na produção de mudas de *Cedrela fissilis* em função dos melhores resultados obtidos para o crescimento e em razões econômicas.

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rLAS® v.5, n.3 Especial (2023)
EIXO III – Ambiente, Sustentabilidades e Implicações na Saúde
ISSN: 2526-219X

Estabelecimento e multiplicação *in vitro* de clones de *Eucalyptus dunnii* e *Eucalyptus benthamii* usando diodos de emissão de luz (LEDs)

Establishment and multiplication in vitro of clones of Eucalyptus dunnii and Eucalyptus benthamii using light-emitting diodes (LEDs)

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RESUMO

A cultura de tecidos tem se apresentado como uma das principais técnicas de propagação vegetativa, tendo importância as medidas de controle e prevenção de contaminação. O uso dos díodos emissores de luz (LEDs), e diferentes colorações dos mesmos, pode se destacar como auxílio para o estabelecimento e multiplicação de explantes desinfestados. O presente capítulo teve como objetivos específicos a desinfestação de explantes de diferentes clones de *Eucalyptus dunnii e Eucalyptus benthamii* submetidos a diferentes tempos de imersão e concentrações de hipoclorito de sódio (NaOCl), variando entre 0,5 a 1,5% e 10 a 20 minutos respectivamente. E também o estabelecimento dos explantes visando sua multiplicação *in vitro*, utilizando LEDs de coloração azul, vermelha e mista com ambas. O uso de NaOCl a concentração de 1,5% com imersão por 10 minutos se mostrou o método mais eficaz de desinfestação do material. Quanto ao estabelecimento e multiplicação dos explantes, o uso de LEDs de luz azul acarretou em maior taxa de brotação durante o estabelecimento e maior aparecimento de calos (não houve brotação nesta fase), dutante a multiplicação.

Palavras-chave: Micropropagação. Eucalipto. Desinfestação.

ABSTRACT

Tissue culture has been presented as one of the main techniques in vegetative propagation, highlighting the importance of measures in controlling and preventing contamination. The use of light-emitting diodes (LEDs), and certain colors, can be highlighted as an aid for the establishment and multiplication of disinfested explants. The specific objectives of the present chapter had were the disinfestation of explants belonging to different clones

of *Eucalyptus dunnii* and *Eucalyptus benthamii* submitted to different immersion times and concentrations of sodium hypochlorite (NaOCl), ranging from 0.5 to 1.5% and 10 to 20 minutes respectively. Also the establishment of explants aiming fot their multiplication *in vitro*, using LEDs of blue, red and mixed coloration. The use of NaOCl at a concentration of 1.5% with immersion for 10 minutes proved to be the most effective method of disinfesting the material. As for the establishment and multiplication of explants, the use of blue light LEDs resulted in a higher rate of sprouting during establishment and a greater appearance of calluses (no sprouting at this stage) during multiplication.

Keywords: Micropropagation. Eucalyptus. Disinfestation.

1 INTRODUÇÃO

A micropropagação é um método cada vez mais utilizado para produção de plantas em larga escala, possibilitando a propagação de espécies difíceis de clonar através de técnicas de propagação mais convencionais, permitindo também entre outras vantagens, assegurar o estado fitossanitário dos propágulos mantidos em condições assépticas. (CANHOTO, 2010)

A micropropagação é uma das principais aplicações de cultura de tecidos vegetais, definida como uma técnica de propagação vegetativa utilizando pequenos propágulos (TRUEMAN *et al*., 2018). Esta técnica visa clonar espécies ou híbridos com altas taxas de crescimento, tolerância a baixas temperaturas e salinidade, e resistência a pragas e doenças (WENDLING *et al*., 2014). A possibilidade de obter muitas microestacas clonais em um curto espaço de tempo e em área reduzida, levou a um aumento da uso comercial da micropropagação (HARTMANN *et al*. 2011).

Através do presente trabalho, se objetivou avaliar o efeito de diferentes tempos de imersão e concentrações de hipoclorito de sódio (NaOCl), na desinfestação de explantes de clones de *Eucalyptus benthamii e Eucalyptus dunnii*, avaliar o estabelecimento e multiplicação in vitro de explantes vindos dos clones, utilizando LEDs de coloração azul, vermelha e mista.

3 METODOLOGIA

Experimento 1 – Clones e desinfestação com NaClO no estabelecimento in vitro

Após a aplicação dos tratamentos de desinfestação, os explantes foram lavados três vezes com água estéril. Os frascos identificados e levados a sala de crescimento usando somente luzes brancas do tipo LED.

Trinta dias após a inoculação dos explantes foram avaliadas a percentagem de sobrevivência dos explantes, contaminação e oxidação, determinadas mediante avaliação visual da presença de fungos e/ou manchas de oxidação no meio de cultura e/ou no explante.

Experimento 2 – Clones e luzes LED no estabelecimento in vitro

Após a inoculação dos explantes, os frascos foram mantidos em sala de cultivo com temperatura de 23ºC ±2 ºC, fotoperíodo de 16 horas e intensidade luminosa de 20 µmol m^{-2} s^{-1}fornecida por lâmpadas fluorescentes de cores branca, azul, vermelha e mista de azul e vermelha.

Trinta dias após a inoculação dos explantes foi realizada a avaliação do estabelecimento *in vitro* (aparecimento de brotações em cada explante) da oxidação fenólica (escurecimento dos explantes) e da contaminação fúngica (aparecimento de bolor e estruturas fúngicas sobre o meio ou explantes), registrados por meio de percentagem.

Experimento 3 – Clones e luzes LED na multiplicação in vitro

Para a fase de multiplicação in vitro, foram utilizadas brotações provenientes de explantes de clones de *E. benthamii* e *E. dunnii* previamente estabelecidos *in vitro*.

Após a inoculação dos explantes, os frascos foram mantidos em sala de cultivo com temperatura de 23ºC ±2 ºC, fotoperíodo de 16 horas e intensidade luminosa de 20 µmol m^{-2} s^{-1}fornecida por lâmpadas fluorescentes de cores branca, azul, vermelha e mista de azul e vermelha.

Trinta dias após a inoculação dos explantes foi avaliada a quantidade de brotações indicando multiplicação, porém, como não houve nenhum sinal de brotação, foi avaliado então, em percentagem, a ocorrência de calos nos explantes.

Análise estatística

Após testar a normalidade dos dados por meio do teste de Kolmogorov-Smirnov os dados foram submetidos à análise de variância. Quando o valor de “F” foi significativo,

dados de tratamentos qualitativos tiveram as médias comparadas por meio do teste de Tukey ao nível de 5% de probabilidade de erro. O programa estatístico SISVAR foi utilizado para a análise estatística dos dados.

3 RESULTADOS E DISCUSSÃO

Experimento 1 – Clones e desinfestação com NaClO no estabelecimento in vitro

Não foi observada interação significativa entre os fatores clones e NaOCl na desinfestação in vitro. Porém, obteve-se efeito significativo para o fator NaOCL (concentração e tempo de imersão) (Tabela 1). Foi observado que o tratamento utilizando 1,5% de NaOCl e 10 minutos de imersão, resultou em maior sobrevivência, com 68% dos explantes sobrevivendo ao processo. Os demais tratamentos envolvendo uso de hipoclorito não diferenciaram entre si, e a testemunha, resultou em menor média (nenhum explante sobrevivendo).

Tabela 1- Sobrevivência (%), contaminação (%) e oxidação fenólica (%) em diferentes clones e desinfestação com NaClO no estabelecimento in vitro de *Eucalyptus benthamii* e *Eucalyptus dunnii.*

Tratamento	% de sobrevivência	% de contaminação	% de oxidação
Testemunha	0% c*	100% d	0% d
0,5% - 20 min	31% b	63% c	5% ab
1% - 10 min	35% b	56% bc	9% ab
1% - 20 min	34% b	46% abc	20% bc
1,5% - 10 min	68% a	26% a	7% ab
1,5% - 20 min	32% b	34% ab	33% c

*Tratamentos com letras iguais não diferem entre si pelo teste de Tukey a 5% de erro.

Para contaminação fúngica (Tabela 1), também foi observada diferença significativa entre os tratamentos com influência do NaOCl, mas não houve efeito entre os clones. O tratamento com 1,5% de NaOCl e 10 min de imersão apresentou menor percentual de contaminação (26%), porém não diferenciou estatisticamente do tratamento com 1,5% e 20 min de imersão e tratamento com 1% e 20 min de imersão. O tratamento testemunha demonstrou maior percentagem de contaminação, sendo que todos seus explantes acabaram sendo contaminados.

Experimento 2 – Clones e luzes LED no estabelecimento in vitro

Não houve interação significativa entre os fatores testados nestes experimentos.

Em nenhuma das três variáveis avaliadas houve variação significativa entre os clones, somente entre os diferentes espectros de luz utilizados. Em relação ao surgimento de brotos nos explantes (Tabela 2), houve efeito significativo entre as diferentes colorações de LEDs, mas não entre os diferentes clones. Os explantes submetidos a luz azul, em geral obtiveram maior percentagem de brotação (64%). As demais cores, e testemunha de luz branca não diferenciaram estatisticamente entre si.

Tabela 2- Sobrevivência (%), contaminação (%) e oxidação fenólica (%) em diferentes clones e colorações de LEDs no estabelecimento in vitro de *Eucalyptus benthamii* e *Eucalyptus dunnii.*

Tratamento	% de brotação	% de contaminação	% de oxidação
Testemunha (Luz Branca)	26% b*	15% ab	59% b
Luz Azul	64% a	11% a	26% a
Luz Vermelha	34% b	21% ab	46% b
Luz Mista	31% b	25% b	45% b

*Tratamentos com letras iguais não diferem entre si de acordo com Tukey a 95% de confiança.

Em termos numéricos, se nota benefício no uso de todas as LEDs coloridas em relação a luz branca da testemunha, sendo que a taxa de brotação foi elevada em todos os casos, porém, o uso de LED azul é recomendado, em comparação ao vermelho ou mesmo ao misto das duas colorações. A taxa de brotação dobrou sob essa coloração quando comparada as outras duas.

A luz azul também foi superior nas demais variaveis analisadas, sendo que suas taxas de contaminação fungica e oxidação ficaram abaixo dos demais tratamentos avaliados, o fato de ser mais próxima da luz verde no espectro de luzes pode ter ajudado.

A luz azul realiza uma série de funções morfogenéticas, além de regular processos metabólicos, influenciar a produção e o crescimento vegetal, influenciar positivamente o desenvolvimento de cloroplastos e a biossíntese de moléculas de clorofila (TAIZ; ZEIGER, 2013; LAZZARINI *et al*., 2017).

Experimento 3 – Clones e luzes LED na multiplicação in vitro

Não houve resposta morfológica neste experimento para a multiplicação, não sendo observado nenhuma emissão de novas brotações. Para a ocorrência de calos, não houve diferença significativa entre os fatores de estudos (Tabela 3). A formação de calos variou entre 13% na luz mista (vermelha/azul) e 25% na luz azul.

Tabela 3- Aparecimento de calos (%) em diferentes clones e colorações de LEDs no estabelecimento in vitro de *Eucalyptus benthamii* e *Eucalyptus dunnii*.

Tratamento	Aparecimento de calos
Testemunha (Luz Branca)	19% a*
Luz Azul	25% a
Luz Vermelha	19%% a
Luz Mista	13% a

*Tratamentos com letras iguais não diferem entre si de acordo com Tukey a 95% de confiança.

4 CONCLUSÃO

O efeito dos LEDs no estabelecimento dos explantes pode ser considerado significativo, principalmente do espectro de coloração azul, que resultou em maior taxa de brotação.

Não houve sucesso na fase de multiplicação como se esperava, apenas surgimento de calos em certos explantes, sem particular diferença entre as luzes utilizadas.

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rLAS® v.5, n.3 Especial (2023)
EIXO III – Ambiente, Sustentabilidades e Implicações na Saúde
ISSN: 2526-219X

Influência da deposição de acículas de *Pinus taeda* sobre a germinação e qualidade de mudas de *Mimosa scabrella* Benth.

Influence of the deposition of Pinus taeda necklace on the germination and quality of seedlings of Mimosa scabrella Benth.

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RESUMO

Este trabalho teve por objetivo analisar os efeitos causados pela deposição de acículas de *Pinus taeda* na germinação e qualidade de mudas de *Mimosa scabrella*, espécie nativa pioneira, com alta importância ecológica e potencial econômico para a região Sul do país. O experimento foi conduzido em casa de vegetação com condições controladas, utilizando-se acículas em dois estados, verdes e secas e com diferentes alturas de deposição das mesmas sobre as sementes, variando de 2,5 a 7,5 cm de altura. Foram avaliadas as variáveis germinação (G) e índice de velocidade de germinação (IVG), além das variáveis biométricas altura, diâmetro de coleto e quantidade de folhas, relacionados a qualidade final das mudas. Em todos os tratamentos houve germinação, sendo que os tratamentos T1 e T3 obtiveram maior valor de G médio, com 88%, seguido por T4, T2, T5, T7 e T6, com 86, 74, 56, 28 e 12%, respectivamente. O T1, testemunha, apresentou maior valor médio para a variável IVG, com 4,6477, seguido por T2, T3, T4, T5, T7 e T6.

Palavras-chave: Bracatinga. Alelopatia. Invasão biológica.

ABSTRACT

The objective of this work was to analyze the effects caused by the deposition of Pinus taeda needles on the germination and quality of seedlings of Mimosa scabrella, a pioneer native species, with high ecological importance and economic potential for the southern region of the country. The experiment was conducted in a greenhouse under controlled conditions, using needles in two states, green and dry, with different deposition heights on the seeds, ranging from 2.5 to 7.5 cm in height. The variables germination (G) and

germination speed index (IVG) were evaluated, in addition to the biometric variables height, stem diameter and number of leaves, related to the final quality of the seedlings. In all treatments there was germination, with treatments T1 and T3 having the highest average G value, with 88%, followed by T4, T2, T5, T7 and T6, with 86, 74, 56, 28 and 12%, respectively. T1, the witness, had the highest mean value for the variable IVG, with 4.6477, followed by T2, T3, T4, T5, T7 and T6.

Keywords: Bracatinga. Allelopathy. Biological invasion.

1 INTRODUÇÃO

No Brasil em 2019, a área de árvores plantadas totalizou 9 milhões de hectares, um aumento de 2,4% em relação a 2018. Desse total, 18% são do gênero *Pinus*, com 1,64 milhão de hectares que são oriundos principalmente da região Sul (IBÁ, 2020).

Espécies de *Pinus* são plantadas em todo o mundo e apresentam valorização comercial crescente, porém, podem produzir substâncias alelopáticas, que em determinadas condições inibem o desenvolvimento de algumas espécies sensíveis de plantas (LOPES *et al*., 2013).

Contaminação ou invasão biológica é o processo de introdução e adaptação de espécies que não fazem parte naturalmente de um dado ecossistema, mas que se naturalizam e passam a provocar mudanças em seu funcionamento (ZILLER, 2000). Muitos são os estudos acerca de invasão biológica no mundo, porém, no Brasil, estas informações não acompanham a expansão das áreas de plantio do gênero *Pinus* no país.

Diante do exposto, considerando a importância do conhecimento do potencial invasor do gênero *Pinus* a e possível influência alelopática das suas acículas na dinâmica do banco de sementes em Floresta Ombrófila Mista, além da existência de uma possível competição por área com a espécie *Mimosa scabrella* que tem alta importância ecológica na tipologia vegetal em que esta encontra-se inserida, o presente trabalho teve por objetivo avaliar a interação entre as espécies, em busca de definir se há influência da deposição das acículas de *Pinus taeda* sob a germinação e crescimento inicial da bracatinga.

2 METODOLOGIA

O presente trabalho foi desenvolvido em casa de vegetação no viveiro florestal do

campus de ciências agroflorestais (CAV) da Universidade do Estado de Santa Catarina (UDESC), no município de Lages durante os meses de dezembro de 2021 e fevereiro de 2022. Lages encontra-se no Planalto Sul Catarinense, o clima da região é *Cfb*, classificação de Koeppen, com temperatura média anual variando de 15 a 16°C e precipitação média anual variando de 1300 a 1500 mm (EPAGRI, 2003).

As sementes utilizadas no experimento foram coletadas dentro de área de produção da EPAGRI no município de Lages e encontravam-se armazenadas sob refrigeração no laboratório de propagação e melhoramento de espécies florestais do CAV - UDESC.

A espécie *Mimosa scabrella* Benth. possui dormência do tipo física, na qual a semente da mesma não permite entrada de água através de seu tegumento, portanto, aplica-se técnica adequada para quebra de dormência das sementes. Para quebra de dormência utilizou-se a técnica de imersão das sementes em água quente, elas foram imersas em água a 80 graus celsius e mantidas resfriando por 8 horas ainda imersas antes de dar início ao processo de semeadura.

As acículas foram obtidas de um plantio experimental de *Pinus taeda* com cinco anos de idade, localizado no centro de Ciências Agroveterinárias da UDESC. Foram coletadas acículas verdes de galhos recém podados e acículas já secas depositadas sob o solo.

O experimento foi instalado no dia 11 de dezembro e a semeadura foi conduzida em bandejas, utilizando-se para cada bandeja, 6 litros de substrato comercial, além de fertilizante do tipo de liberação controlada (FLC), na concentração de $4g.L^{-1}$. Foram alocadas 50 sementes em cada bandeja e após isso, cada bandeja recebeu o respectivo tratamento de cobertura, sendo uma bandeja testemunha, onde as sementes foram cobertas com uma fina camada de substrato e as demais cobertas com as acículas, sendo três bandejas com acículas secas e três com acículas verdes, com a altura desta camada de cobertura variando de 2,5 - 7,5 cm, conforme ordenamento dos tratamentos: T1 – Testemunha, sem presença de acículas; T2 - Camada de 2,5 cm de acículas verdes; T3 - Camada de 5 cm de acículas verdes; T4 - Camada de 7,5 cm de acículas verdes; T5 – Camada de 2,5 cm de acículas secas; T6 - Camada de 5 cm de acículas secas, T7 - Camada de 7,5 cm de acículas secas.

O delineamento experimental utilizado foi o inteiramente casualizado, com 7

tratamentos, totalizando 350 sementes analisadas. Foram avaliados inicialmente dois parâmetros, a germinação (G) e o índice de velocidade de germinação (IVG) para cada tratamento no período de 30 dias. Os dados obtidos foram tabulados e analisados através do *software* Microsoft Excel®. Para realização dos cálculos de germinação e velocidade de germinação foram utilizadas as seguintes fórmulas respectivamente (MAGUIRE, 1962):

Germinação (G) calculada pela fórmula

$$G = (N/100) \times 100$$

onde:

N = número de sementes germinadas ao final do teste.

Índice de velocidade de germinação (IVG) calculado pela fórmula

$$IVG = \sum (ni /ti)$$

onde:

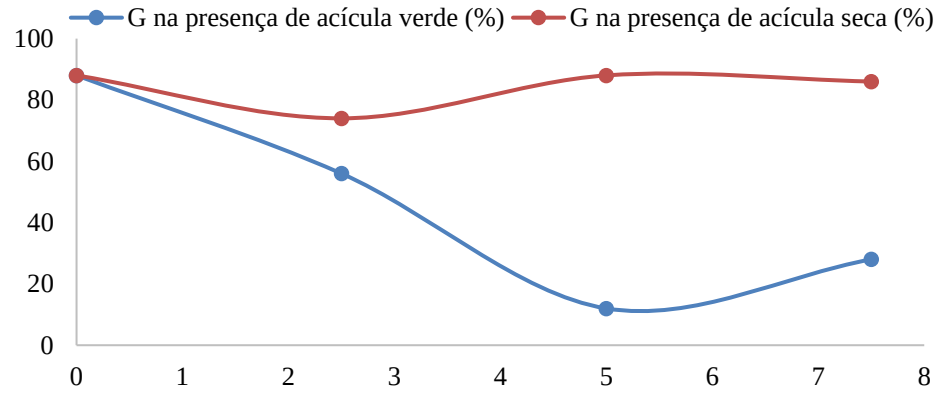
ni = número de sementes que germinaram no tempo ‘i’;

ti = tempo após instalação do teste.

3 RESULTADOS

Após 30 dias da instalação do experimento, foi possível observar que houve germinação das sementes em todos os tratamentos analisados, tendo esta iniciado ao 6º dia e cessado ao 18° dia após semeadura. A germinação média mínima observada foi de 12% para o tratamento com camada de 5 cm de acículas verdes (T6), enquanto a máxima foi de 88% para o tratamento testemunha, sem deposição de acículas (T1) e tratamento com camada de 5 cm de acículas secas (T3) (Figura 1).

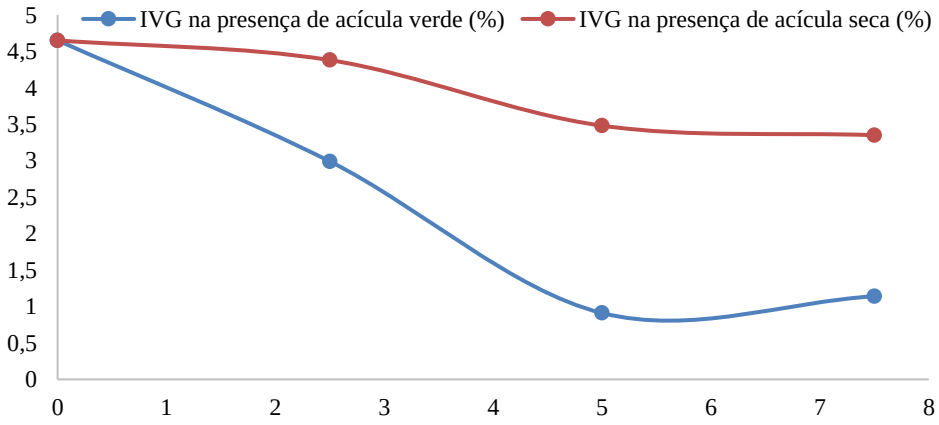
Figura 1 - Relação entre a germinação das sementes de *Mimosa Scabrella* e altura da camada de acículas, secas e verdes.



Fonte: Autores.

As médias encontradas para índice de velocidade de germinação (IVG) (Figura 2) também foram superiores para os tratamentos que utilizaram acículas secas ou não utilizaram acículas, sendo a maior média do tratamento T1, seguido por T2, T3 e T4, com 4,65, 4,38, 3,48 e 3,35 respectivamente.

Figura 2. Relação entre o índice de velocidade de germinação das sementes de *Mimosa Scabrella* e altura da camada de acículas, secas e verdes.



Fonte: próprios autores.

4 DISCUSSÃO

Wielewicki *et al*. (2006), propõe como padrão de germinação para a espécie Mimosa scabrella, 71%, baseando-se neste padrão, tanto o tratamento testemunha, T1, quanto os tratamentos que utilizaram acículas secas, T2, T3 e T4 encontram-se acima do padrão proposto, enquanto os tratamentos com presença de acículas verdes, T5, T6 e T7

encontram-se todos abaixo do padrão esperado de germinação.

Pereira (2015) ressalta os efeitos inibitórios de extratos obtidos de acículas verdes de *Pinus elliottii* na germinação, velocidade de germinação e crescimento inicial de plântulas de alface, tal característica auxilia o entendimento de sua alta capacidade invasora e a menor regeneração de plantas nativas no sub-bosque de talhões de *P. elliottii* no sul do Brasil.

A redução sútil e uniforme observada na curva que relaciona altura da deposição de acículas secas com o IVG pode ser explicada pelo tempo que as plântulas demoram para ultrapassar a barreira física formada pelas acículas e serem então contabilizadas, não sendo necessariamente associada a uma relação direta entre as acículas neste estado com a velocidade de germinação da espécie bracatinga.

5 CONCLUSÃO

É possível inferir, através deste experimento, que as acículas verdes de *Pinus taeda* possuem efeito alelopático e afetam diretamente a germinação e velocidade de germinação das sementes de *Mimosa scabrella*, principalmente se presentes em grande quantidade, como visto nos tratamentos com deposição superior a 2,5 cm, enquanto as acículas no estado seco não têm tamanha influência.

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Ciência Rural, Santa Maria, v.53:11, e20220365, 2023
http://doi.org/10.1590/0103-8478cr20220365
ISSNe 1678-4596
FORESTRY SCIENCE

Light-emitting diodes (LEDs) in development and response to water stress in *Eucalyptus benthamii* seedlings (Myrtaceae)

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ABSTRACT: Plants use light as a source of energy in the process of photosynthesis. Different levels of luminosity cause physiological and morphological changes in the plant, and its success depends on its adaptation to these different levels. Light emitting diodes (LED) have been proposed as a light source in controlled environments. The present research evaluated physiological and morphological aspects in *Eucalyptus benthamii* (Myrtaceae) seedlings kept under different colors of LED lamps and submitted to water stress. The experiment was carried out in a completely randomized design, in which the treatments were defined as: T1 (control, with white light); T2 (blue light); T3 (red light) and T4 (mixed blue and red light). Red light resulted in the best response to most morphological variables in plant growth. In response to water stress, blue light LEDs resulted in a better seedling response, with slower reduction of photosynthetic rate and other variables. This may indicate the possibility of reducing water deficit damage in seedlings acclimated to blue light prior to field planting.
Key words: seedling acclimatization, light supplementation, photosynthesis.

Uso de diodosemissores de luz (LEDs) no desenvolvimento e resposta a estresse hídrico em mudas de *Eucalyptus benthamii* (Myrtaceae)

RESUMO: As plantas utilizam a luz como fonte de energia no processo de fotossíntese. Diferentes níveis de luminosidade causam mudanças fisiológicas e morfológicas na planta, e o seu sucesso depende da sua adaptação a esses diferentes níveis. Diodos de emissão de luz (LED) têm sido propostos como fonte luminosa para ambientes controlados. O objetivo do presente trabalho foi avaliar aspectos fisiológicos e morfológicos em mudas de *Eucalyptus benthamii* (Myrtaceae) mantidas sob diferentes cores de lâmpadas LED e submetidas ao estresse hídrico. Foram realizados dois experimentos, sendo que um avaliou o efeito de diferentes cores de LEDs no crescimento das mudas e outro analisou o efeito das luzes na aclimatação das mudas e após exposição a deficit hídrico. O experiment foi conduzido em delineamento inteiramente casualizado, em que os tratamentos foram definidos como T1 (controle, com luz branca); T2 (luz azul); T3 (luz vermelha) e T4 (luz azul e vermelha mista). A luz vermelha resultou em melhor resposta para a maioria das variáveis morfológicas no crescimento das plantas. Emresposta ao estresse hídrico, os LEDs de luz azul resultaram em uma melhor resposta das mudas, reduzindo mais lentamente a taxa fotossintética e demais variáveis. Isso pode indicar a possibilidade de reduzir os danos de deficit hídrico nas mudas aclimatadas com luz azul anteriormente ao plantio a campo.
Palavras-chave: aclimatação de mudas, suplementação de luz, fotossíntese.

INTRODUCTION

Plants use light as a source of energy in the process of photosynthesis and respond to this light energy according to its intensity, wavelength, and direction in which it is being emitted. Plants perceive light through photoreceptors, such as phytochromes and cryptochromes, and respond to these receptors generating a series of specific physiological responses (MUNEER et al., 2014).

Light-emitting diode (LED) lamps are often used in place of fluorescent lamps, as LED light sources are more efficient, versatile, and economically viable for *in vitro* plant growth and regeneration, offering alternatives to obtain positive results in the field on a commercial scale (MONTEUUIS, 2015).

Different studies indicate better growth of plants under LED lighting conditions (HUNG et al., 2015; FERREIRA et al., 2017; LERIN et al., 2019). However, the effect of light sources on the morphogenesis of the species and clones of interest needs to be evaluated for protocol optimization. The red and blue wavelengths correspond to the absorption peak of chlorophyll a (660 nm) and chlorophyll b (460 nm) (TAIZ & ZEIGER, 2013).

According to DONG et al. (2014) and SAMUOLIENĖ et al. (2013) the great challenge of cultivation with artificial light is to provide controlled

Received 06.27.22 Approved 01.04.23 Returned by the author 03.27.23
CR-2022-0365.R2
Editors: Leandro Souza da Silva Mauricio Hunsche

light intensities in sufficient quantity and quality for the development of plants. Light-emitting diodes (LED) have been proposed as a light source for controlled environments in agricultural facilities or in plant growth chambers. They have desirable characteristics, such as the ability to control the spectral composition, long durability, ability to emit specific wavelengths, relatively cold emission surfaces, in addition to having a reduced size, which facilitates handling and installation in growth chambers (LI et al., 2010; MUNEER et al., 2014).

One of the main factors that contribute to the decrease in seedling development, in addition to causing negative morphological, physiological, and nutritional changes, which influence the seedling's ability to resist adverse environmental conditions; and consequently, its quality is water stress (LISAR et al., 2012). The water stress to which a plant is subjected can be characterized as water deficit, more common, or as water saturation (flooding). Water deficiency stress induces a wide range of physiological and biochemical changes in plants; blocking cell growth and photosynthesis, in addition to changes in respiration, these being the first effects (VELÁZQUEZ & HERNÁNDEZ, 2013).

Taking these points into account, our hypothesis is that plants grown in different light lengths may show greater growth and/or tolerance to physiological and morphological aspects when subjected to water stress. In this way, supplementation with artificial light could be used as a form of pre-acclimatization to the field of seedlings aiming at greater hardening. Thus, the objective of the present research evaluated physiological and morphological aspects in *E. benthamii* seedlings kept under different colors of LED lamps and submitted to water stress.

MATERIALS AND METHODS

Clonal seedlings (a commercial clone) of *E. benthamii* developed and cultivated by a forestry company in the region were used. The clone was selected based on growth, shape and adaptation to the cold climate of the growing region. Clonal seedlings were chosen to avoid genetic variation between plants. The seedlings with approximately 120 days of age, produced at Forest Nursery in the municipality of Otacílio Costa (SC/Brazil) measuring an average of 25 cm in height and 2 mm stem diameter, were kept for 15 days in a shade house (50% shading), with temperature varying between 15 to 28 ºC and 70% of air humidity.

For the experiment testing the growth of plants subjected to different LED lights, the seedlings were transferred to 2L pots, which were filled with commercial substrate based on Pinus bark, with the addition of 6 g L^{-1} of Osmocote® (19-06-10) with 3-4 months of release. The pots were allocated in the growth room (25±2 ºC and 60±10 % relative humidity) being divided into 4 treatments, using 8 replicates of one seedling each.

The experiment was carried out in a completely randomized design, in which the treatments were defined as: T1 (control, with white light); T2 (blue light); T3 (red light) and T4 (mixed blue and red light). The specifications of each lamp were: (i) white lamps - 36", 1W, AC85 - 265 V and (ii) colored lamps - 36", 1W, AC85 - 265 V. Figure 1 shows the installation scheme of the experiments, showing the lights used. Each shelf was sealed with a blackout curtain to prevent light from interfering with another treatment. Three lamps were allocated per bench, three white in the control treatment, and two colored for one white in the colored light treatments.

Biometric evaluations of height and diameter were carried out in two stages, the first being on the day of implementation, and the second at the end of the experimental period, 33 days after implantation. The height of the seedlings was determined with the aid of a millimeter ruler, measuring from the base of the plant to the insertion of the highest leaf. The stem diameter was determined with the aid of a digital caliper. The measurements were carried out in the region of the neck of the seedlings. The data from these evaluations were used to obtain the increment in height and diameter of the neck, as well as the height-diameter ratio (h/d).

At the end of the experimental period, using the destructive method, the total biomass of the plants was manually collected. The samples were divided into root and shoot and placed in paper bags kept in an oven with forced air circulation (60 ± 3 °C) until reaching constant mass (grams) after 96 hours. Afterwards, the samples were weighted to determine the dry matter contents of the components on a precision scale (0.01 g). The Dickson quality index (DQI) was also determined. This was determined as a function of shoot height (H), collar diameter (DC), shoot dry matter weight (PMSPA) and root dry matter weight (PMSR), using the formula (DICKSON et al., 1960).

To evaluate the response of the seedlings to water stress, they were kept in 55 cm^3 tubes and placed in the growth room (25±2 ºC and 60±10 % relative humidity), under different lights. The experiment was carried out in a completely randomized design, in which the treatments were defined as: T1 (control, with white light); T2 (blue light); T3 (red light)

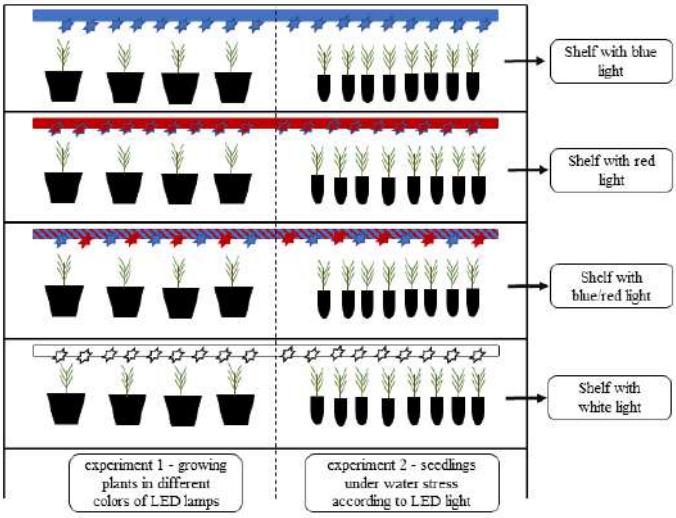


Figure 1 - Scheme of installation of the experiments, showing the LED (light-emitting diodes) lights used.

and T4 (mixed blue and red light), with the same characteristics of the lamps presented above. Fourteen replicates (one plant each) were used per treatment. The evaluation of the experiment was carried out after 14 days of acclimatization of the seedlings, and they were kept in the growth room.

After the initial measurement, irrigation of the plants was suspended for 24 hours to induce stress. During these 24 hours, measurements were taken at previously defined time intervals, initially every two hours, to define the effect of stress on the seedlings during this period. To measure photosynthetic variables, five seedlings were selected for each treatment, which were performed with the portable photosynthesis measurer IRGA (Infra-Red Gas Analyzer), model LI-6400 (LI-COR). Photosynthetic analyzes were performed 24, 30 and 48 hours after stress induction, respecting the randomness between treatments. The photosynthetically active radiation (PAR) used during the measurements was 700 µmol of photons m^{-2} s^{-1}, provided by an artificial light source (LI-6400-40), and the percentage of blue light was 10% of the PAR. The atmospheric concentration of CO_2 ranged from 390 to 400 µmol mol^{-1}.

With the IRGA, the variables of net photosynthetic rate (A - µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (Gs - mol m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}), intercellular concentration of CO_2 in the mesophyll (Ci - µmol CO_2) and current external CO_2 concentration (Ca - µmol CO_2) were measured. These variables later made it possible to calculate the ratios of intercellular concentration of CO_2 in the mesophyll over the current external CO_2 concentration (Ci/Ca- µmol CO_2) and the plant water use efficiency (WUE – [µmol m^{-2} s^{-1}] $^{-1}$). WUE is a measure of the carbon gained by plants through photosynthesis relative to the water lost through transpiration, defined a A/E.

The normality of the data was ascertained with the Shapiro-Wilk test as was the homogeneity of variances with Bartlett's test. They were then submitted to an analysis of variance (ANOVA). And with significant F values ($P < 0.05$), Tukey's range test ($P < 0.05$) was used to find the differences of means. As a complementary analysis, the data were submitted to the elaboration of matrices of variance and covariance to proceed with the analysis of principal components (PCA).

RESULTS

In the evaluation of morphological variables (Table 1), diameter increment at root height (IDAC), height/diameter ratio (h/d) and root dry mass (MSR) showed no significant difference between treatments on the different LED lamps used in plant growth. The other variables showed a significant difference depending on the color of the LED used.

The seedlings under blue light showed a greater increase in height (HI), however, they did not statistically differ from the seedlings under red and

Table 1 - Morphologic variables obtained in the evaluation of *Eucalyptus benthamii* seedlings, according to the different LED lights (light-emitting diodes).

Treatment	----------Variables----------						
	HI(cm)	SDI(mm)	h/d	SDM(g)	RDM(g)	TDM(g)	DQI
Control Group (White Light)	5.48 b*	0.95	13.74	1.60 c	0.76	2.37 c	0.15 b
Blue Light	14.10 a	1.16	14.44	2.80 ab	0.95	3.76 ab	0.22 b
Red Light	10.38 ab	1.46	12.31	3.72 a	1.00	4.73 a	0.30 a
Mixed Light	8.22 ab	0.95	14.1	2.58 bc	0.80	3.38 bc	0.20 b

*Equal letters don't differ between treatments according to Tukey test at 5% probability. Where: HI: Height increment; SDI: Stem diameter increment; h/d: Height/diameter ratio; SDM: Shoot dry mass; RDM: Root dry mass; TDM: Total dry mass; DQI: Dickson quality index.

mixed light. The seedlings under red light showed heavier aerial dry mass (MSA) and total dry mass (MST), not statistically different from the seedlings under blue light in both variables. In Dickson's quality index (DQI), seedlings under red light also showed higher value, statistically different from all other treatments. The significant increase in the height increase of the seedlings under colored LEDs stands out immediately, in the comparison between blue and red light from the control.

Principal component analysis (PCA) showed that the first two principal components account for 78.2% of the total variance. The first component and the second component account for 58.5% and 19.6% of the total variance, respectively. It can also be seen from the PCA that the blue light corresponds to and explains growth variables such as HI, SDM and TDM. Already red light on DQI and SDI (Figure 2).

In the response of seedlings to water stress, a significant difference was observed between treatments in all evaluations carried out during the 48 hours of follow-up after the induction of water stress (Figure 3).

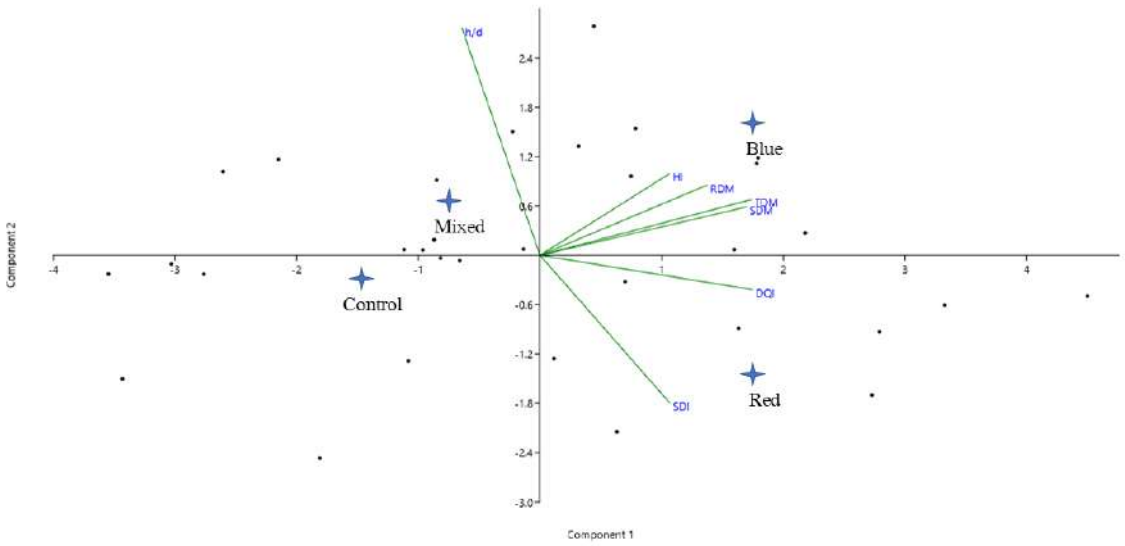


Figure 2 - Principal component analysis (PCA) of the variables analyzed in relation to LED (light-emitting diodes) light treatments on the growth of *Eucalyptus benthamii* seedlings. Where: HI: Height increment; SDI: Stem diameter increment; h/d: Height/diameter ratio; SDM: Shoot dry mass; RDM: Root dry mass; TDM: Total dry mass; DQI: Dickson quality index.

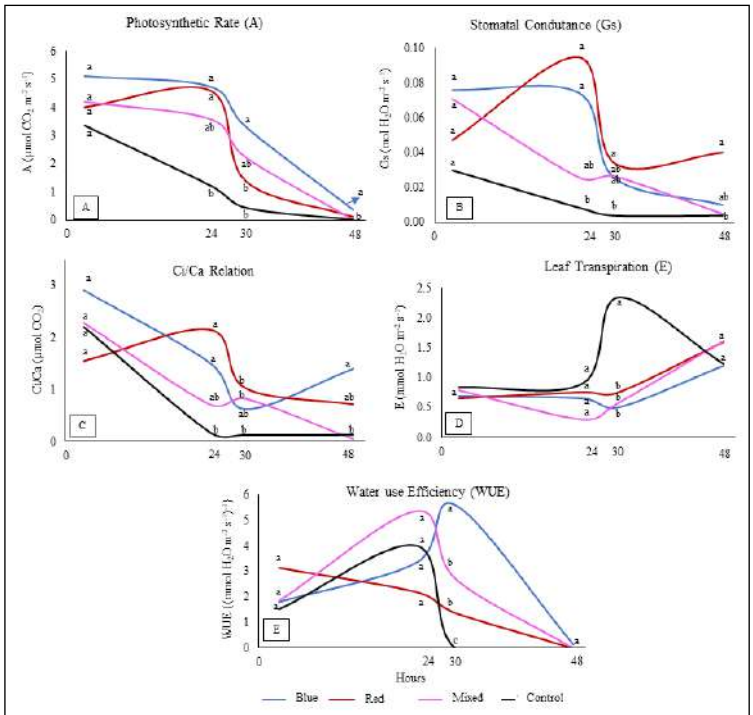


Figure 3 - A) Photosynthetic rate (µmol CO2 m-2 s-1), B) Stomatal conductance (H2O m-2 s-1), C) Ci/Ca ratio - Ci/Ca (Intercellular CO2 concentration/ external CO2 concentration ratio in µmol CO2), D) Leaf transpiration (H2O m-2 s-1), E) Water use efficiency – WUE (mmol H2O m-2 s-1) in *Eucalyptus benthamii* seedlings under water stress according to LED (light-emitting diodes) light during the experiment. Letters correspond to differences between treatment groups by Tukey test.

For photosynthesis (Figure 3a) there was no significant difference between treatments in the evaluation performed before induction. After 24 hours a significant drop in photosynthesis under white light (control), a moderate drop in seedlings under mixed light and a slight reduction in both blue and red light can be seen. After 30 hours, photosynthesis of seedlings under white light approaches zero, and finally hits zero after 48 hours, demonstrating the rapid effect of stress on seedling metabolism. The photosynthesis under mixed light drops after 30 hours, ending after 48 hours, also at zero. Until 30 hours, the drop in photosynthesis under blue light is slight, remaining almost three times higher than the photosynthetic rate of seedlings under red light, which feel greater effect from the same point onwards, both end the experiment with photosynthesis close to zero, but not completely zeroed.

For stomatal conductance (Figure 3b), the drop in seedlings under blue light is lighter, with these being noticeable after 30 hours of drought, while the others show effects after 24 hours for white and mixed light, and 30 hours for red light. The seedlings under white and mixed light ended the experiment with zero conductance.

For external and internal carbon ratio (Ci/Ca) (Figure 3c), in the seedlings under blue light, the drop was slight, being noticeable after 30 hours, and showing recovering slightly after 48 hours of drought. The ratio in seedlings under red light had a slight increase after 24 hours, and a strong drop after 30, also recovering slightly, but falling again after 48 hours, the same behavior was observed in seedlings under mixed light. The ratio in seedlings under white light dropped sharply after 24 hours and remained stable after that.

Transpiration (Figure 3d) remained stable in all treatments up to 24 hours. After 30 hours, the transpiration of seedlings under white light increased sharply and recovered slightly after 48 hours. The transpiration of the other seedlings remained stable until 48 hours of drought, when a significant effect of stress on this variable was observed.

The water use efficiency (WUE) (Figure 3e) of all seedlings remained stable until 24 hours of drought. After 30 hours, only the seedlings under blue light did not show a drop in this variable, and the efficiency of the seedlings under white light had already reached zero at that point. After 48 hours, only the seedlings under blue light had not zeroed in terms of WUE.

Principal component analysis (PCA) showed that the first two principal components account for 87.8% of the total variance (Figure 4). The first component and the second component account for 65.3% and 22.5% of the total variance, respectively. Blue and red light explain most of the variables such as photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (H_2O m^{-2} s^{-1}) and leaf transpiration (H_2O m^{-2} s^{-1}). Blue and red lights (mixed) have a greater influence on the *WUE* variable.

DISCUSSION

We observed that in the growth of *E. benthamii* seedlings, supplementation with blue and red light promoted an improvement in morphological characteristics such as increase in height, dry mass and DQI. Studies indicate that red light promotes leaf and stem elongation via stimulation of gibberellin biosynthesis, through the control of mitosis and cell proliferation (MANIVANNAN et al., 2015).

In the production of raspberry seedlings cultivars Batum and Dorman Red, ROCHA et al. (2013) observed that the fresh mass of the plant grown under the red LEDs presented superior results than the plants grown under the blue and green LEDs. BELLO-BELLO et al. (2016), evaluating the quality of light (fluorescent lamp, white LEDs, red LEDs, blue LEDs, and blue plus red LEDs) in the growth of shoots of *Vanilla planifolia* Andrews, reported that the highest average fresh mass per plant was obtained with the treatment consisting of blue LEDs plus red LEDs.

Plant growth, leaf water potential and crop production are critically limited or even suppressed by water restriction (HOSSAIN et al. 2016). It was reported that the treatment of two *Melissa officinalis* genotypes with LED lights significantly improved tolerance to water stress (AHMADI et al. 2019) probably by maintaining leaf turgor pressure at satisfactory levels and attenuating the effects of stress. Furthermore, promising results indicated that drought tolerance in two lemon balm genotypes was increased by LED pretreatment, since the relative water content in LED-treated plants remained like that of seedlings kept in a greenhouse (AHMADI et al. 2019).

The smaller decrease in photosynthesis of plants conducted under blue light during the water stress process may be related to the physiological process that occurs. Blue light plays a crucial role in

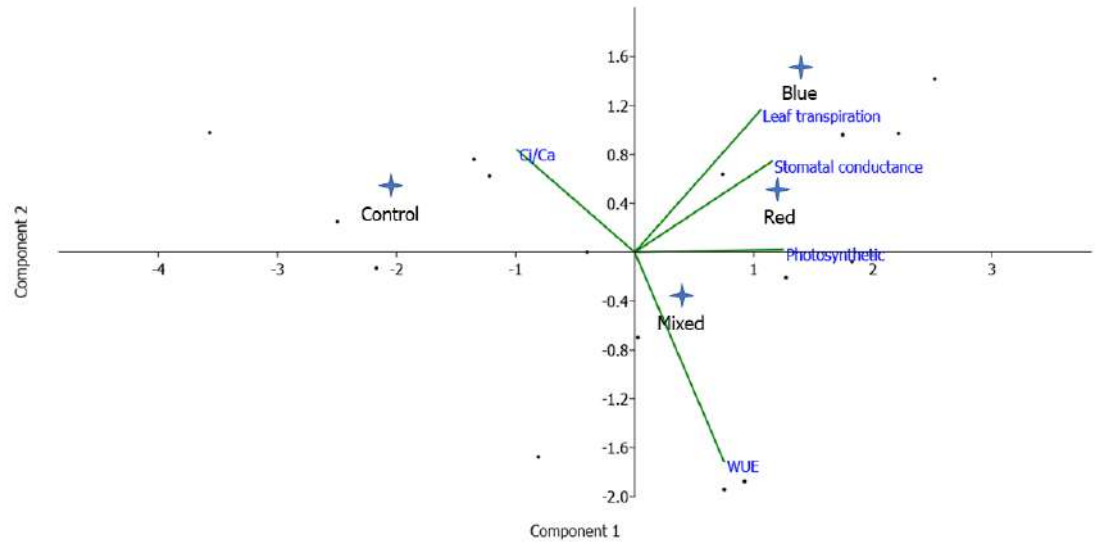


Figure 4 - Principal component analysis (PCA) of the variables analyzed in relation to LED (light-emitting diodes) light treatments under water stress in function to LED light of *Eucalyptus benthamii* seedlings. Where: Photosynthetic rate (μmol CO2 m-2 s-1), Stomatal conductance (H2O m-2 s-1), Ci/Ca ratio - Ci/Ca (Intercellular CO2 concentration/external CO2 concentration ratio in μmol CO2), Leaf transpiration (H2O m-2 s-1), and Water use efficiency – WUE (mmol H2O m-2 s-1).

water relations and gas exchange; and consequently, in growth and tolerance to stress factors. Furthermore, it exerts a positive and coordinated influence on the plant genome and plastid, which influences the development of chloroplasts in plant cells and the synthesis of chlorophyll. The absorption of blue light by chlorophylls promotes a higher energy state than that of red light due to the higher energy existing in blue light (TAIZ & ZEIGER, 2013).

Among the various narrow spectrum lights, blue and red LEDs are mainly used for plant growth because their wavelengths of approximately 460 and 660 nm respectively are highly effective for chlorophyll absorption, which results in optimal photosynthetic efficiency (JOHKAN et al. 2010; GUPTA & JATOTHU, 2013).

The experiment indicated advantages in using colored LEDs regarding the response of seedlings to water stress, and seedlings under white LEDs showed more severe and faster physiological effects. However, the use of blue LEDs can be recommended, and the seedlings of this treatment resisted for longer, and when there were drops, these were lighter.

CONCLUSION

The red light resulted in greater development in most of the evaluated traits, and in the traits in which it did not obtain better results, it did not statistically differ from the seedlings under blue light.

In response to water stress, blue light LEDs resulted in a better response of seedlings, slower reducing photosynthetic rates such as photosynthesis. This may indicate the possibility of treating the seedlings before planting in the field, to reduce possible effects of water deficit.

ACKNOWLEDGEMENTS

The authors thank the Universidade do Estado de Santa Catarina (UDESC), for the institutional support for this research. To FAPESC for funding the research group Management of Planted and Natural Forests (PAP2019031000055) and was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001."

DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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ISSN 1980-5098
ACESSO ABERTO

Ci. Fl., Santa Maria, v. 33, n. 1, e69093, p. 1-25, jan./mar. 2023 • https://doi.org/10.5902/1980509869093
Submissão: 25/01/2022 • Aprovação: 29/11/2022 • Publicação: 10/04/2023

Artigos

Resgate vegetativo, estabelecimento *in vitro* e estaquia de *Drimys brasiliensis* Miers

Vegetative rescue, *in vitro* establishment and cutting technique of *Drimys brasiliensis* Miers

Jaiton Jaime das Neves Silva[I], Marcio Carlos Navroski[I], Marina Gabriela Cardoso de Aquino[I], Lucas Denega[I], Pedro Henrique Tavares da Fonseca[I], Luciana Magda de Oliveira[I], Mariane de Oliveira Pereira[I]

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RESUMO

Drimys brasiliensis Miers, conhecida popularmente por cataia, é uma espécie arbórea nativa da Mata Atlântica que apresenta importância fitoquímica, fitoterapêutica, aromática e econômica, com amplo uso na medicina popular. É considerada uma espécie de difícil multiplicação via sementes, apresentando dormência por imaturidade embrionária. Assim, objetivou-se com este estudo analisar o resgate vegetativo, estabelecimento *in vitro* e a propagação via estaquia de *Drimys brasiliensis*. Para o resgate, realizou-se a aplicação das técnicas de anelamento a 30 e 90 cm do solo, semianelamento a 30 cm do solo e galhos podados acondicionados vertical e horizontalmente, sendo avaliada a capacidade de emissão de brotações. Com as brotações obtidas pelo resgate vegetativo, foram confeccionados os explantes utilizados para o estabelecimento *in vitro*, avaliando diferentes tempos de imersão em hipoclorito de sódio 1,0 % (v/v) (0, 10, 15 e 20 minutos). Foram avaliadas as percentagens de contaminação total, bacteriana, fúngica e oxidação fenólica. Na estaquia foram testadas brotações oriundas do resgate vegetativo, da parte aérea de indivíduo adulto e de galhos destacados e acondicionados em mini-túnel. Avaliaram-se a porcentagem de sobrevivência das estacas, porcentagem de estacas com calos e porcentagem de enraizamento. Em relação ao resgate vegetativo, a técnica de galhos podados resultou na indução de brotações, destacando os galhos acondicionados verticalmente (100%). No estabelecimento *in vitro*, a imersão em hipoclorito de sódio (1%) por 20 minutos se mostrou eficiente no controle da contaminação por fungos, entretanto, não foram observadas diferenças significativas em relação ao controle de contaminação bacteriana. Na estaquia, o uso de estacas provenientes de galhos podados ou anelamento/semianelamento mostrou potencial para estas técnicas na clonagem da espécie, obtendo resultados próximos a 10% de enraizamento. No geral, a espécie apresenta potencial de propagação vegetativa, contudo, são necessários ajustes nos protocolos de estabelecimento *in vitro* e estaquia.

Palavras-chave: Propagação vegetativa; Rejuvenescimento; Cultivo *in vitro*; Espécie nativa

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ABSTRACT

Drimys brasiliensis Miers, popularly known as cataia, is a tree species native to the Atlantic Forest that has phytochemical, phytotherapeutic, aromatic and economic importance, with wide use in folk medicine. It is considered a species of difficult multiplication via seeds, presenting dormancy due to embryonic immaturity. Thus, the objective of this study was to analyze the vegetative rescue, *in vitro* establishment and propagation by way of cuttings of *Drimys brasiliensis*. For the rescue, the girdling techniques were applied at 30 and 90 cm from the ground, semi-girdling at 30 cm from the ground and pruned branches conditioned vertically and horizontally, being evaluated the shoot emission capacity. With the shoots obtained by vegetative rescue, the explants used for *in vitro* establishment were made, evaluating different immersion times in sodium hypochlorite 1.0 % (v/v) (0, 10, 15 and 20 minutes). The percentages of total, bacterial, fungal contamination and phenolic oxidation were evaluated. On the cuttings, shoots from vegetative rescue, from the aerial part of an adult individual and from detached branches were tested and placed in a mini-tunnel. The percentage of cuttings survival, percentage of cuttings with callus and percentage of rooting were evaluated. Regarding the vegetative rescue, the technique of pruned branches resulted in the induction of shoots, highlighting the branches conditioned vertically (100%). In the *in vitro* establishment, the immersion in sodium hypochlorite (1%) for 20 minutes proved to be efficient in the control of fungal contamination, however, no significant differences were observed in relation to the control of bacterial contamination. In cuttings, the use of cuttings from pruned branches or girdling/semi-girdling showed potential for these techniques in the cloning of the species, obtaining results close to 10% rooting. In general, the species has the potential for vegetative propagation, however, adjustments are necessary in the in vitro establishment and cutting protocols.

Keywords: Vegetative propagation; Rejuvenation; *in vitro* cultivation; Native species

1 INTRODUÇÃO

Drimys brasiliensis Miers., conhecida popularmente como cataia ou casca-d'anta, é uma espécie nativa da Floresta Ombrófila Mista da Mata Atlântica pertencente à família Winteraceae (BACKES; NARDINO, 1998; ABREU *et al*., 2005). A espécie tem ocorrência em vários Estados do Brasil, principalmente em regiões montanhosas do Sul e Sudeste (MARCHIORI, 1997). A casca-d'anta tem várias possíveis utilizações, como madeireiro, no paisagismo e, principalmente, medicinal (SIMÕES *et al*., 1986).

As sementes de *D. brasiliensis* apresentam dormência, em virtude da imaturidade embrionária que, por sua vez, é devido aos seus embriões serem rudimentares, necessitando de um período adicional para completar o seu desenvolvimento até que estejam aptas para germinação. Isso acaba dificultando

a análise dessas sementes e, consequentemente, a produção de mudas da espécie (ABREU *et al*., 2005). Contudo, devido à crescente demanda pelo cultivo de espécies florestais que apresentem algum potencial econômico, ou ainda visando a recuperação de sistemas degradados, diversos estudos têm sido desenvolvidos especialmente relacionados à seleção de genótipos mais adaptados às diversas condições ambientais, maior produtividade e superioridade genética e fisiológica (WENDLING; BRONDANI, 2015) e à propagação vegetativa.

O uso de espécies nativas, visando um retorno econômico ou com o objetivo de recuperação de sistemas degradados, tem sido uma atividade rentável e de grande importância. Entretanto, a falta de conhecimento sobre as técnicas na produção de mudas de plantas nativas e, em algumas situações, a dificuldade de propagação pela via sexuada, em virtude da inviabilidade de suas sementes ou da necessidade de produzir um material com características mais homogêneas, indicam a propagação vegetativa como uma possível alternativa para a multiplicação dessas espécies (DIAS *et al*., 2015).

Com o objetivo de maximizar as características de interesse, é necessário selecionar materiais genéticos superiores. No entanto, tendo em vista a alta variabilidade dos povoamentos e a consequente presença de características genéticas indesejáveis (SOUZA *et al*., 2009), tornam-se indispensáveis estudos para seleção e propagação vegetativa da espécie. As matrizes selecionadas e, posteriormente, propagadas assexuadamente dão origem aos clones, nesse processo inicialmente ocorre o resgate do material superior. Para tanto, a primeira etapa após a seleção da matriz é a promoção de seu rejuvenescimento mediante a indução de brotações juvenis, sendo esse um material fisiologicamente mais apto ao enraizamento e com maior vigor de crescimento (WENDLING *et al*., 2015). A indução de brotações basais se apresenta como a forma mais eficiente de obter brotos juvenis de árvores adultas mediante o corte raso da planta-matriz (WENDLING *et al*., 2014). Entretanto, a

desvantagem do uso deste método de resgate vegetativo é a perda de copa ou mesmo a perda total do genótipo selecionado (BITENCOURT *et al*., 2009; WENDLING *et al*., 2013), o que justifica a busca por alternativas que possibilitem o rejuvenescimento e revigoramento da planta-matriz, sem que a mesma seja perdida, principalmente em pesquisas envolvendo espécies nativas.

A micropropagação é uma das técnicas das cultura de tecidos mais difundida, amplamente estudada e com maiores aplicações práticas comprovadas (LUZ *et al*., 2014). Essa técnica tem como principal vantagem a possibilidade de obter várias plantas a partir de um explante inicial, independente da estação do ano. Assim, reduz o tempo e a área necessária para a propagação, melhora as condições sanitárias, reproduz indivíduos genotipicamente idênticos à planta-matriz e multiplica vegetativamente espécies que apresentam dificuldade de propagação por outros métodos (ERIG; SCHUCH, 2005). Dentre as fases da micropropagação, o estabelecimento é considerado uma das mais importantes do processo, exigindo maiores cuidados, principalmente com relação à contaminação dos explantes. Outra técnica de propagação vegetativa bastante utilizada é a estaquia (WENDLING; BRONDANI, 2015), que pode propiciar não somente a obtenção de indivíduos idênticos à planta matriz, mas também tem o potencial de aumentar a obtenção de tecidos juvenis, a uniformidade e vigor na produção, maximizando o potencial de estabelecimento quando levadas à campo, podendo ainda ser uma ferramenta importante para a propagação de espécies de difícil enraizamento (TOSTA *et al*., 2012; BADILLA *et al*., 2016).

Apesar da propagação vegetativa ter potencial para ser uma técnica eficaz na produção de mudas, são necessárias pesquisas que aumentem a sua eficiência, principalmente em espécies como a *D. brasiliensis*, a qual apesar de apresentar um grande potencial não é amplamente estudada. Portanto, objetivo geral da pesquisa foi avaliar a espécie *D. brasiliensis* quanto ao seu resgate vegetativo, estabelecimento *in vitro* e a propagação vegetativa via estaquia.

2 MATERIAIS E MÉTODOS

2.1 Resgate vegetativo

O material vegetativo foi coletado nos municípios de Urupema e São Joaquim - Santa Catarina, em florestas nativas de propriedades privadas. O município de Urupema localiza-se nas coordenadas geográficas 27°57'14"S, 49°52'27"W e São Joaquim nas coordenadas 28°17'19"S, 49°55'54"W (SANTA CATARINA, 2021). O clima de ambos os municípios é classificado como Cfb (ALVARES *et al*., 2013), com temperatura média anual em Urupema de 14,1°C e 14,0°C em São Joaquim.

No experimento de resgate vegetativo, foram selecionadas 56 árvores, sendo 29 em Urupema e 27 em São Joaquim, nas quais foram aplicados diferentes métodos de resgate vegetativo (Tabela 1).

Tabela 1 – Métodos de resgate vegetativos (tratamentos) e seus respectivos números de repetições testados para a espécie *Drimys brasiliensis* em dois municípios do estado de Santa Catarina

Município	Tratamento	Nº de repetições (indivíduos)
Urupema (SC)	Anelamento (100%)	15
	Semianelamento (50%)	14
São Joaquim (SC)	Anelamento (30 cm)	9
	Anelamento (90 cm)	9
	Galhos podados/horizontal	9
	Galhos podados/vertical	9

Fonte: Autores (2021)

Para ambos os experimentos, as árvores selecionadas apresentavam boas condições estruturais físicas, nutricionais e fitossanitárias, além de obedecer a um distanciamento mínimo de 50 m entre si, evitando-se indivíduos com coeficientes de parentesco mais elevado. As técnicas de resgate vegetativo foram aplicadas em

dezembro de 2019 e os experimentos nos dois locais de coleta o delineamento utilizado foi inteiramente casualizado. A não padronização dos tratamentos nas duas áreas deu-se em virtude do número limitado de matrizes disponíveis, objetivando-se realizar diferentes estudos de métodos de resgate.

No experimento instalado em Urupema, foram aplicados dois tratamentos, contendo quinze repetições (cada indivíduo constituiu-se de uma repetição) para o anelamento e quatorze repetições para o semianelamento, ambos aplicados a 30 cm do solo. A diferença se deu pela impossibilidade de mais indivíduos no mesmo local e com as mesmas condições. Para os procedimentos de anelamento e semianelamento, foram seccionadas duas linhas transversais ao tronco, em que foi cortada somente a espessura da casca de cada árvore e, posteriormente, removeu-se o anel de 2 cm de largura entre as linhas seccionadas em 100% da circunferência da árvore no anelamento e em 50% da circunferência no semianelamento.

Em São Joaquim, foram aplicados quatro tratamentos (Tabela 1) com nove repetições para todos os tratamentos. O procedimento de anelamento seguiu a descrição anterior sendo realizado a duas alturas (30 e 90 cm de altura do solo). Para o tratamento de coleta de galhos podados, estes foram obtidos de nove matrizes diferentes, sendo retirados dois galhos de cada matriz, um acondicionado verticalmente e outro horizontalmente.

Os galhos tiveram suas extremidades envolvidas em plástico, a fim de evitar a perda excessiva de umidade (Figura 1). Posteriormente, foram transportados para o Viveiro Florestal localizado no Centro de Ciências Agroveterinárias (CAV/UDESC) em Lages – Santa Catarina, sendo armazenados em mini-tunel (estufim), com quatro irrigações diárias de cinco minutos cada.

Para a indução de brotações em campo, foram realizadas duas avaliações. A primeira aos 180 dias e a segunda aos 390 dias, após a aplicação das técnicas de resgate. Foram quantificados o número de árvores brotadas, o número de brotações por indivíduos, além da mortalidade. Foi ainda realizado o cálculo de correlação

de Pearson a 5% de erro, a fim de avaliar a possível relação entre a porcentagem de mortalidade e o diâmetro das árvores. Para os galhos podados, cada galho foi considerado um indivíduo, sendo igual avaliado quanto ao brotamento, número de brotações e mortalidade.

Figura 1 – Diferentes métodos de resgate vegetativo aplicados em *Drimys brasiliensis*: anelamento a 30 cm do solo (a), anelamento a 90 cm do solo (b), semianelamento a 30 cm do solo (c), galhos podados acondicionados verticalmente (d) e galhos podados acondicionados horizontalmente (e)



Fonte: Autores (2021)

2.2 Estabelecimento *in vitro*

Os explantes utilizados no estabelecimento *in vitro* de *D. brasiliensis* foram obtidos a partir de brotações induzidas pelas técnicas de anelamento e semianelamento realizadas no município de Urupema. As brotações foram seccionadas em explantes de 2,0 – 2,5 cm de comprimento, contendo, pelo menos, um par de gemas axilares.

Os explantes foram lavados em água corrente, por cerca de 30 minutos, a fim de lixiviar substâncias fenólicas e reduzir contaminantes superficiais. Após essa higienização inicial, visando a desinfestação, os explantes foram submersos em detergente neutro (2 mL L^{-1}), por 1 minuto. Em seguida, os explantes foram imersos em solução de etanol a 70%, por 30 segundos e, posteriormente, imersos em solução de hipoclorito de sódio a 1,0 % (v/v) durante três diferentes tempos de imersão: 0, 10, 15 e 20 minutos. Após a aplicação do hipoclorito de sódio, os explantes foram lavados três vezes com água estéril.

Os explantes foram introduzidos em frascos de vidro com capacidade para 150 ml, nos quais foram adicionados igualmente 30 ml de meio de cultura MS (MURASHIGE; SKOOG, 1962) com 50% da concentração de sais, 30 g L^{-1} de sacarose e 6 g L^{-1} de ágar. Foi adicionado, ainda, 250 mg L^{-1} de polivinilpirrolidona (PVP), com a finalidade de reduzir a oxidação fenólica. O pH do meio de cultura foi ajustado para 5,8 ± 0,1, antes da adição do ágar, e em seguida autoclavados à temperatura de 121 ºC (1,5 kgf cm^{-2}) por 20 minutos. A fase de estabelecimento foi realizada em sala de crescimento, com temperatura 25 ± 2 °C e fotoperíodo de 16 horas de luz, a uma intensidade luminosa de 30 µmol m^{-2} s^{-1}.

Cinco dias após a introdução dos explantes, iniciaram-se as avaliações diárias do experimento, durante os 10 dias subsequentes, obtendo-se informações sobre a porcentagem de contaminação total, porcentagem de contaminação bacteriana, porcentagem de contaminação fúngica e a porcentagem de oxidação fenólica. O delineamento experimental utilizado foi o inteiramente casualizado com quatro tratamentos e três repetições, sendo cada repetição representada por dez frascos contendo três explante cada.

2.3 Estaquia

A coleta do material vegetativo utilizado nos experimentos de estaquia foi realizada somente na população localizada no município de São Joaquim – SC. Os tratamentos testados foram compostos por diferentes origens de brotações, sendo: brotações obtidas através da aplicação das técnicas de resgate (anelamento e semianelamento); brotações retiradas de partes áreas de um indivíduo adulto e brotações de galhos podados acondicionados em mini-tunel. Para o tratamento com estacas coletadas de indivíduo adulto, este possuía 17,3 de DAP e 5,70 de altura. Para esse tratamento selecionou-se apenas uma árvore matriz, para a retirada das brotações, a fim de anular a variação genotípica e de modo a considerar somente as variações causadas pelos diferentes tratamentos.

As estacas foram submetidas a uma dose padrão de AIB, sendo essa de 6000 mg $^{L-1}$. As bases das estacas foram imergidas em solução de AIB (solução hidroalcóolica de 50%) por 15 segundos. As bandejas com as estacas foram acondicionadas em mini-tunel, por 180 dias, com temperatura no intervalo de 20 e 30°C, umidade relativa do ar (UR) superior a 80%, e irrigadas com auxílio de nebulizadores.

O experimento foi realizado em delineamento inteiramente casualizado, contendo três repetições de nove estacas cada. As variáveis que foram estudadas quanto à utilização de estacas obtidas por meio de diferentes métodos de resgate de *D. brasiliensis* foram: porcentagem de sobrevivência, de formação de calos e enraizamento das estacas, além do número de raízes das estacas enraizadas. Foram consideradas estacas sobreviventes aquelas que apresentassem lenho vivo, com folhas velhas ou brotações novas. Para quantificação de calos, foram observados na base da estaca a presença de estruturas arredondadas e esbranquiçadas indicando a existência de calos.

2.4 Análise estatística

Após a verificação da normalidade dos dados por meio do teste de Kolmogorov-Smirnov e da homogeneidade pelo teste de Bartlett, realizou-se a análise de variância. Quando necessário, os dados foram transformados pela função $(x + 0,5)^{0,5}$ e as médias foram comparadas pelo teste de Scott-Knott, a 5% de probabilidade. Para as análises, foi utilizado o pacote estatístico SISVAR (FERREIRA, 2011).

3 RESULTADOS E DISCUSSÃO

3.1 Resgate vegetativo

Em relação à sobrevivência, nos indivíduos analisados no município de Urupema, foi possível observar a mortalidade de árvores em que foram aplicadas as técnicas de anelamento e semianelamento a 30 cm do solo (Tabela 2).

Tabela 2 – Porcentagem de mortalidade em resposta a diferentes métodos de resgate vegetativo aplicados à espécie *Drimys brasiliensis* em duas áreas distintas

Experimento	Tratamento	Mortalidade (%)	
		180 dias	390 dias
% de anelamento (Urupema)	Anelamento (100%)	16,7 a*	22,2 a
	Semianelamento (50%)	9,1 a	9,1 a
	Média	12,9	16,7
	CV%	23,6	25,1
	p-valor	0,5821	0,3817
Método/resgate (São Joaquim)	Anelamento (30 cm)	23,8 b	33,3 b
	Anelamento (90 cm)	0,0 a	0,0 a
	Galhos podados/horizontal	0,0 a	0,0 a
	Galhos podados/vertical	0,0 a	0,0 a
	Média	6,0	8,3
	CV%	21,56	23,1
	p-valor	0,1499	0,0424

Fonte: Autores (2021)

Em que: *Médias seguidas de mesma letra não diferem entre si pelo teste de médias Tukey (P<0,05).

Quanto às técnicas aplicadas nas árvores na área de São Joaquim, o tratamento a 30 cm do solo foi o único método que apresentou mortalidade. Esses resultados denotam que a espécie pode não suportar a retirada de um anel, principalmente no diâmetro total da árvore, sem comprometer sua sobrevivência. Santos Junior *et al*. (2021) constaram que o anelamento possibilitou a obtenção brotações de *Cedrela fissilis* sem causar a morte das árvores-matrizes. Diante disso,

a metodologia de aplicação dessas técnicas de resgate vegetativo pode não ser indicada para o resgate vegetativo da espécie *D. brasiliensis*.

Foi ainda realizado o cálculo de correlação de Pearson, a fim de avaliar se há relação entre as variáveis diâmetro à altura do peito (DAP) e mortalidade, entretanto, não houve correlação significativa entre as variáveis (r = -0,17 a um nível de significância de 0,05). Desse modo, a mortalidade pode estar atribuída a outros fatores, como por exemplo genético, haja vista que os métodos de resgate foram aplicados de forma padronizada com relação à altura do solo, espessura e profundidade do anelamento.

Em relação às técnicas de resgate vegetativo testadas em Urupema, houve diferença significativa na porcentagem de árvores brotadas após 180 dias entre o anelamento e semianelamento, ambos aplicados a 30 cm do solo. O anelamento apresentou resultados superiores em ambos os períodos de avaliação (Tabela 3). Menos de 10% das árvores submetidas ao semianelamento apresentaram brotações aos 180 dias. Contudo, o semianelamento alcançou-se quase 30% de brotação aos 390 dias.

Tabela 3 – Porcentagem de árvores brotadas em resposta a diferentes métodos de resgate vegetativo aplicados à espécie *Drimys brasiliensis* em duas áreas distintas

Método/resgate Experimento	**Tratamento**	**Árvores brotadas (%)**	
		180 dias	**390 dias**
% de anelamento (Urupema)	Anelamento (100%)	55,6a*	61,1 a
	Semianelamento (50%)	9,1 b	27,3 b
	Média	32,4	44,2
	CV%	25,5	26,5
	p-valor	0,0111	0,0818
Método/resgate (São Joaquim)	Anelamento (30 cm)	33,3 b	38,1 a
	Anelamento (90 cm)	25,0 b	50,0 a
	Galhos podados/horizontal	62,5 ab	25,0 a
	Galhos podados/vertical	100,0 a	75,0 a
	Média	55,2	44,1
	CV%	23,6	24,9
	p-valor	0,0052	0,0297

Fonte: Autores (2021)

Em que: *Médias seguidas de mesma letra não diferem entre si pelo teste de médias Tukey (P<0.05).

O maior número de árvores brotadas por meio do anelamento pode ser atribuída a quebra de dominância apical causada pelo corte total do floema, que afeta o balanço hormonal entre auxina e citocinina, fotossimilados e metabolismo na área anelada (DIAS *et al*., 2015), diferentemente do tratamento de semianelamento em que ocorre a retirada parcial da circunferência da árvore, não sendo suficiente para causa o desbalanço hormonal necessário para a indução de brotações. No presente estudo, o semianelamento mostrou certo potencial de resgate após um maior período e após a aplicação das técnicas de resgate (390 dias), contudo, comparando-se ao anelamento, o resultado foi praticamente a metade de árvores brotadas. Carvalho (2020) estudando resgate de matrizes e propagação vegetativa de *Plathymenia reticulata* Benth. obteve somente 40% de suas matrizes com emissão de brotos, ao empregar a técnica de semianelamento, além de terem ocorrido em menor número, assemelhando-se aos resultados obtidos no presente trabalho.

Quanto ao método de resgate, testados em São Joaquin, as técnicas de anelamento não diferenciaram significativamente entre si na avaliação realizada aos 390 dias (Tabela 3). Aos 180 dias foi notada diferença entre os tratamentos, sendo que o resgate por galhos podados, especialmente os acondicionados verticalmente, mostrou ser o método mais eficiente na indução de brotações epicórmicas de árvores de *D. brasiliensis*, visto que todos os galhos podados que se encontravam na casa de vegetação emitiram brotações após 180 dias de acondicionamento. O semianelamento a 30 cm do solo, além de não ter se mostrado viável para a indução de brotações ao analisar a porcentagem de árvores brotadas, também apresentou baixa média de brotações por indivíduos (0,3) (Tabela 4).

Além da baixa aptidão da espécie à emissão de brotações, as técnicas foram aplicadas no mês de dezembro, período em que se inicia o verão, podendo atribuir à elevada temperatura o reduzido número de brotações obtidas. Segundo Hartmann *et al*. (2011), no verão há menor quantidade de reservas no caule, devido à alta atividade metabólica da parte aérea, dificultando a retomada do crescimento vegetativo. Ferriani *et al*. (2011) observaram no estudo sobre a espécie *Piptocarpha angustifolia*

Dusén ex Malme um maior número de emissão de brotos durante o período da primavera. Além disso, possivelmente o vigor fisiológico e a idade ontogenética de algumas plantas têm influenciado negativamente a emissão de brotações, visto que as matrizes em que foram aplicadas as técnicas de resgate estão em condições naturais sem aplicação de fertilizantes ou qualquer tratamento silvicultura que favoreça seu crescimento (WENDLING *et al*., 2014).

Tabela 4 – Número médio de brotações por indivíduo em resposta a diferentes métodos de resgate vegetativo aplicados à espécie *Drimys brasiliensis* em duas áreas distintas

Experimento	**Tratamento**	**Nº de brotações por indivíduo**	
		180 dias	**390 dias**
% de anelamento (Urupema)	Anelamento (100%)	3,3 a*	1,5 a
	Semianelamento (50%)	0,3 b	0,3 b
	Média	1,8	0,9
	CV%	61,6	41,7
	p-valor	0,0108	0,0177
Método/resgate (São Joaquim)	Anelamento (30 cm)	1,0 b	0,7 b
	Anelamento (90 cm)	1,5 b	2,0 ab
	Galhos podados/horizontal	4,8 b	0,7 b
	Galhos podados/vertical	13,6 a	3,9 a
	Média	43,5	11,0
	CV%	45,6	41,4
	p-valor	0,0000	0,0129

Fonte: Autores (2021)

Em que: *Médias seguidas de mesma letra não diferem entre si pelo teste de médias Tukey (P<0,05).

A técnica de galhos podados, além de ter apresentado maior porcentagem de brotações, também apresentou maior número de brotações (13,6 por árvore). Essa técnica também foi estudada por Almeida *et al*. (2007) ao abordarem a propagação vegetativa de *Eucalyptus cloeziana*, Wendling *et al*. (2009) com *Araucaria angustifolia* e Meneguzzi (2017) ao tratar sobre o resgate da espécie *Persea willdenovii* Kosterm., destacam que o acondicionamento em casa de vegetação desenvolve um microclima favorável para a sobrevivência e emissão de brotos em galhos podados.

Foi observada uma diminuição quanto ao número de brotações emitidas aos 390 dias quando comparado ao número de brotos emitidos aos 180 dias após o acondicionamento dos galhos em mini-tunel. Wendling *et al*. (2009), ao avaliarem brotações epicórmicas de *Araucaria angustifólia*, observaram uma diminuição no número de brotações no período de 60 a 90 dias após o acondicionamento dos galhos, com subsequente mortalidade de quase 100% do material, ocorrido após 120 dias. Essa redução pode estar associada ao esgotamento das reservas presentes nos galhos, competição por água, nutrientes, espaço e luz entre os brotos (WENDLING *et al*., 2009; DIAS *et al*., 2015).

O método de galhos podados é recente e pouco difundido, apesar disso, em geral, pode proporcionar brotações precoces e em maior número e tamanho, quando comparado a outras técnicas. Além disso, possibilita o acompanhamento do desenvolvimento destas brotações em ambiente controlado oferecido pela casa de vegetação.

3.2 Estabelecimento *in vitro*

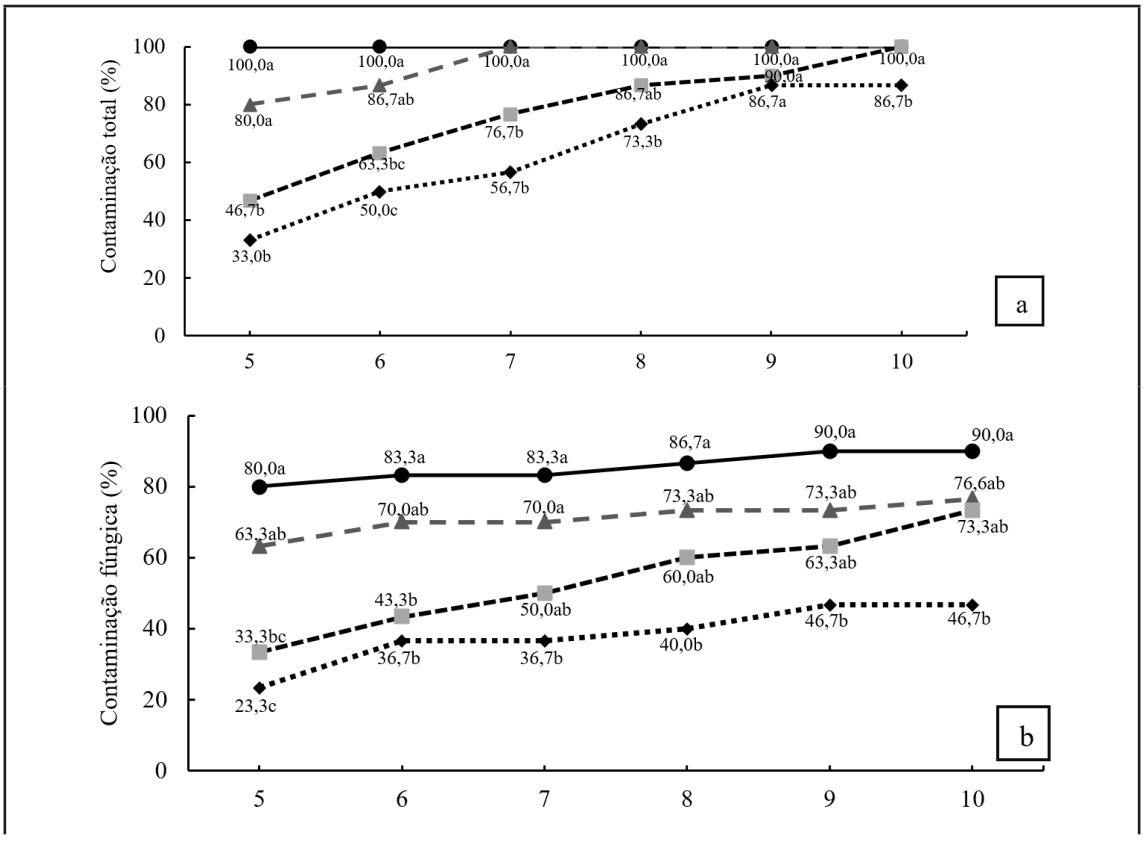
Em relação ao estabelecimento *in vitro*, todos os tratamentos apresentaram contaminação após iniciadas as avaliações diárias do quinto ao décimo dia, após a introdução dos explantes (Figura 2).

Os resultados mostram que na primeira avaliação, ocorrida ao quinto dia após a introdução, o tratamento em que os explantes não foram submetidos à imersão em hipoclorito de sódio apresentou 100% de contaminação, enquanto o tratamento com 20 minutos apresentou 86,7% ao final das avaliações, diferindo estatisticamente dos demais tratamentos (Figura 2a). Os tratamentos testados neste estudo não foram eficazes no controle da contaminação dos explantes introduzidos *in vitro* para *D. brasiliensis*, considerando que todos apresentaram percentagens acima do índice aceitável de contaminação que é igual a 10% (GEORGE, 1993; HARTMANN *et al*., 2011). De acordo com Erig e Schuch (2005), as plantas lenhosas possuem dificuldades

para o estabelecimento *in vitro*, principalmente em virtude da contaminação por microrganismos, sendo a contaminação causada por fungos e bactérias um dos maiores entraves encontrados inicialmente na fase de estabelecimento do explante *in vitro* em especial na superfície dos tecidos de folhas, gemas e segmentos nodais (TEIXEIRA, 2005). Além disso, na assepsia dos explantes não ocorre a exposição dos microrganismos endógenos aos agentes desinfestantes (XAVIER *et al*., 2013).

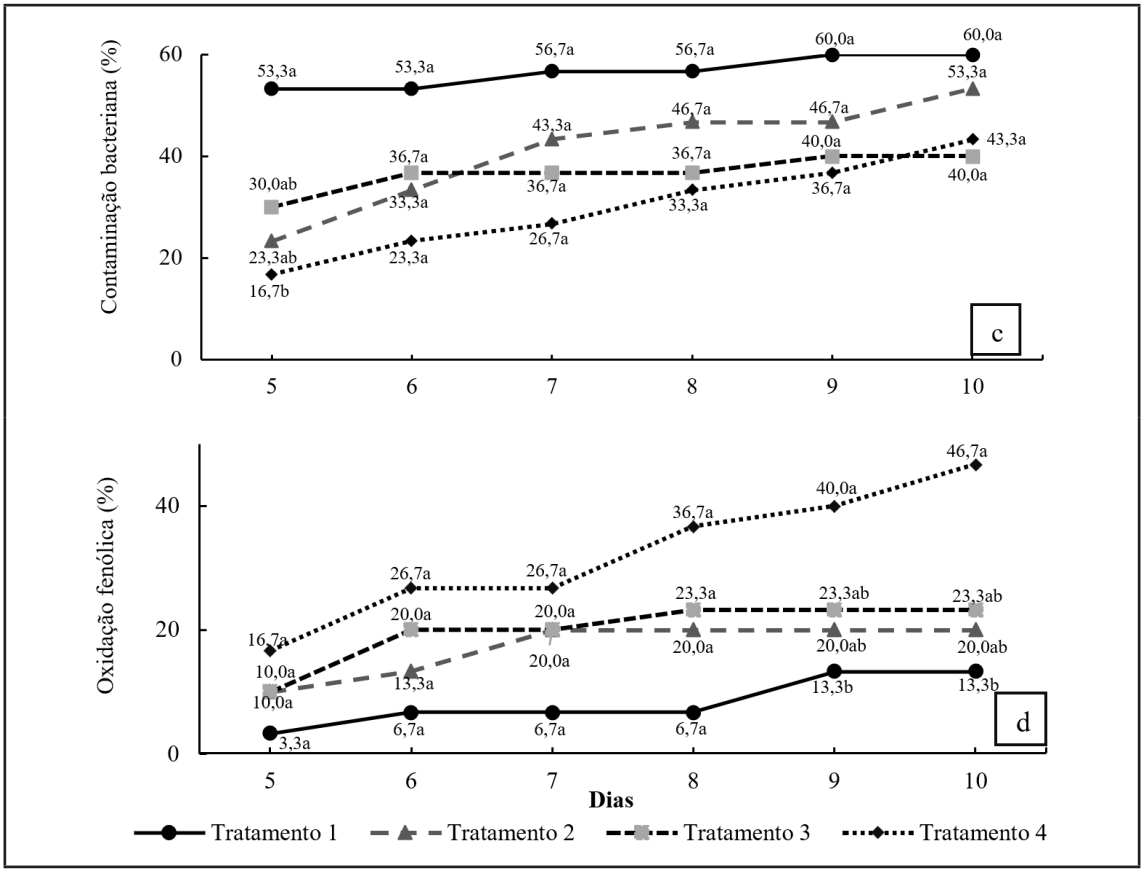
Os tratamentos com tempo de imersão de 0 e 20 minutos foram os que apresentaram a maior e a menor porcentagem de contaminação, respectivamente, durante todo o período de avaliação. A imersão por 20 minutos, apesar do aumento na contaminação fúngica (46,7%), ainda permaneceu como o melhor tratamento, sendo verificada a diferença estatística quando comparado aos demais (Figura 2b).

Figura 2 – Porcentagens de contaminação total (a), fúngica (b), bactericida (c) e oxidação fenólica (d) no estabelecimento *in vitro* de explantes de *Drimys brasiliensis* submetidas a diferentes tempos de imersão em solução de hipoclorito de sódio a 1,0 % (v/v)



Continua ...

Figura 2 – Conclusão



Fonte: Autores (2021)

Em que: *Médias seguidas de mesma letra não diferem entre si pelo teste de médias Tukey (P<0,05). Tratamento 1: 0 minutos de imersão; Tratamento 2: 10 minutos de imersão; Tratamento 3: 15 minutos de imersão; Tratamento 4: 20 minutos de imersão.

Quanto à contaminação causada por bactérias (Figura 2c), os tratamentos avaliados apresentaram menores percentagens em comparação à contaminação por fungos. Inicialmente, os quatro tratamentos apresentaram porcentagens de contaminação por bactérias, no entanto, o tratamento em que não houve imersão em hipoclorito de sódio apresentou maior porcentagem de contaminação (53,3%), enquanto o tratamento de maior tempo de imersão (20 minutos) apresentou a menor porcentagem (16,7%). Contudo, a partir do segundo dia de avaliação, nenhum dos tratamentos apresentou diferenças significativas. Esses resultados sugerem que o uso da solução de hipoclorito de sódio, para os tempos de imersão e concentração analisadas neste estudo, não possui influência quanto à contaminação bacteriana para a espécie estudada.

Esses resultados indicam que possivelmente os microrganismos estariam impregnados em locais onde o hipoclorito de sódio não teve alcance, haja vista que esse processo remove bactérias localizadas na superfície dos explantes (NADHA *et al*., 2012). Com isso, torna-se necessária a realização de novos ensaios, a fim de se testar outras concentrações e tempos de imersão e, possivelmente, outros agentes desinfestantes.

Outro fator que pode contribuir para o insucesso no estabelecimento *in vitro* é a utilização de material vegetativo coletado em campo, situação em que ocorre alta exposição a agentes contaminantes exógenos e endofíticos (PORFÍRIO *et al*., 2016). Alguns autores citam que intervenções feitas visando a prevenção contra agentes contaminantes têm influenciado positivamente, de forma a otimizar a etapa seguinte de desinfestação em laboratório, promovendo assim melhor assepsia dos explantes.

Quanto à oxidação fenólica (Figura 2d) do material vegetativo utilizado no estabelecimento *in vitro*, não houve diferença estatística entre os tratamentos no período do quinto ao oitavo dia de avaliação, contudo, ao final das avaliações realizadas ao décimo dia, o tratamento de maior tempo imersão em solução de hipoclorito (tratamento 4) foi responsável pela maior porcentagem de oxidação fenólica (46,7%), diferenciando-se estatisticamente do tratamento 1, o qual não se utilizou a solução de hipoclorito na assepsia dos explantes.

Embora, geralmente, apresente maior eficiência quanto à contaminação, um maior tempo de imersão pode afetar a viabilidade dos explantes, reduzindo-os por meio da perda de material por fitotoxidez (BARRUETO; ZIMMERMANN, 2006). O uso de agentes desinfetantes, como o hipoclorito de sódio pode potencializar o aumento do estresse oxidativo (OLIVEIRA *et al*., 2021). Os resultados corroboram com o trabalho desenvolvido por Ferreira (2021), na micropropagação de *Theobroma grandiflorum* a partir de explantes florais, os quais foram imersos em hipoclorito de sódio durante 20 e 30 minutos nas concentrações 0,25 e 0,50%, sendo recomendado pelos autores o uso da menor concentração de hipoclorito de sódio (0,25%) com 20 minutos de imersão dos explantes, a fim de reduzir a oxidação fenólica dos tecidos dos explantes.

Além das dificuldades citadas anteriormente, outros fatores podem contribuir para a oxidação fenólica dos explantes, como o tempo elevado de transporte do campo ao laboratório e a tendência natural que algumas espécies possuem. De acordo com Yoshiko *et al*. (2001), quando a espécie possui alta suscetibilidade natural à oxidação fenólica, independente do tempo de imersão em solução desinfetante, a oxidação fenólica ocorrerá.

3.3 Estaquia

Com relação à estaquia, houve diferença significativa entre a origem das brotações (método de resgate) para a porcentagem de calos, sobrevivência e enraizamento. As maiores médias de percentagens de sobrevivência, formação de calos e enraizamento foram obtidos nos tratamentos de rejuvenescimento através de anelamento/semianelamento e galhos podados (Tabela 5).

Observou-se a formação de calos apenas para os tratamentos com estacas herbáceas obtidas através das técnicas anelamento/semianelamento e galhos podados. A superioridade das estacas herbáceas na formação de calos, além da menor lignificação dos tecidos, pode estar relacionada ao teor de auxina endógena no propágulo, visto que as estacas herbáceas estão próximas das gemas apicais, regiões de síntese de auxinas, resultando em maior concentração endógena desse hormônio, estando, assim, mais propensa ao enraizamento adventício (HARTMANN *et al*., 2011). Aguiar *et al*. (2021), contrariando os resultados do presente estudo, avaliaram a estaquia das espécies *Calliandra brevipes* e *Calliandra tweedii* utilizando estacas semilenhoas em que observaram a presença de calos nas duas espécies (4,2% e 13,3%, respectivamente), também foi observado que a maior parte das estacas enraizadas possuía calos.

Tabela 5 – Porcentagem de formação de calos, sobrevivência (%), enraizamento (%) e número de raízes em estacas de *Drimys brasiliensis* em função de diferentes métodos de resgate

Método de resgate	Calos (%)	Sobrevivência (%)	Enraizamento (%)	Nº de raízes
Parte aérea	0,0 b	33,3 b*	0,0 b	0,0 a
Anelamento e semianelamento	33,3 a	54,2 ab	9,0 a	3,0 a
Galhos podados	48,0 a	68,0 a	14,8 a	3,8 a

Fonte: Autores (2021)

Em que: *Médias seguidas de mesma letra não diferem entre si pelo teste de médias Tukey (P<0,05).

Os resultados obtidos para a porcentagem de sobrevivência e enraizamento, além do número de raízes das estacas de *D. brasiliensis* indicam que o sucesso da estaquia de árvores adultas pode estar diretamente relacionado ao grau de lignificação das estacas, visto que os tratamentos em que foram utilizadas estacas herbáceas obtidas das técnicas de resgate vegetativo (anelamento/semianelamento e galhos podados) obtiveram melhores resultados quando comparados ao tratamento em que as estacas utilizadas possuíam características semilenhosas.

Os resultados para diferentes tipos de estaca podem variar conforme a espécie. No entanto, é possível identificar uma tendência para melhores resultados quando utilizadas estacas herbáceas para a multiplicação vegetativa por estaquia, visto que estacas herbáceas apresentam maior concentração de auxinas endógenas (FOLARODI *et al*., 2021). Em alguns casos, estacas lenhosas e semilenhosas podem ser mais vantajosas, pois reduzem a desidratação do material, entretanto, podem acumular inibidores de enraizamento (XAVIER *et al*, 2013; HARTMANN *et al*., 2011). Hussain *et al*. (2017) avaliando a estaquia da espécie *Rubus* spp. com o uso de diferentes estacas com diferentes níveis de lignificação (herbáceas, semilenhosas e lenhosas) observaram melhores resultados relacionados à sobrevivência e enraizamento para estacas herbáceas.

Um fator importante para o sucesso da estaquia é a idade ontogenética da árvore-matriz que pode influenciar negativamente os resultados, havendo necessidade de rejuvenescimento desse material, visto que as plantas possuem um gradiente de juvenilidade no sentido ápice/base, logo, ramos maduros tendem a ter menor concentração de auxina (WENDLING *et al*., 2012; WENDLING *et al*., 2015). Além disso, outros fatores exógenos, como a utilização de reguladores vegetais, também podem influenciar a sobrevivência e enraizamento das estacas (HARTMANN *et al*., 2011; ZHANG *et al*., 2016; STUEPP *et al*., 2018).

No geral as técnicas de rejuvenescimento, como galhos podados e anelamento/semianelamento mostraram potencial de clonagem da espécie, apresentando boa qualidade do enraizamento e potencial para geração de mudas de qualidade (Figura 3). Contudo, o empecilho está na baixa produção de brotações obtidas, podendo ser o maior limitante. O uso de galhos podados pode ser uma boa alternativa para a geração destes brotos, não correndo o risco de haver mortalidade de plantas e permitindo maior controle dos fatores ambientais para elevar a quantidade de brotos.

Figura 3 – Estaca enraizada de *Drimys brasiliensis* obtida do rejuvenescimento por anelamento após 180 dias em ambiente de enraizamento



Fonte: Autores (2021)

4 CONCLUSÕES

A técnica de semianelamento não se mostrou uma boa alternativa para a indução de brotações. Os tratamentos em que as árvores foram completamente aneladas a 30 cm do solo, de modo geral, apresentaram uma resposta positiva da espécie *D. brasiliensis* com relação à emissão de brotações, no entanto, causaram a morte de parte dos indivíduos.

O método de galhos podados apresentou melhores resultados considerando as três variáveis avaliadas, quando comparadas aos métodos de anelamento e semianelamento, sendo uma alternativa viável para o resgate de *D. brasiliensis*.

O hipoclorito de sódio demonstrou ser uma alternativa viável para o controle inicial da contaminação fúngica por maiores tempos de exposição. Enquanto, para a contaminação bacteriana, não foi observada influência significativa quando utilizado.

Na estaquia, recomenda-se o uso de estacas submetidas ao resgate vegetativo por galhos podados ou anelamento/semianelamento, mostrando serem técnicas potenciais na clonagem da espécie.

AGRADECIMENTOS

Os autores agradecem ao BNDES pelo financiamento do projeto “Mais Florestas com Araucária” com financiamento de bolsa ao autor principal, à Fundação Certi pela condução do projeto e a FAPESC – pelo financiamento do grupo de pesquisa Gestão de Recursos Florestais Nº: FAPESC 27/2020.

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Como citar este artigo

Silva, J. J. N.; Navroski, M. C.; Aquino, M. G. C.; Denega, L.; Fonseca, P. H. T.; Oliveira, L. M.; Pereira, M. O. Resgate vegetativo, estabelecimento in vitro e estaquia de Drimys brasiliensis Miers. Ciência Florestal, Santa Maria, v. 33, n. 1, e69093, p. 1-25, 2023. DOI 10.5902/1980509869093. Disponível em: https://doi.org/10.5902/1980509869093.

VEGETATIVE RESCUE AND PROPAGATION OF NATIVE *Ilex paraguariensis* POPULATIONS IN SANTA CATARINA STATE, BRAZIL

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ABSTRACT – When producing *Ilex paraguariensis* seedlings through vegetative propagation, selecting the correct populations and genotypes, and obtaining juvenile material, may be fundamental for its success. Therefore, this search aimed to test its vegetative propagation through cutting and rescue using detached branches of different populations in Santa Catarina state, Brazil. Thus, two experiments were installed in September 2019 in order to test: I) the cuttings of four populations belonging to the municipalities of Catanduvas (CT), Painel (PL), Três Barras (TB), and Urupema (UR), using ten randomly chosen genotypes from each, and; II) the epicormic sprouting of detached branches from these same populations. Both experiments were carried out in Lages, Santa Catarina. In February 2020, the cutting was evaluated according to the percentages of survival, callus, rooting, new sprouts, and original leaves permanence. The branches were evaluated every 30 days after storing, observing the percentages of sprouting branches, number of sprouts, and length of sprouts in centimeters. As for cutting, there was a low survival percentage in all populations (<15%), but some genotypes presented greater vigor, such as TB1 (46%), TB7, and TB3 (both 28%). Most live cuttings presented calluses (>70%), characteristic of material of high maturity. Rooting was low for populations (<1.5%) and genotypes (<10%). Branches presented sprouts up to 60 days, with TB presenting the highest total number of sprouts (approximately 300) and the greatest average length (2.8 cm). In general, survival and rooting of cuttings were affected by both populations and genotypes, highlighting TB. A similar response was observed for the branches' sprouting. New studies with more populations, further analyses of the branches' characteristics, and better storage conditions are recommended.

Keywords: Cutting; Detached branches; Erva-mate.

RESGATE E PROPAGAÇÃO VEGETATIVA DE POPULAÇÕES NATIVAS DE Ilex paraguariensis NO ESTADO DE SANTA CATARINA, BRASIL

*RESUMO – Quando produzidas mudas de **Ilex paraguariensis** através da propagação vegetativa, a seleção correta das populações, genótipos, e ser capaz de obter material juvenil, são fundamentais para seu sucesso. Assim, o objetivo desta pesquisa foi testar sua propagação vegetativa por estaquia e resgate utilizando galhos destacados de diferentes populações do estado de Santa Catarina, Brasil. Desta forma, dois experimentos foram instalados em setembro de 2019 para testar: I) a estaquia de quatro populações, pertencentes aos municípios de Catanduvas (CT), Painel (PL), Três Barras (TB) e Urupema (UR), utilizando dez genótipos escolhidos aleatoriamente de cada, e; II) a brotação epicórmica de galhos destacados destas mesmas populações. Ambos os experimentos foram conduzidos em Lages, Santa Catarina. Em fevereiro de 2020, a estaquia foi avaliada de acordo com as porcentagens de: sobrevivência, calos, enraizamento, novas brotações e permanência das folhas originais. Os galhos foram avaliados a cada 30 dias após seu acondicionamento observando as porcentagens de galhos com brotações, número de brotações e comprimento em centímetros. Para a estaquia, houve baixa*

sobrevivência em todas as populações (<15%), mas com maior vigor em alguns genótipos, tais como TB1 (46%), TB7 e TB3 (ambos 28%). A maior parte das estacas vivas apresentou calo, característico de material de elevada maturidade. O enraizamento foi baixo nos níveis de população (<1,5%) e genótipo (<10%). Os galhos apresentaram brotações até 60 dias, com TB apresentando o maior número total de brotações (aproximadamente 300) e de maior comprimento médio (2,8 cm). Em termos gerais, a sobrevivência e o enraizamento de estacas foram afetados por ambos população e genótipo, destacando-se TB. Uma resposta similar foi observada para a brotação de galhos. Novos estudos contendo mais populações, análises mais aprofundadas das características dos galhos e melhores condições de acondicionamento são recomendadas..

Palavras-Chave: Estaquia; Galhos destacados; Erva-mate.

1. INTRODUCTION

The species *Ilex paraguariensis* Saint Hilaire (Aquifoliaceae), popularly known as erva-mate or yerba-mate, is widely distributed within the Mixed Rainforest, encompassing Brazil, Argentina, Uruguay, and Paraguay. It is an evergreen and shade-tolerant climax species, which is usually found associated with *Araucaria angustifolia* (Bertol) Kuntze adult individuals, capable of growing and regenerating easily when the shrub and herbaceous strata are thinned (Carvalho, 2003).

In the Brazilian territory, the production of this species is conducted in native areas, enriched or not, or cultivated in plantations, mainly on the state of Paraná and Santa Catarina (Barbosa et al., 2020). It has a wide consumer market, from products that undergo little processing, such as teas from its leaves (Santin et al., 2019), a more intensive processing, such as energy drinks, alcoholic beverages, foods, to its use in the pharmaceutical industry, such as medicines and beauty products, due to its phytochemicals, mainly theobromine and caffeine (Cardozo Junior and Morand, 2016). However, it is a species that can present different amounts of phytochemicals according to the population and genotype (Santin et al., 2017; Vieira et al., 2021), whereas morphological and physiological differences can also be observed (Wendling and Brondani, 2015).

These differences between plants occur due to their sexual propagation, which guarantees a greater genetic variance and range, capable of producing different characteristics within the same species (Xavier et al., 2013). On the other hand, the standardization of its products is affected by that, causing lower productivity and quality, hence, loss of income. Thus, since it is critical that plants be standardized, the correct selection of seedlings is also fundamental, with two options of propagation: resorting to seeds (through sexual propagation) or clones (through vegetative propagation).

The use of *I. paraguariensis* seeds for seedling production can be problematic, in addition to the heterogeneity caused by genetic variation, there are issues regarding emptiness and, for full seeds, physical and morphological dormancies. (Xavier et al., 2013). The morphological dormancy, whereas the embryo is still under development even though being detached from the mother plant, can be considered as the main problem of production of seedlings through seeds, caused by the long periods and variance of germination (Wendling and Brondani, 2015). Consequently, growers and producers must use the available materials, regardless of their genetic quality, hampering standardization, i.e., production and quality.

Focusing on overcoming these challenges, several experiments with this species have been carried out using techniques of vegetative propagation (Wendling et al., 2013; Stuepp et al., 2016, 2017a, 2017b, 2018; Nascimento et al., 2019, 2020, 2022). The main advantage of this technique is the capability to directly select individuals with superior characteristics, enabling the homogenization of plantations and the quality of the final product. Even though there are current studies about this subject, there is a lack of specific vegetative propagation protocols for this species, since a high variation of rooting between individuals is observed (Wendling and Brondani, 2015), which may also be present in same populations (Nascimento et al., 2020, 2022).

Currently, the main challenge to vegetative propagation is to overcome the maturation of *I. paraguariensis* plant material, using techniques

that do not require the tree cutting (Wendling et al., 2013; Stuepp et al., 2018), aiming more sustainable procedures that maintain the production. The vegetal materials considered as mature are those that, due to a combination of physiological and ontogenetical characteristics, caused and enhanced according to the increase of age, may lose their totipotency capability and are no longer able to efficiently differentiate its tissues, thus, to produce roots. Furthermore, it is common that the expression of desired plant characteristics to occur only after reaching a certain maturity degree, narrowing the use of its material. Even though, the use of propagules from mature sources can still achieve certain rooting levels, but these are considerably lower in relation to materials of juvenile characteristics (Stuepp et al., 2017a, 2017b; Nascimento et al., 2018).

To this purpose, many rescue techniques can be used to overcome the maturation factor. Essentially, it is necessary to stimulate the development of the dormant buds located in the lower regions of the plant, as this material originated during the first years of plant growth, thus, being ontogenetically younger, i. e., of juvenile characteristics. Among many techniques, the sprouting of physiologically active branches can be also used (Wendling et al., 2013; Nascimento et al., 2018). These branches, after being collected from selected plants, can be stored in a greenhouse under favorable conditions for their sprouting using their energy reserves, working as a continuous source of propagules for further vegetative propagation during its lifespan. Once properly stored, the branches enable the production of juvenile sprouts with higher vigor when compared to those from the canopy, increasing the rooting success (Wendling et al., 2013).

As exposed, two main questions were highlighted in this search. I) Do populations and genotypes influence the vegetative propagation of *I. paraguariensis*? If so, is it possible to use mature material for this purpose? II) Are the *I. paraguariensis* detached branches able to sprout and produce reliable quantities of epicormic sprouts for their vegetative propagation? If so, are they influenced by different populations? For that reason, the objective of this study was to evaluate the vegetative propagation of this species by the technique of cutting considering four different populations and the genotypes within, as well as its vegetative rescue through the sprouting of detached branches considering these same populations.

2. MATERIAL AND METHODS

2.1 Cuttings of different populations and genotypes

Plant material was collected (September 2019) from different regions of Santa Catarina state (SC) on four native production areas (Figure 1), being: Catanduvas (CT) (28°95'67" S; 52°78'22" W), Painel (PL) (27°84'97" S; 49°93'54" W), Três Barras (TB) (26°12'19" S; 50°21'62" W) and Urupema (UR) (27°96'52" S; 49°83'92" W). All locations belong to a *Cfb* climate, with average yearly temperature between 14.1 °C and 17.9 ° C, and with well-distributed yearly rainfall between 1,634 mm and 1,895 mm.

The mother plants were selected according to their sanity and phenotypic similarity, taking into consideration their diameter, height (visually) and proportion of their areal part, totaling ten individuals from each location. The individuals were selected with at least 50 m apart to ensure greater genetic diversity within the same population. As a native area criterion, only shaded mother plants from other higher strata species of plants were chosen, such as from *Araucaria angsutifolia* (Bertol.) Kuntze. Aiming to reduce the effect of physiological age, the mother plants selected from the same population had similar diameters. The averages (and standard error) of diameter obtained were 9.5 (± 0.7) cm for CT; 8.2 (± 0.9) cm for PL; 7.8 (± 0.7) cm for TB; and 6.5 (± 0.6) cm for UR.

From the previously selected mother plants, the canopy sprouts produced in the year were collected, i. e., a high maturity level material. To reduce the influence of ontogenetic age between individuals, the sprouts were collected above the diameter at breast height (DBH – 1.3 m), up to 1.9 m, approximately. The sprouts were obtained with pruning shears and stored in a Styrofoam box with cooled water to avoid excessive dehydration during transportation to the Forest Nursery at the University of Santa Catarina State (UDESC) in Lages (SC) (27°47'33" S; 50°18'04" W). This municipality has *Cfb* climate and an average yearly rainfall of 1,441 mm.

After transportation, the sprouts were cut into cuttings with at least one leaf of reduced area (50%) to avoid excessive transpiration. Rectangular trays (20

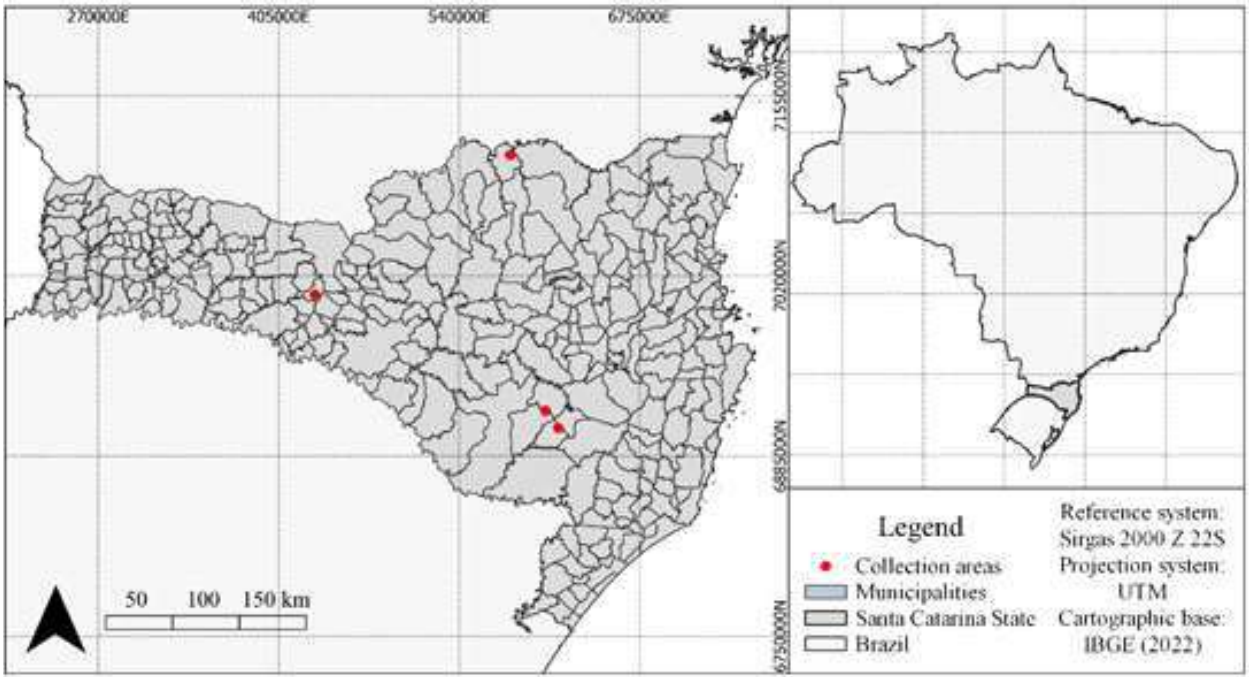


Figure 1 – Collection areas of *I. paraguariensis* vegetal material in the municipalities of Catanduvas (West), Três Barras (North), Urupema (South/bottom) and Painel (South/upper), in Santa Catarina state, Brazil.
Figura 1 *– Áreas de coleta de material vegetal de* ***I. paraguariensis*** *nos municípios de Catanduvas (oeste), Três Barras (norte), Urupema (sul/inferior) e Painel (sul/superior), no estado de Santa Catarina, Brasil.*

L) were used as containers, containing a homogeneous mixture of commercial substrate (mixture of *Pinus* spp. Bark, ashes and peat, pH: 5.4, density: 310 kg m^{-3}) and thin vermiculite (1:1 v/v), with the addition of 6 g L^{-1} of controlled release fertilizer (N[15]-P[9]-K[12]). The purpose of using controlled release fertilizer was only to guarantee the minimum nutritional for rooted cuttings. The trays were filled to approximately 2/3 of their total volume with the prepared substrate mixture, undergoing irrigation (field capacity) to provide support for the cuttings. Each tray was representative of a genotype from a specific population.

The trays containing the cuttings were placed in a mini tunnel system with automatic micro-sprinkler irrigation, composed of four daily irrigations (9:00 AM, 12:00 PM, 3:00 PM and 6:00 PM) of five minutes each, inserted inside a greenhouse with shading (50%). The temperature and relative humidity were measured using a datalogger every ten minutes (Figure 2).

Both genotypes and populations were considered as treatments. At 150 days of implantation of the experiment (February 2020), the cuttings were evaluated in percentages of their survival, rooting, callus formation, presence of new sprouts and permanence of original leaves. Alive cuttings were considered those still presenting green material without any signs of oxidation, rooted or not, with or without leaves and sprouts. Calluses were considered present when alive cuttings had any proportions of an indifferent mass material at their bottom, rooted or not. Rooted cuttings were the ones with well-formed roots, either with high secondary root number or with a root primordium of at least 0.5 cm. New sprouts were considered when one or more new leaves were present, formed after the cutting process. Original leaves were the ones maintained during the cutting process, evaluated according to their permanency or not at the final evaluation.

The experiment was carried out in a completely randomized design, evaluating separately into population and genotypes. The experiment was implemented at the end of September, as there is the beginning of the return of the increase in temperatures (reactivation of vegetative growth) in this period. On average, 50 cuttings were produced for genotype treatments (individuals) and approximately 500 for

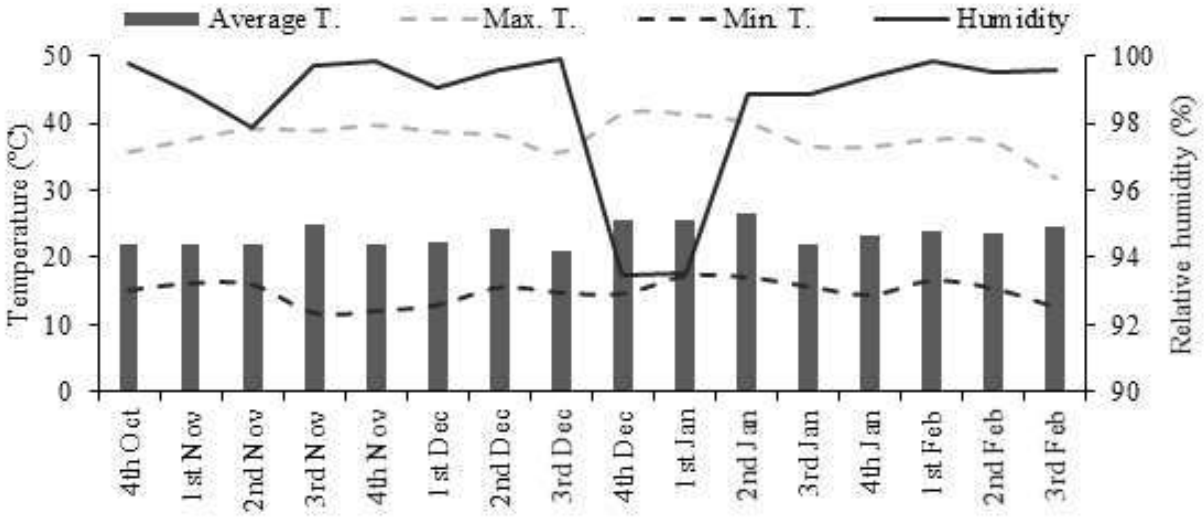


Figure 2 – Average, maximum, minimum temperatures (°C) and relative humidity (%) according to the weeks of *I. paraguariensis* vegetative propagation experiments.
Figura 2*– Temperatura média, máxima, mínima (°C) e umidade relativa (%) de a acordo com as semanas dos experimentos de propagação vegetativa de* ***I. paraguariensis****.*

population treatments. Each repetition consisted of ten cuttings, totaling five repetitions per individual.

2.2 Epicormic sprouting from detached branches

Branches from the same previous mother plants were also cut and collected, keeping the same genotypes. They were collected with a manual pruning saw, removing the remaining sprouts and leaves. Longer branches were divided into smaller ones, separated individually, varying between 20 cm and 50 cm long. Morphologically, two types of branches were obtained, but were not considered as treatments in this experiment. The first type being green materials or with some initial degree of lignification, formed from the buds of the year of the mother plants' canopy (tip), with a general average diameter (and standard error) of 13.3 (± 0.2) mm. The second type being a woody, bifurcated material of larger diameters of general average (and standard error) of 32.9 (± 0.4) mm, since they did not present branches with buds of the year at the collection time (mainly the material from TB and UR). Both typologies were collected above DBH.

The ends of the branches were sealed using plastic and tape to prevent excessive dehydration, stored in 50 L trash bags, lightly humidified and sealed. They were transported to the Forest Nursery of the University of the State of Santa Catarina (UDESC) in Lages (SC) and were staked in the vertical position, following their natural disposition, in trays (20 L) containing vermiculite, and were stored under the same conditions as the cutting experiment. Each tray with branches represented one population.

Since the number of branches may not have been statistically adequate to be evaluated at the genotype level, the experiment was carried out considering only the populations. The beginning and finishing of sprouting were evaluated every 30 days, observing the number of branches with new sprouts in percentage, the number of sprouts produced, and the length of sprouts in centimeters. In addition, three categories were determined for possible uses of these sprouts (adapted from Wendling et al., 2013), considering: greater than or equal to 4 cm, which could be used for cutting; between 4 cm and greater than or equal to 2 cm, which could be used for micropropagation, and; less than 2 cm, without immediate use.

The experiment was carried out in a completely randomized design, in a 4x2 factorial scheme, with "A" being the four populations (CT, PL, TB and UR) and "B" the evaluation periods (every 30 days), consisting of two evaluations. Each branch was considered a repetition, totaling ten repetitions for each population.

2.3 Statistical analysis

The data normality through was verified the Shapiro-Wilk test (p<0.05) and transformed by the Box Cox test if necessary. Then, the data was submitted to ANOVA followed by the Scott-Knott average test (p<0.05). The statistics were performed using the R statistical software (R CORE TEAM, 2020).

Aiming a higher reliability to the analyses performed, an Experimental Accuracy (EA%) in percentage was calculated for significant treatments by ANOVA (Equation 1). It evaluates the degree of confidence in the experiment, observing values for selection purposes, considering the ranges: less than or equal to 50% (low); between 50% and 70% (moderate); between 70% and 90% (high), and; greater than 90% (very high) (Navroski et al., 2013). EA% is given by the formula:

$$EA\% = \sqrt{(1-(1/Fcal))} \times 100 \quad \text{(Eq. 1)}$$

Whereas: $Fcal = F$ value obtained in the ratio of the mean squares of the treatment ($QMtreat$) and residue ($QMres$), from analysis of variance (ANOVA).

3. RESULTS

3.1 Cuttings of different genotypes and populations

There was no statistical significance for the rooting of cuttings for the populations. For the genotypes, all variables were significant, but only 17 genotypes presented live cuttings at the end of the experiment (Table 1). The results of EA% were considered at least high (>70%) for all variables in both schemes.

The survival of cuttings was below 15% considering the populations and varied between

Table 1 – Percentages of survival, callus formation, rooting, new sprouts, and permanence of original leaves of *I. paraguariensis* cuttings of four native populations of Santa Catarina state, Brazil.
Tabela 1 *– Porcentagens de sobrevivência, formação de calos, enraizamento, novas brotações e permanência das folhas originais de estacas de quatro populações nativas de* ***I. paraguariensis*** *do estado de Santa Catarina, Brasil.*

Population	Survival	Callus formation	Rooting	New sprouts	Permanence of original leaves
	%				
CT	1.4 c*	1.2 c	0.0	1.0 b	0.4 b
PL	2.6 b	2.2 c	1.0	2.2 b	0.4 b
TB1	2.2 a	6.8 a	1.2	8.6 a	2.6 a
UR	3.0 b	3.0 b	0.6	2.0 b	1.4 a
p	<0.0001	<0.0001	0.1190	<0.0001	0.0017
EA%	99.6	96.3	ns	98.7	87.8
Genotypes**	%				
CT 1	2.0 f	0.0 d	0.0 c	2.0 f	0.0 b
CT 5	2.0 f	2.0 d	0.0 c	2.0 f	0.0 b
CT 6	4.0 f	4.0 d	0.0 c	4.0 e	0.0 b
CT 9	2.0 f	2.0 d	0.0 c	0.0 f	2.0 b
CT 10	4.0 f	4.0 d	0.0 c	2.0 f	2.0 b
PL 6	6.0 e	6.0 d	2.0 c	6.0 e	0.0 b
PL 7	20.0 c	16.0 b	8.0 a	16.0 c	4.0 b
TB 1	46.0 a	32.0 a	6.0 a	32.0 a	12.0 a
TB 2	12.0 d	4.0 d	2.0 c	8.0 d	2.0 b
TB 3	28.0 b	20.0 b	4.0 b	18.0 c	8.0 a
TB 6	2.0 f	2.0 d	0.0 c	2.0 f	0.0 b
TB 7	28.0 b	10.0 c	0.0 c	26.0 b	4.0 b
TB 8	6.0 e	0.0 d	0.0 c	2.0 f	0.0 b
UR 5	2.0 f	2.0 d	0.0 c	0.0 f	0.0 b
UR 8	4.0 f	4.0 d	0.0 c	0.0 f	2.0 b
UR 9	12.0 d	12.0 c	2.0 c	10.0 d	8.0 a
UR 10	12.0 d	12.0 c	4.0 b	8.0 d	4.0 b
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
EA%	99.2	96.2	75.2	97.4	86.5

CT: Catanduvas; PL: Painel; TB: Três Barras; UR: Urupema; EA%: Experimental Accuracy in percentage; ns: not significant; *Averages followed by the same letter do not differ from each other according to the Scott-Knott average test (p<0.05); **Only the surviving genotypes are present, 17 out of 40.
*CT: Catanduvas; PL: Painel; TB: Três Barras; UR: Urupema; EA%: Acurácia Experimental em porcentagem; ns: não significativo; *Médias seguidas de mesma letra não se diferem de acordo com o teste de médias Scott-Knott (p<0,05); **Somente os genótipos sobreviventes estão presentes, 17 de 40.*

46% and 2% for the surviving genotypes. The TB population presented the highest survival percentage, at least four times higher than the others. This population presented six genotypes alive, highlighting TB1, TB3 and TB7, all of them with at least 25% of survival. The populations of CT had the lowest survival average (five genotypes alive), followed by PL (two genotypes) and UR (four genotypes).

Callus formation was similar to the survival percentages of the CT, PL and UR populations, which indicates that most of the alive cuttings presented calluses (less than 0.4% of difference between variables). This same relation was observed at the genotype level in all populations. On the other hand, the population of TB did not follow this perspective, with approximately 50% of cuttings with calluses.

Rooting did not reach 2% and 10% for the population and genotypes, respectively. The CT population did not obtain any rooted cuttings, while the populations of UR, PL and TB presented 0.6%, 1.0% and 1.2%, respectively, without statistically significance. For the genotypes, only seven (out of 40) presented at least one rooted cutting, highlighting the genotypes of PL7 and TB1, with more than 5% rooting.

Regarding the presence of new sprouts and permanence of original leaves, both presented a similar response to the callus formation. For the populations of CT, UR and PL, almost every cutting alive presented new sprouts and leaves, while TB presented a percentage nearly two-thirds of its survival percentage. Furthermore, the TB population and its genotypes also presented low results of original leaves permanency when compared to survival.

3.2 Epicormic sprouting from detached branches

Epicormic sprouting started approximately 20 days after the branches were staked in the mini tunnel system. However, only two evaluations (30 and 60 days) occurred, as sprouting and former sprouts were no longer detected on further evaluations.

A significant factorial interaction between the populations and the evaluation periods for sprouting and number of sprouts was detected, while the interaction for the length of sprouts was not detected (Table 2). For this variable, only the populations were statistically different (p<0.0001; EA%=97.1) while the evaluations were not (p=0.5198; EA%=ns).

The first evaluation provided the best results for branches with sprouts as well as for number of sprouts, with TB and UR producing more than 25 sprouts on average, while other populations presented half as many sprouts for this period. However, there was a drastic decrease in sprouting and on the average number of sprouts during the second evaluation, mainly for UR, which had the greatest decrease among all populations.

Table 2 – Percentage of branches with new sprouts, number of sprouts and length of sprouts in centimeters of *I. paraguariensis* detached branches of four native populations of Santa Catarina state, Brazil.
Tabela 2 *– Porcentagem de galhos com novas brotações, número de brotações e comprimento de brotações em centímetros de galhos destacados de quatro populações nativas de* ***I. paraguariensis*** *do estado de Santa Catarina, Brasil.*

Evaluation	Population				
	CT	PL	TB	UR	Average
Branches with new sprouts (%) (p=0.0004; EA%=91.9)					
30 days	100.0 aA*	100.0 aA	90.0 aA	100.0 aA	98.0
60 days	20.0 bB	20.0 bB	70.0 aA	10.0 bB	34.0
Average	60.0	60.0	80.0	55.0	66.0
Number of sprouts (p=0.0006; EA%=91.6)					
30 days	12.8 bA	12.8 bA	28.8 aA	26.9 aA	19.4
60 days	0.7 bB	0.6 bB	7.5 aB	0.1 bB	2.4
Average	6.8	6.7	18.2	13.5	10.9
Length of sprouts (cm) (p=0.6523; EA%=ns)					
30 days	1.0	0.9	2.8	0.7	1.2
60 days	0.6	0.9	3.0	0.1	1.1
Average	0.8 b	0.9 b	2.9 a	0.4 b	1.2

CT: Catanduvas; PL: Painel; TB: Três Barras; UR: Urupema; EA%: Experimental Accuracy in percentage; ns: not significant; *Averages followed by the same letter do not differ from each other according to the Scott-Knott average test (p<0.05).
CT: Catanduvas; PL: Painel; TB: Três Barras; UR: Urupema; EA%: Acurácia Experimental em porcentagem; ns: não significativo; *Médias seguidas de mesma letra não se diferem de acordo com o teste de médias Scott-Knott (p<0,05).

As for length, the TB population presented sprouts at least three times longer than all other populations. The CT and PL populations presented sprouts of nearly 1 cm during both evaluations. Meanwhile, UR had the shortest sprouts, meaning that this population had a high number of sprouts of short length during the first evaluation.

Regarding the categorization the epicormic sprouts according to their length, these presented considerable differences between the evaluation periods of the present study (Figure 3). During the first evaluation, only TB presented a higher quantity of sprouts longer than 4 cm as well as between 2 cm and 4 cm (both approximately 35 sprouts). For the second evaluation, even though the branches of all populations presented a considerable decrease in the sprouts number, the ones that remained continued their development. In this scenario, TB had an increase of the quantity of sprouts longer than 4 cm (approximately 57).

4. DISCUSSION

4.1 Cuttings from different genotypes and populations

EA% was considered at least high (>70%) for both populations and genotypes. EA% analysis aims to asses, alternatively, the degree of confidence in the experiment, in the performed analyses and in the statistics applied. It may also be used as a criterion for further selection of among different materials (Navroski et al., 2013). Nascimento et al., (2022) highlights elevated EA% (>90%) values for the same variables tested in the present search, in two populations of the same species. As well as in the present search, the authors also observed a high EA% (>80%) for their genotypes, thus indicating that the conducted analyses were carried out with precision and reliability.

Even though the plant material of *I. paraguariensis* was collected at the best time for its survival and rooting according to literature, which is spring (Stuepp et al., 2017a; Sá et al., 2018), they presented little adaptation to the new climate conditions. This was possibly affected by the unfavorable conditions during the experiment found inside the mini tunnel (Figure 1). Although humidity (>90%) was ideal for the maintenance of propagules (Xavier et al., 2013), the excessive temperatures during daytime (sometimes above 40 °C) and the thermal amplitude between day and night (up to 25 °C) were possibly the main reason for the low survival and further results.

Even though rooting environments of low technological investments may not present significant

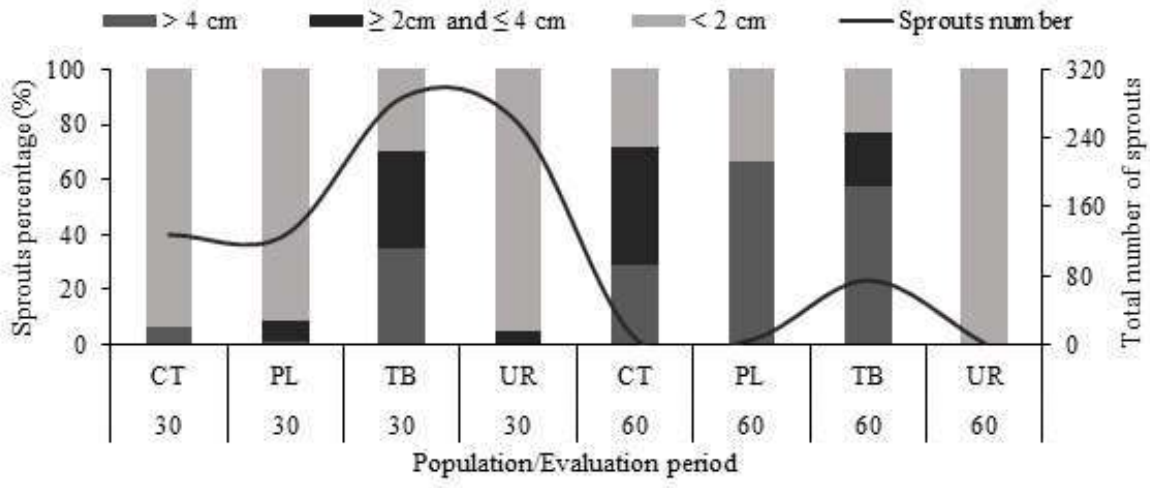


Figure 3 – Total number and percentage of sprouts in three categories of *I. paraguariensis* detached branches of four native populations of Santa Catarina state, Brazil, in two evaluations periods; CT: Catanduvas, SC; PL: Painel, SC; TB: Três Barras, SC; UR: Urupema, SC.

***Figura 3** – Número total e porcentagem de brotações em três categorias de galhos destacados de quatro populações nativas de **I. paraguariensis** do estado de Santa Catarina, Brasil, em dois períodos de avaliação; CT: Catanduvas, SC; PL: Painel, SC; TB: Três Barras, SC; UR: Urupema, SC.*

differences in the maintenance of *I. paraguariensis* cuttings from the ones with more technological advancements, they are more susceptible to external environmental variations, mainly temperature (Stuepp et al., 2017a). According to Nascimento et al., (2020), when submitted to two different rooting environments, one with temperature control (adapted for *Eucalyptus* sp. cutting) and the other being a simplified structure, *I. paraguariensis* cuttings presented a higher survival percentage inside the non-controlled temperature environment (approximately 20% more). It is still unknown for completely the survival behavior this species' cuttings regarding temperature variance, besides the necessity of a low variance, which was not present in this experiment.

This variation in survival can also be a direct reflection of the plant material origin, easily observed between and within the populations of the study. It is possible that origin places with lower annual temperatures and lower solar incidence, such as PL and UR, produce materials with greater sensitivity to environmental factors of the rooting environment. The genetics of the mother plant seems to directly influence the survival of propagules (Vieira et al., 2021), but it can be favored or affected according to the climatic conditions of the mini tunnel. Corroborating with this perspective, Nascimento et al., (2022) observed that the survival of a genotype can vary up to 15% and 20% for UR and TB populations, respectively, according to the rooting environment, while the differences considering genotypes may be higher, up to 50% between the most and least adapted.

For rooting, it is possible that the high degree of maturation of the propagules used, which was observed through the presence of calluses on cuttings, may have been the main factor for the low rooting. The ontogenetic age is a crucial factor for the rooting success of propagules, as a reinvigorated material present a higher possibility due to its higher vigor (Stuepp et al., 2018). Since no reinvigoration techniques were used for the rescue of vegetative material, it is possible that the sprouts collected from the canopy used for cutting presented a higher maturation degree. Cuttings of *I. paraguariensis* tend to have their rooting directly, hardly occurring after the formation of calluses (Stuepp et al., 2017a), and this factor can be easily observed in the present search. However, the low rooting came directly from cuttings that had calluses, indicating that, even though it is initially an undifferentiated tissue, it can still produce roots if remained for a longer period inside the rooting environment (Stuepp et al., 2017b).

Even though the hindering environmental conditions of the present work as well as the use of mature material, some genotypes still presented rooted cuttings, mainly PL7, TB1 and TB3. Recent searches (Nascimento et al., 2020, 2022; Vieira et al., 2021) show a great variance of rooting from individuals of the same population and established clones. This relation is easily seen in the present study, with a great variability of rooting results from individuals in the same populations. When the containers used for the cutting of *I. paraguariensis* is considered, there is the formation of longer and adventitious roots if the material has more contact with soil, especially when using 1.5 L and 3 L containers (Pimentel et al., 2017). Even though 20 L trays were used as containers in the present search, rooting and root development was minimal.

The presence of leaves in cuttings is crucial for their rooting, since these organs are responsible for the production of auxins (Taiz et al., 2015). In general, there was a considerable sprouting of the surviving cuttings from all populations and genotypes. It is possible that the production of new sprouts and leaves are high dependent on the genotype used, being also favored or affected by the rooting environment.

However, the same did not happen for the original leaves, as most of the cuttings did not present them in the final evaluation, which may also justify the low rooting. The loss of the original leaves and the production of new sprouts may be a response of an excessive stress undergone by the cuttings, using their remnant energy on new aerial organs. The collection of *I. paraguariensis* vegetative material for propagation during spring can maintain more than 75% of cuttings with leaves, considering a rooting environment with irrigation though nebulization (Sá et al., 2018). Therefore, the leaf fall in the present study may be due to the high stress conditions of the rooting environment, as previously cited, being increased or decreased according to the genotype tested.

4.2 Epicormic sprouting from detached branches

Even though some factors were not considered when this experiment was carried out, I. e., the two

branches' typologies, their ontogenetic age and the storing position of the branches (vertical or horizontal), it is important to cite the possible consequences of these post assessed conditions.

The two types of branches, which had singular shapes and diameters, may have caused the differences of sprouting and number of sprouts observed (Nascimento et al., 2018). The thicker and more lignified branches were of larger diameters (, what possibly allowed a greater number of buds to be stored during their development, enabling higher sprouting percentages and number of sprouts for the populations of TB and UR. Materials with good surface areas have greater chances to sprout and in higher quantities, as they develop at a slow pace, thus storing more dormant buds (Stuepp et al., 2016).

The ontogenetic age may have affected the development of the branches as well, as canopy material may have had lower vegetative vigor than those obtained in the lower parts of the mother plants. This might be true between the two typologies, but not between populations, as branches from similar heights of the mother plants were obtained between TB and UR, which diverged on the results. Therefore, it is possible that the determining factor was the populations themselves, resulting in a higher/lower vigor and amounts of energy reserves for those materials. Furthermore, the storing environment also had a crucial role in the development of the sprouts, as this material may have oxidized faster for UR, a material of higher sensitivity. when compared to TB, due to the higher temperatures of the mini tunnel system (Xavier et al., 2013).

The branches' storing position can affect their useful life, in which Nascimento et al., (2018) tested the epicormic sprouting of *I. paraguariensis*' branches stored horizontally, observing their development up to 300 days. However, Wendling et al., (2013) determined that vertically stored sprouts can produce good quality sprouts up to 70 days, being a similar result to the present search. The relation between the branches' diameter and storing position could allow new interpretations and results, being an interesting subject to be tested in this species in new experiments.

Regarding the classification of the sprouts according to their length, it is possible to notice in the initial evaluation a greater number of sprouts shorter than 4 cm without an immediate use for cutting, similar to the results obtained by Wendling et al., (2013). However, sprouts between 2 cm and 4 cm could still be used for micropropagation, allowing an alternative way for the vegetative propagation of the species. Since plant improvement is a not easy task for *I. paraguariensis* due to its problems with conventional methods of selection by seeds (Wendling and Brondani, 2015), the vegetative rescue of this species is fundamental for this purpose. Therefore, as branches are easy to obtain and to produce sprouts, evaluating the sprouting variables in these materials may prove essential to the formulation of new vegetative propagation protocols.

5. CONCLUSION

The first hypothesis of this search was confirmed. A possible superiority was detected according to certain populations and genotypes for vegetative propagation of *I. paraguariensis*, highlighting Três Barras (TB). However, it is possible that the association between material maturity and excess of temperature hampered the survival of cuttings from all origins.

Both conditions of the second hypothesis of this search were confirmed. *I. paraguariensis*' detached branches produced different quantities of sprouts according to the populations. On the other hand, they were also probably affected by the different branch typologies, the harsh conditions of the storage environment, and the vertical/horizontal storage sense, which were not considered in this search.

Thus, it is recommended: I) New studies with different populations and genotypes for the vegetative propagation through cutting, testing materials of juvenile characteristics to confirm with certainty any superiorities; II) New studies with different populations for the vegetative rescue through detached branches, considering the typologies and the two storing senses. Furthermore, both recommendations can be followed by better environmental conditions for material storing.

AUTHOR CONTRIBUTIONS

Conceptualization: Nascimento B, Navroski MC, Pereira, MO, Mantovani, A. Performed the analyses: Nascimento B, Sá ACS, Corrêa BJS, Schilisting T. Analysis of results: Nascimento B, Navroski MC,

Mantovani A. Statistical analysis: Nascimento B, Navroski MC. Writing-original draft: Nascimento B. Writing-review and editing: Nascimento B. Supervision and coordination of research: Navroski MC, Mantovani A.

6. ACKNOWLEDGMENTS

We are thankful for the financial support of the FAPESC institution, process number 23038.013359/2017-71, which made this search possible. We are also grateful for the Laboratory of Propagation and Forestry Improvement and Forest Ecology for providing the means of this search as well as for all the intellectual support.

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Schatz Sá et al. *New Zealand Journal of Forestry Science* (2024) 54:17
https://doi.org/10.33494/nzjfs542024x326x
E-ISSN: 1179-5395
published on-line: 20/09/2024

RESEARCH ARTICLE Open Access

New Zealand Journal of Forestry Science

A broad analysis of vegetative rescue and propagation of *Moquiniastrum polymorphum* (Less.) G. Sancho

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(Received for publication 2 August 2023; accepted in revised form 1 July 2024)
Editor: Euan Mason

Abstract

Background: The primary method for propagating forest species is through seeds, which is cost-effective and ensures genetic adaptability to environmental changes. However, germination issues and genetic variability can hinder standardisation of productivity. In the case of *Moquiniastrum polymorphum*, a species known for its remarkable wood quality and pharmacological potential, seedling production and genetic improvement efforts have been limited. This study focused on the rescue and vegetative propagation of *M. polymorphum*, which are crucial steps for species selection and genetic enhancement.

Methods: Protocols were tested to rescue and propagate propagules from different individuals collected in Lages, Santa Catarina (2020/2022). The vegetative rescue tests were: (I) epicormic sprouting induction through girdling techniques and detached branches; and (II) influence of individuals, disposition sense and time on the epicormic sprouting of detached branches. Vegetative propagation analyses included: (I) cutting according to individuals; and (II) relation between rooting environments and individuals on cutting.

Results: The results indicated that the girdling techniques were not efficient for vegetative rescue, as only 8% of individuals produced epicormic sprouts. In contrast, detached branches showed a much higher success rate of 80% for epicormic sprouting, revealing significant differences in sprout development among individuals over time. Vertically oriented branches produced nearly three times more sprouts compared to horizontally oriented branches. Regarding vegetative propagation, certain individuals exhibited remarkable rooting rates of over 75%. However, no conclusive results were obtained when using epicormic materials or when considering different rooting environments.

Conclusions: Given the significance of rescue and vegetative propagation in plant genetic improvement and the limited research addressing these aspects in *M. polymorphum*, this study holds substantial importance for future investigations. It is recommended to expand rescue and vegetative propagation studies to encompass additional populations, different individuals, and rooting environments, further advancing our understanding and efforts to enhance this species.

Keywords: cambará, detached branches, girdling, rooting environment

Introduction

Moquiniastrum polymorphum (Less.) G. Sancho (Asteraceae), previously known as *Gochnatia polymorpha*, is a native tree species found in various forest typologies in Brazil, particularly in the Cerrado biome, Ombrophilous Forest, and Mixed Ombrophilous Forest. From an ecological perspective, it is important in both primary and secondary succession processes and thrives in nutrient-poor areas, making it a recommended choice for reforesting degraded regions (Lorenzi 2014). It is valued for its wood, which is moderately heavy, hard, and compact, rendering it well-suited for challenging environmental conditions (Faria et al. 2019). Furthermore, recent research suggests notable

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pharmacological potential, as extracts derived from its leaves have demonstrated phytotoxic effects on other plants (Pinto & Kolb 2015) and are being explored as alternative treatments for inflammatory ailments (De Moraes et al. 2019) and oxidative diseases (Bastos et al. 2022).

Despite its ecological and economic significance, *M. polymorphum* has a low germination rate. Even though seed dormancy does not occur, germination is still reported to be relatively low, ranging between 30% and 50% (Lorenzi 2014). Within the Asteraceae family, germination issues are common, and this may be attributed to factors such as seed emptiness (Chaves & Ramalho 1996). Moreover, there have been few studies on seed propagation of *M. polymorphum* (Faria et al. 2019), regardless of the importance of this method for natural selection and adaptation to adverse environmental conditions over time.

In addition to low germination rates, producing plants from seeds does not guarantee the desired silvicultural characteristics because it involves cross-fertilisation and results in completely new individuals with unique genetic makeups. In order to achieve standardisation in growth and production, the implementation of vegetative propagation techniques is necessary. Vegetative propagation takes advantage of the totipotency of plant cells, theoretically allowing the production of an entire new plant from a single vegetal cell (Xavier et al. 2013). These techniques enable direct selection of individuals with desired traits, establishing clonal gardens to produce a large quantity of individuals (Oliveira et al. 2016). However, there is currently very little research on the use of vegetative propagation for *M. polymorphum*, despite its potential for crucial vegetative improvement of the species.

Several factors directly influence the success of vegetative propagation, particularly when using a cutting technique. This method is efficient and straightforward, as it enables the selection of mature individuals and the production of significant quantities of new seedlings within relatively short timeframes (Xavier et al. 2013). Some of these influential factors include: (i) selecting the correct individual; (ii) overcoming the maturation of the mother plant; and (iii) determining the optimal rooting environment.

Firstly, rooting potential varies with genotype (Pimentel et al. 2019). Several studies have shown contrasting percentages of rooting among propagules from different genotypes within the same population (Nascimento et al. 2020), as well as among established clones (Vieira et al. 2021). On the other hand, determining such characteristics can be challenging, as they are primarily influenced by genetic and physiological factors rather than easily identifiable phenotypic traits. This complexity may hinder the process of direct selection.

Desired phenotypic characteristics in forest species often become more pronounced after certain levels of maturity of the mother plant. This poses a challenge for material collection and propagation (Wendling et al. 2014; Stuepp et al. 2018; Nascimento et al. 2018). Nonetheless, this maturation process can be reversed by using juvenile material, also referred to as ontogenetic young, which is commonly found in the dormant buds located in the lower parts of the plant's stem. Specific techniques can be applied to stimulate the development of these buds into new sprouts, enabling their collection and subsequent propagation (Stuepp et al. 2015; 2017; Nascimento et al. 2018).

One common technique to induce the production of epicormic sprouts, considered as reinvigorated material, is stem girdling, which involves interrupting the sap flow and creating an imbalance between auxin and cytokinin. This disruption promotes the development of buds below the girdled area (Santin et al. 2008). Another technique involves the use of detached branches, which are stored under optimal greenhouse conditions to stimulate epicormic sprouting. This approach facilitates subsequent steps in vegetative propagation (Wendling et al. 2013). Although not generally recommended, even ontogenetic old material, such as sprouts from canopies of trees, can still exhibit significant rooting when subjected to ideal conditions (Stuepp et al. 2017; Nascimento et al. 2020;2022).

Another crucial factor for successful vegetative propagation is the rooting environment. An efficient rooting environment should maintain a stable temperature range with minimal fluctuations and provide constant high humidity throughout the rooting process (Xavier et al. 2013). However, there is a limited number of current studies that specifically assess this variable for native tree species. In contrast to exotic forestry species like the *Eucalyptus* genus, the research on rooting environments for native species in Brazil is relatively scarce. Moreover, it's important to note that environments with temperature and humidity control, tailored to specific species, may not be suitable for others, as each species has its own unique requirements and characteristics (Nascimento et al. 2022).

Given the information presented above, several important questions arise. These questions include: Are there variations in vegetative rescue and propagation based on different individuals? Is the use of reinvigorated materials necessary? Can certain rooting environments be more suitable than others? Therefore, the objective of this study was to investigate the vegetative rescue of the species using girdling and detached branch techniques, as well as its propagation through cuttings, taking into consideration individuals and rooting environments.

Methods

Population determination and individual selection

The studied individuals were located in an experimental site of the University of the State of Santa Catarina (UDESC) in the municipality of Lages, Santa Catarina state (27°45'39" S and 50°04'36" W). The forest type is characterised as Mixed Ombrophilous Forest, also known as Araucaria Forest, with characteristics of a secondary forest in different stages of succession. According to the Köppen's classification, the climate of

the region is classified as *Cfb*, with a temperate climate and mild summers. Rainfall is evenly distributed throughout the year, without a dry season. The region has an average annual temperature of 17.0 °C and an average annual precipitation of 1,500 mm.

A forest inventory was previously conducted in the experimental area, mapping a total of 302 individuals of *M. polymorphum*. Among them, some criteria were used to select those for the experiments. Firstly, only individuals with a diameter at breast height (DBH) greater than 5 cm were selected, based on the higher availability of plant material, and ensuring their resilience in the application of abrasive treatments. Diameters were obtained using a measuring tape around the circumference at breast height (CBH, 1.3 m), which was then divided by π to obtain the DBH value. Secondly, after determining desired diameters, individuals with the greatest possible distance between them were chosen to ensure higher genetic diversity within the population of the species. Finally, only individuals free from pests and diseases, and phenotypically similar were included in the final selection. Hence, 26 individuals of *M. polymorphum* were identified for the vegetative rescue and propagation experiments (Figure 1 and Table 1). It is emphasised that the selection of individuals was carried out randomly, in accordance with the previously mentioned guidelines.

Two main items were considered in the experimental layout. The first part of the study involved the application of rescue techniques using the production of epicormic sprouts, and the experiments in this section consisted of: (I) epicormic sprouting induction through girdling techniques and detached branches; and (II) influence of individuals, disposition sense and time on the epicormic sprouting of detached branches. The second part focused on vegetative propagation using cuttings. This portion of the study involved: (I) exploratory analyses considering individuals and epicormic material; and (II) cutting according to rooting environments and individuals.

1. Epicormic sprouting induction through girdling techniques and detached branches

Nineteen *M. polymorphum* individuals were submitted to three different vegetative rescue techniques in September 2020. These techniques were: complete girdling (100% of the bark circumference), semi-girdling (50% of the bark circumference), and collection of detached branches.

The complete girdling technique was applied to the following individuals: 2, 4, 47, 68, 80, and 116, totaling six individuals. This technique involves the complete removal of the bark in a 5 cm wide strip around the circumference of the stem (100% of a ring), starting

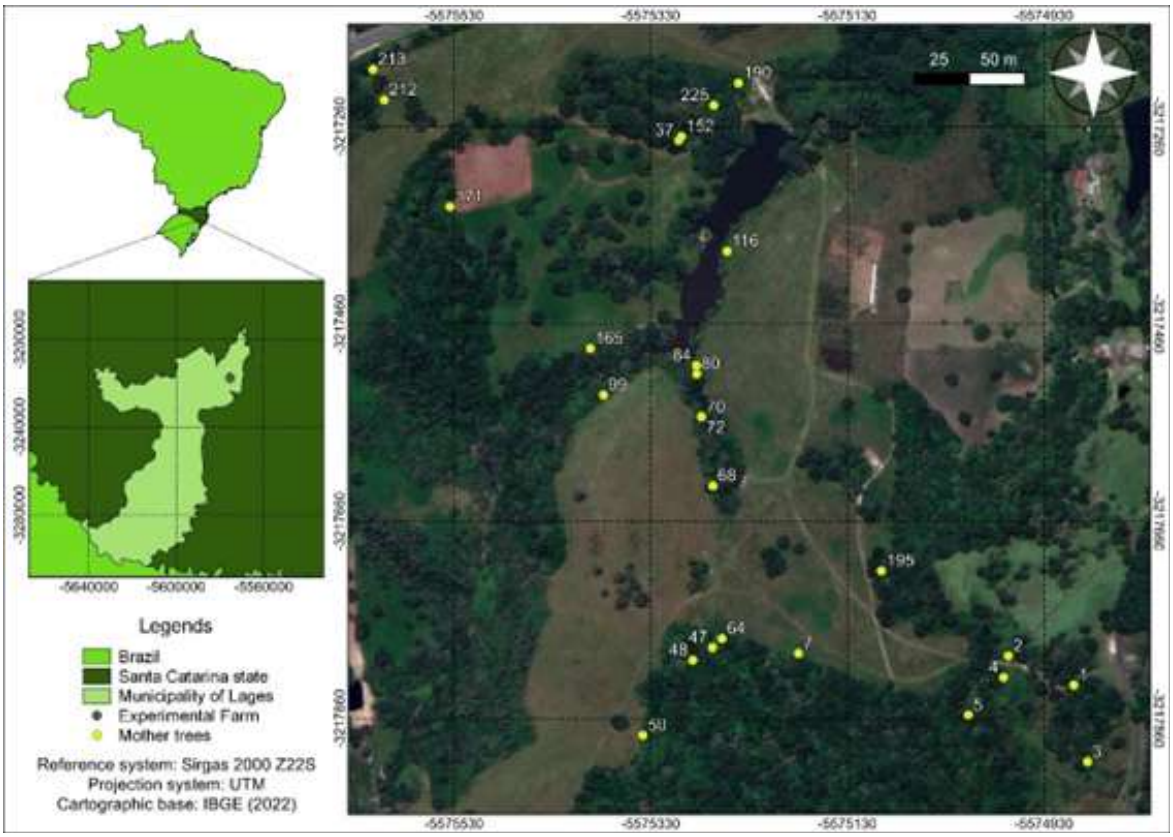


FIGURE 1: Location map of the experimental farm of the University of Santa Catarina state in Lages, Santa Catarina state, and the location of 26 out of 302 *M. polymorphum* selected trees.

TABLE 1: Dendrometric variables of each *M. polymorphum* individual tested in different vegetative rescue and propagation experiments.

***M. polymorphum* Individual**		**Property**		**Testing regime(s)[3]**
Number	**Identification code**	**CBH[1] (cm)**	**DBH[2] (cm)**	
N. 1	1	153.7	48.9	*Rb*
N. 2	2	115.5	36.8	*Rg, Rb*
N. 3	3	159.8	50.9	*Gn, Re*
N. 4	4	132.0	42.0	*Rg, Rb, Gn*
N. 5	5	58.5	18.6	*Rs*
N. 6	7	21.5	6.8	*Gn, Re*
N. 7	37	160.0	50.9	*Rs, Rb*
N. 8	47	98.0	31.2	*Rg*
N. 9	48	54.0	17.2	*Rs*
N. 10	50	159.8	50.9	*Gn, Re*
N. 11	64	85.0	27.1	*Gn, Re*
N. 12	68	79.0	25.1	*Rg, Rb, Gn, Re*
N. 13	70	58.0	18.5	*Gn, Re*
N. 14	72	49.8	15.9	*Rs, Rb*
N. 15	80	166.4	53.0	*Rg, Gn, Re*
N. 16	84	103.0	32.8	*Rs, Rb, Gn, Re*
N. 17	99	16.6	5.3	*Gn*
N. 18	116	32.1	10.2	*Rg*
N. 19	152	32.3	10.3	*Gn, Re*
N. 20	165	18.9	6.0	*Gn*
N. 21	171	52.5	16.7	*Gn, Re*
N. 22	190	64.6	20.6	*Gn, Re*
N. 23	195	142.0	45.2	*Rs*
N. 24	212	38.5	12.3	*Gn*
N. 25	213	36.0	11.5	*Gn, Re*
N. 26	225	40.9	13.0	*Gn*

[1] CBH = Circumference at breast height in centimeters
[2] DBH = Diameter at breast height in centimeters
[3] *Rb* = Vegetative rescue using detached branches; *Rg* = Vegetative rescue using complete girdling; *Rs* = Vegetative rescue using semi-girdling; *Gn* = Vegetative propagation according to individuals; and *Re* = Vegetative propagation according to the rooting environment.

from 20 cm above the soil level. Careful attention was given to ensure that only the superficial part of the bark was removed, without damaging the underlying wood, with a well-sharpened machete.

The semi-girdling technique was applied to the following individuals: 1, 5, 37, 68, 72, and 84, totalling six individuals. This technique followed the same principles as the complete girdling technique, with the only difference being that only half of the length of the bark ring was removed (50% of the ring). The procedure ensured that the bark was carefully removed along a specific section of the stem circumference, while leaving the other half intact.

Detached branches were collected from a total of seven individuals: 1, 2, 4, 37, 68, 72, and 84. For each individual, three branches were carefully selected and pruned using a chainsaw, at an estimated height varying from 1.5 m to 2.5 m. The collected branches, which were between 30 cm and 50 cm long, were then prepared by removing any remaining sprouts and leaves. To prevent excessive moisture loss, the branches were placed in a Styrofoam box filled with cooled water. Then they were transported to the forest nursery of the University of Santa Catarina State, located in Lages, Santa Catarina state. Upon arrival at the nursery, the branches were vertically staked into containers filled with fine vermiculite to provide support. The upper cut section of the branches was sealed with plastic film, aiming to prolong their lifespan. To create an optimal environment for sprouting, all material was kept in a mini tunnel system inserted in a shaded greenhouse (50%). The mini tunnel system employed micro sprinkler irrigation, which consisted of ten irrigation cycles lasting ten minutes each.

Following the girdling treatments, sprouting success was checked at 384 days. For the detached branches, a single evaluation was conducted at 70 days after the

treatment application. The variables examined included tree or branch survival percentage, sprouting percentage, number of sprouts, and length of sprouts in centimeters.

To determine tree or branch survival, living trees were identified as those that showed no signs of decay or deterioration, such as leaf loss or drying symptoms, despite the initial injury caused by the girdling technique. Similarly, living branches were recognised by their unchanged coloration, absence of fungal growth, and lack of drying conditions. Effective sprouting for trees was determined by the presence of new sprouts measuring at least 0.5 cm in length below the girdled area. For branches, any new sprouts of at least 0.5 cm, regardless of their position, were considered as evidence of effective sprouting. Only sprouts directly originating from the tree's or branch's stem were counted when assessing the number of sprouts. Secondary sprouts that emerged from primary sprouts were not included in the count. The length of sprouts was measured using either a millimeter ruler or a millimeter tape, ensuring accurate measurements of the sprout's elongation.

The experiment was carried out using a completely randomised design, which included three different vegetative rescue treatments: complete girdling, semi-girdling, and collection of detached branches. Each tree subjected to the girdling techniques was considered as a repetition, resulting in six repetitions for each treatment. In the case of the detached branches, three branches were collected from each tree, and the average of the three branches was considered as a single repetition. Therefore, there were a total of seven repetitions for the detached branches treatment.

2. Influence of individuals, disposition and time on the epicormic sprouting of detached branches

From the previously mentioned *M. polymorphum* individuals, an additional two sets of three branches were collected from each individual during the same collection period. The branches were subjected to the same transportation and preparation procedures as described earlier, and they were kept under the same climatic conditions within the mini tunnel system. Furthermore, the same variables under the same conditions were also assessed.

In this experiment, three different conditions were evaluated in relation to the sprouting of the branches. The first condition was the effect of the different individuals on the sprouting response. The second condition examined the influence of the disposition of the branches on sprouting, considering branches maintained in the vertical and horizontal positions. For the branches maintained horizontally, those were laid over containers of tubes, avoiding direct contact with the ground. For this condition, both sides of these branches were sealed with plastic film. Lastly, the evaluation periods were another factor that was assessed. The branches were evaluated every ten days for a total of nine assessments.

This experiment followed a completely randomised design and involved two patterns of evaluation. In the first pattern, a factorial scheme of 7 x 2 was employed. Factor A comprised the seven individuals that were tested, while factor B represented the two different branch dispositions: vertical and horizontal. For this condition, a single evaluation was considered at 70 days after the experiment implementation. In the second pattern, a factorial scheme of 2 x 9 was implemented. Factor A represented the two branch dispositions (vertical and horizontal), and factor B comprised the evaluation periods, with measurements taken at intervals of ten days. The population values were considered in this condition.

3. Exploratory analyses considering individuals and epicormic material

In the second part of the study three experiments were conducted concurrently in November 2020 to investigate the rooting potential of *M. polymorphum* cuttings. The aim was to understand the factors of individuals and ontogenetic age influencing rooting success and to optimise the vegetative propagation of the species, taking into consideration two simple analyses.

Experiment I aimed to evaluate the rooting ability of cuttings derived from different individuals. Canopy sprouts were selected as the source material for the cuttings. Material was collected from a total of 17 individuals, at an estimated heigh varying from 1.5 m to 2.5 m, represented by the following individuals: 3, 4, 7, 50, 64, 68, 70, 80, 84, 99, 152, 165, 171, 190, 212, 213 and 225. These individuals were considered as a population sample for the subsequent experiment.

Experiment II involved a comparison between cuttings derived from canopy sprouts and epicormic sprouts obtained from detached branches. The epicormic sprouts were provided by the previously experiments of detached branch tests, approximately 90 days after the previous test installation. The aim was to assess whether there were any differences in the rooting potential between these two types of cuttings, considering their ontogenetic age, with canopy sprouts representing older growth and epicormic sprouts representing younger growth. For canopy material, the population results obtained from Experiment I were considered.

The sprouts, whether obtained from the canopies of the trees or the detached branches, were collected using pruning shears. Sprouts collected from the canopy were carefully stored in a Styrofoam box filled with cooled water to prevent excessive moisture loss during transportation. These boxed sprouts were then transported to the forest nursery of the University of Santa Catarina state. Epicormic sprouts, on the other hand, were obtained from the branches that were already present and maintained at the nursery. These sprouts were collected on-site and processed immediately.

To prepare the sprouts for rooting, they were carefully sectioned into cuttings using a bevel cut of approximately 8 cm (with a tolerance of ± 2 cm). During the sectioning process, one or two leaves were retained, with a reduction of approximately 50% of the total leaf area. This was done to prevent excessive transpiration. To ensure the quality and standardisation of the cuttings, the apical part and/or the most lignified

sections of the sprouts were discarded. This step aimed to provide uniformity in the sectioned material used for the experiment. Prior to implantation in the substrate cuttings were kept in cooled water to maintain their hydration and prevent desiccation.

The prepared cuttings were inserted into tubes with a volume of 180 cm^3. These tubes were filled with a commercial substrate consisting of a mixture of peat, vermiculite, organic waste classified as "A," and limestone. The substrate had a density of 130 kg m^{-3}, a water retention capacity of 300% m/m, and a pH of 5.5. To provide essential nutrients for the development of the rooted cuttings, 6 g L^{-1} of a controlled-release fertiliser with a composition of 15-9-12 (NPK) was added to the substrate. This addition aimed to ensure the continuous supply of nutrients during the rooting and subsequent growth stages of the cuttings. All trays containing the cuttings were placed in the mini tunnel system mentioned earlier.

The following variables were evaluated after 90 days for both experiments in percentage: survival, callus formation, rooting, presence of new sprouts, and retention of original leaves. The percentage values for all variables were calculated based on the total number of cuttings used in the experiments. Live cuttings were defined as those demonstrating signs of vitality, including live wood, mature leaves, or young sprouts, irrespective of their successful rooting outcome. Callus formation was ascertained by the presence of an undifferentiated mass of cells at the base of the cuttings, regardless of their rooting status. Rooted cuttings were identified when they exhibited the development of root primordia measuring at least 5.0 mm in length. Cuttings with new sprouts were characterised by the emergence of fresh leaves subsequent to the cutting process. The assessment of original leaves involved determining their continued presence on the cuttings at the time of evaluation.

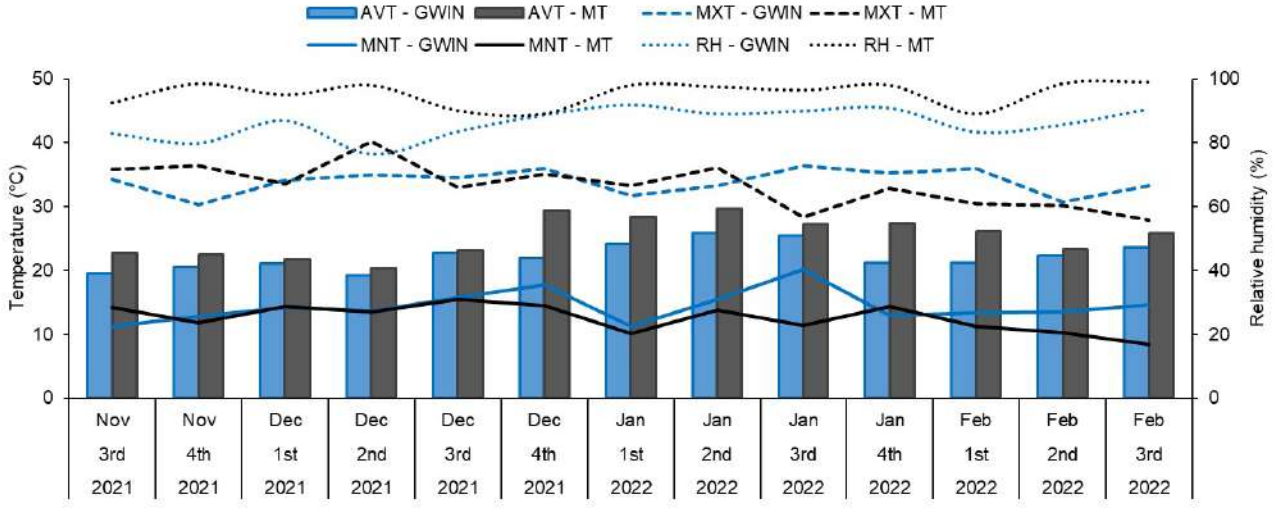
Both experiments conducted in this section followed a completely randomised design, each with its specific response variables. In experiment I the focus was solely on the cutting process of the 17 individuals. Each individual was represented by 18 repetitions, and within each repetition, nine cuttings were assessed, resulting in a total of 162 cuttings per individuals. Experiment II involved the comparison between canopy and epicormic material obtained from detached branches. Due to the limited availability of suitable sprouts for cutting in the detached branches, the number of repetitions was reduced to six. Within each repetition, seven cuttings were examined, resulting in a total of 42 cuttings.

4. Cutting according to rooting environments and individuals

In the third part of the study the rooting of *M. polymorphum* cuttings was tested using seven individuals: 3, 7, 50, 64, 80, and 190; and two distinct rooting environments. The two rooting environments assessed were as follows (Figure 2):

(I) Mini Tunnel System (MT): The MT was a simplified structure located within a shade house at the Forest Nursery of UDESC in Lages, Santa Catarina state (this structure was previously mentioned, used for storing the detached branches in a previous methodology). The MT featured automatic irrigation using micro sprinklers, with four daily irrigation sessions scheduled at 9:00 AM, 12:00 PM, 3:00 PM, and 6:00 PM. Each irrigation session lasted for ten minutes. The characteristics and setup of the MT were previously described by Pereira et al. (2019).

FIGURE 2: Temperature (°C) and relative humidity (%) of the mini tunnel system (MT) and the greenhouse with intermittent nebulisation (GWIN) during the weeks of experiment of *M. polymorphum* individuals cuttings.



AVT: Average temperature; MXT: Maximum week's temperature assessed; MNT: Minimum week's temperature assessed; RH: Relative humidity.

(II) Greenhouse with Intermittent Nebulization (GWIN): The GWIN represented a more advanced technological structure situated in a forestry company located in the municipality of Otacílio Costa, Santa Catarina state. The GWIN was equipped with automatic temperature control, maintaining temperatures between 20 °C and 30 °C using a thermostat. The humidity levels in the GWIN were consistently maintained above 70.0%. The details and specifications of the GWIN system were previously documented by Nascimento et al. (2020; 2022).

All the plant material utilised in these analyses was collected from the canopies of the trees in November 2021. The transportation, preparation of the material, establishment of the experimental area, and evaluation of the variables followed the same protocols and procedures as the previous cutting experiments. After material preparation, one set of repetitions of each individual was transported to GWIN. During transportation, the material was frequently spayed with water to avoid its excessive transpiration.

This analysis followed a completely randomised design with a factorial scheme of 7 x 2. The factor A represented the seven individuals tested, and the factor B represented the two rooting environments (MT and GWIN). Each individual was represented by six repetitions, and each repetition consisted of nine cuttings, resulting in a total of 54 cuttings for each rooting environment.

Statistical analyses

All data were subjected to preliminary analyses to ensure they met the assumptions required for statistical tests and, if required, a Box-Cox transformation was used. A Shapiro-Wilk test ($P > 0.05$) was conducted to assess normality of the data, while a Bartlett test ($P > 0.05$) was used to examine homogeneity of variance. Once these assumptions were confirmed, analysis of variance (ANOVA, $P < 0.05$) was performed. For experiments with a single factor, One-way ANOVAs were employed, while two-way ANOVAs were used for experiments with multiple factors. With statistical significance being met, post-hoc comparisons were conducted using the Scott-Knott test ($P < 0.05$). Additionally, for variables that involved multiple evaluations of the detached branches, a regression analysis of the dependent variables was performed, taking into consideration the adjusted R^2 value. The statistical software SISVAR, version 5.6 (Ferreira 2011), was utilised for all statistical analyses.

Summary of experiments

Figure 3 provides a visual representation that highlights the scope and design of each experiment, including the aspects evaluated and the evaluation time.

FIGURE 3: Vegetative rescue and propagation experiments of *Moquiniastrum polymorphum* assessing: (1) Epicormic sprouting induction through girdling techniques and detached branches; (2) Influence of individuals, disposition sense and time on the epicormic sprouting of detached branches; (3) Exploratory analyses considering individuals and epicormic material; and (4) Cutting according to adventitious rooting environments and individuals.

Session		Experiments	Evaluation time	Design
1	I	Complete girdling Semi-girdling Detached branches	→ For 384 days (3x) → At 70 days	→ Single factor Vegetative rescue techniques Population evaluated
2	I	Different individuals X Branches disposition (vertical and horizontal)	→ At 70 days	→ Factorial 7X2 1st factor = individuals 2nd factor = disposition
	II	Branches disposition (vertical and horizontal) X Evaluation periods	→ For 90 days (10x)	→ Factorial 2X9 1st factor = disposition 2nd factor = number of evaluations
3	I II	17 individuals Materials source (canopy and branch sprouts)	→ At 90 days	→ Single factor 17 individuals (assessed separately) → Population evaluated
4	I	Different individuals X Rooting environment	→ At 90 days	→ Factorial 7 X 2 1st factor = individuals 2º factor = rooting environments

Results

1. Epicormic sprouting induction through girdling techniques and detached branches

The statistical analysis was not conducted in this experiment due to unfavorable results obtained from the girdling techniques. As a result, a meaningful comparison between treatments could not be performed (Table 2).

The selected trees underwent two assessments prior to the final evaluation, conducted at 32 and 209 days, respectively. However, no signs of sprouting were observed during these assessments. It was only during the final evaluation at 384 days that a single girdled mother tree with one epicormic sprout measuring approximately 47 cm was identified. In contrast, detached branches demonstrated a considerable potential for rescuing epicormic material, with over 80% of the branches sprouting. On average, each branch produced more than 6 sprouts measuring approximately 3 cm.

2. Influence of individuals, disposition and time on the epicormic sprouting of detached branches

None of the variables assessed showed significant differences for both factors ($P > 0.05$). However, individuals ($P = 0.0119$) and disposition ($P < 0.0001$) were significant for sprouting, while only individuals ($P = 0.0062$) was significant for the number of sprouts, and disposition ($P = 0.0001$) was significant for the length of sprouts (Table 3).

Sprouting was nearly six times more prevalent in vertically stored branches, with over 80% of branches exhibiting epicormic sprouts, compared to horizontally stored branches. Among the individuals, almost 80% of horizontally stored branches did not sprout, with only individuals 4 and 84 showing signs of sprouting. Furthermore, these two individuals displayed statistically superior sprouting compared to the others, with over 65% sprouting.

Regarding the number of sprouts, only individual 84 demonstrated superiority, with more than twice as many sprouts as individual 2. Although not statistically significant in the factorial scheme, it is worth noting that vertically stored branches had a higher number of sprouts compared to horizontally stored branches, with an average difference of nearly eight sprouts. Therefore, vertically stored branches facilitated almost nine times more effective sprout development compared to horizontally stored branches. These conditions are further illustrated when examining the sprouting and sprout development over time within the population (Figure 4).

Sprouting was observed at the 20-day evaluation in half of the materials from vertical branches, with an average of nearly 2 sprouts, but with a length of less than 1 cm. On the other hand, a single horizontally stored branch showed negligible sprouting at the third evaluation for both number and length.

Maximum sprouting was detected at 80 days for vertical branches, with 90% of the materials exhibiting sprouts. In contrast, maximum sprouting achieved for horizontal branches was 14% at 60 days, which remained constant until the final evaluation. The maximum average number of sprouts observed was 6.1 at the fifth evaluation for vertical branches, nearly three times higher than the maximum obtained for horizontal branches, which was 2.5 for the same period. Additionally, the maximum average length for vertical branches was 4.3 cm at 80 days, whereas the highest average for horizontal branches was almost eight times lower, at 0.6 cm on average during the same period.

3. Exploratory analyses of individuals and epicormic material

Considering the cutting of *M. polymorphum* individuals, all assessed variables showed statistically significant differences ($p<0.0001$). Notably, significant variations were observed among the 17 individuals that were tested (Table 4).

Individual 152 demonstrated exceptional performance, displaying superior values compared to other individuals. It achieved survival, and rooting rates close to 80%, indicating a high success rate in vegetative propagation. Almost 95% of the surviving cuttings of

TABLE 2: Survival, sprouting, number of sprouts and length of sprouts of *Moquiniastrum polymorphum* selected trees and detached branches according to three vegetative rescue techniques, in the municipality of Lages, Santa Catarina state, Brazil.

Rescue technique	Days of evaluation	Survival (%)	Sprouting (%)	Number of sprouts	Length of sprouts (cm)
Girdling	32	100	0.0	0.0	0.0
	209	100	0.0	0.0	0.0
	384	100	16.7	7.8	4.1
Semi-girdling	32	100	0.0	0.0	0.0
	209	100	0.0	0.0	0.0
	384	100	0.0	0.0	0.0
Branches	70	100	83.4 ± 6.2*	6.1 ± 0.3	3.4 ± 0.5

* Standard error.

TABLE 3: Sprouting, number of sprouts and length of sprouts of *Moquiniastrum polymorphum* detached branches from different individuals, in the municipality of Lages, Santa Catarina state, Brazil.

Parameter	Individual							
	1	**2**	**4**	**37**	**68**	**72**	**84**	**Average**
Disposition	Sprouting (%)							
Vertical	83.3†	100.0	100.0	33.3	66.7	100.0	100.0	83.3 a
Horizontal	0.0	0.0	33.3	0.0	0.0	0.0	66.7	14.3 b
Average*	41.7 b	50.0 b	66.7 a	16.7 b	33.4 b	50.0 b	83.4 a	48.8
Disposition	Number of sprouts							
Vertical	4.0	10.3	7.3	1.3	5.0	5.3	9.6	6.1
Horizontal	0.0	0.0	0.3	0.0	0.0	0.0	17.3	2.5
Average*	2.0 b	5.2 b	3.8 b	0.7 b	2.5 b	2.7 b	13.5 a	4.3
Disposition	Length of sprouts (cm)							
Vertical	7.1	1.4	4.4	0.7	1.8	4.5	4.3	3.5 a
Horizontal	0.0	0.0	0.5	0.0	0.0	0.0	2.1	0.4 b
Average*	3.6	0.7	2.5	0.4	0.9	2.3	3.2	1.9

*Averages followed by the same letter do not differ according to the Scott-Knott average test; † Standard error was not added due to the low number of repetitions.

this individual developed roots. Furthermore, by the end of the evaluation period, all living cuttings of individuals 152 had produced new sprouts, with nearly 80% retaining their original leaves.

Although individual 152 exhibited a high level of rooting success, a similar pattern of development was observed for most other individuals as well. Individuals 4, 7, 70, 84, 165, 190, 213, and 225, for instance, demonstrated a rooting success rate of at least 50% among the surviving cuttings. This suggests a substantial rooting capability within the *M. polymorphum* species, highlighting the need to optimise conditions for material survival. Additionally, the callus formation also followed this favorable pattern, indicating a high level of maturity in the materials used.

When examining the cutting performance based on the origin of the material, no statistically significant differences were observed (p>0.05) (Table 5). Overall, the survival rate reached approximately 22%, with half of the surviving cuttings successfully rooting. Only 5% of the living cuttings did not exhibit callus formation, while nearly 60% of the cuttings developed new sprouts and maintained their original leaves.

4. Performance of cuttings in relation to rooting environments and individuals

No significant difference was found between the individuals and rooting environments for any of the variables assessed (Table 6).

In general, individuals selected for this experiment showed lower survival and rooting performance compared to previous tests in this study. However, individuals 3, 7, and 190 exhibited the highest survival rates, all above 8%, at least two times higher than the others. This trend was also observed for callus formation and sprouting.

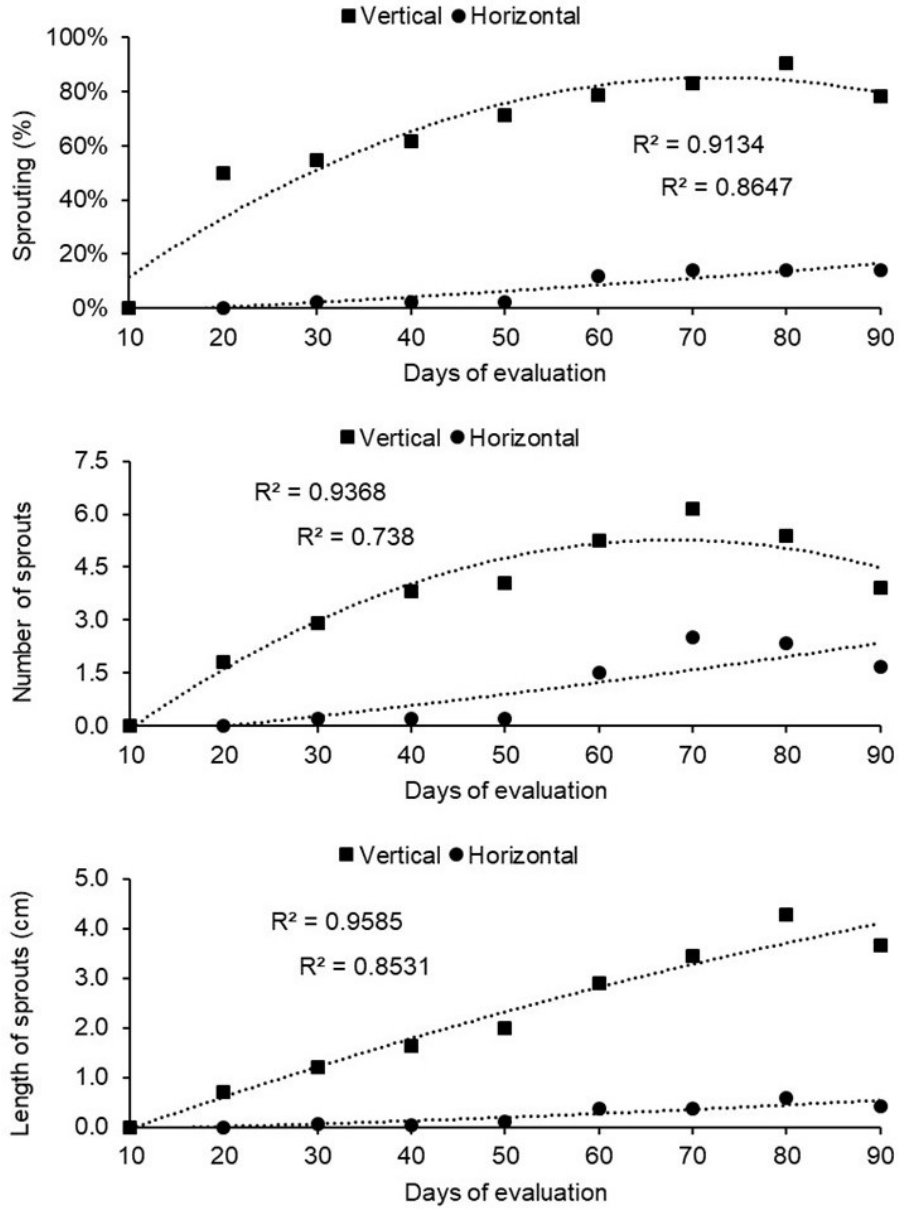
Discussion

1. Epicormic sprouting induction through girdling techniques and detached branches

Several factors could have contributed to the absence of sprouting through the girdling technique. These factors included inadequate injury to induce hormonal imbalance, the thickness of the bark hindering sprout development, or incorrect timing of the implementation. Additionally, the height of the injury and the number of dormant buds play a significant role. Stuepp et al. (2016) discovered that in *Ilex paraguariensis* St. Hill, sprouting was more successful when the bark was removed above 60 cm, resulting in nearly three times more sprouts compared to removal at 15 cm. The authors suggest that a greater surface area on the stem allows for more dormant buds, thereby facilitating more effective sprouting. This observation is supported by the sprouting observed in the detached branches in this study, which had a minimum length of 30 cm, resulting in a higher surface area and contact with dormant buds. Furthermore, dormant buds tend to accumulate as the plant grows taller (Hartmann et al. 2014), suggesting that there may have been an insufficient number of dormant buds below 20 cm in the girdled stems. This condition also explains the sprouting of the detached branches, which were collected between 1.5 m and 2.5 m.

Despite the absence of sprouting, the removal of bark did not cause harmful symptoms or lead to the mortality of the mother trees. This finding aligns with previous studies on girdled *I. paraguariensis* and *Cedrela fissilis* Vell. mother trees, where researchers observed the resilience of the trees and subsequent recovery of the injured area over time (Wendling et al. 2013; Stuepp et al. 2016; Nascimento et al. 2018; Santos Junior et al.

FIGURE 4: Sprouting, number of sprouts and length of sprouts of *Moquiniastrum polymorphum* detached branches according to evaluation periods, in the municipality of Lages, Santa Catarina state, Brazil.



2021). These results are consistent with those obtained in this study, as the girdled *M. polymorphum* mother trees did not exhibit any symptoms of senescence during or after the evaluation periods.

2. Influence of individuals, disposition sense and time on the epicormic sprouting of detached branches

The results indicated that individual was a key factor for branch sprouting. Silva et al. (2021) also observed a similar response in detached branches of *Paratecoma peroba* (Record) Kuhlm, as substantial differences in sprouting were observed among mother trees, varying up to 40%. However, determining this factor is challenging, as it involves not only phenotypic characteristics but also hormones, dormant buds, and plant vigor. According to Hartmann et al. (2014), the percentage of sprouts in branches is possibly related to the endogenous levels of auxin and cytokinins present in the branches, regardless to the number of dormant buds.

The accumulation of higher quantities of cytokinins relative to auxins is a pivotal factor influencing the success of the sprouting technique. However, this may not exert a substantial influence on horizontally stored branches. The high percentage of sprouts in vertical branches can be explained by the ease of hormonal imbalance with an accumulation of cytokinins, as the branches were kept in their natural orientation (Taiz & Zeiger 2018; Hartmann et al. 2014), which may promote better development of dormant buds. In contrast, horizontal branches require rearrangement in the direction and orientation of these hormones for proper functioning, as there is a deviation

TABLE 4: Average* percentage survival, callus formation, rooting, sprouting and original leaves permanency of *Moquiniastrum polymorphum* canopy cuttings according to different individuals, in the municipality of Lages, Santa Catarina state, Brazil.

Individual	Survival (%)		Callus formation (%)		Rooting (%)		Sprouting (%)		Original leaves (%)	
3	8.0 ± 3.9†	d	6.8 ± 3.9	c	1.2 ± 0.7	c	2.5 ± 1.8	d	7.4 ± 3.7	C
4	3.7 ± 1.9	d	1.9 ± 1.3	c	3.1 ± 1.5	c	7.4 ± 4.8	d	2.5 ± 1.8	C
7	4.9 ± 1.8	d	4.3 ± 1.5	c	2.5 ± 1.6	c	2.5 ± 0.8	d	4.3 ± 1.8	C
50	2.5 ± 1.8	d	1.9 ± 1.3	c	1.2 ± 1.2	c	2.5 ± 1.8	d	1.9 ± 1.3	C
64	6.2 ± 3.1	d	5.6 ± 2.9	c	0.0	c	6.2 ± 3.1	d	1.2 ± 0.8	C
68	33.3 ± 4.1	c	33.3 ± 4.1	b	5.6 ± 2.5	c	29.6 ± 5.5	c	13.0 ± 3.4	C
70	1.2 ± 1.2	d	0.6 ± 0.6	c	0.6 ± 0.6	c	0.0	d	0.0	C
80	7.4 ± 3.7	d	7.4 ± 3.7	c	0.0	c	0.0	d	7.4 ± 3.7	C
84	11.1 ± 4.9	d	10.2 ± 4.9	c	8.3 ± 4.0	c	7.4 ± 3.4	d	4.6 ± 2.2	C
99	12.9 ± 6.0	d	11.1 ± 6.0	c	0.0	c	7.4 ± 4.7	d	5.6 ± 5.6	C
152	79.6 ± 13.6	a	75.9 ± 13.9	a	77.8 ± 13.1	a	79.6 ± 13.6	a	64.8 ± 13.9	A
165	16.7 ± 4.8	d	16.7 ± 4.8	c	9.3 ± 4.5	c	13.0 ± 5.3	d	9.3 ± 3.4	C
171	27.8 ± 4.8	c	26.0 ± 5.5	c	0.0	c	24.1 ± 6.0	c	5.6 ± 3.8	C
190	20.4 ± 10.2	c	20.4 ± 10.2	c	11.1 ± 5.0	c	16.0 ± 8.0	c	9.3 ± 7.4	C
212	12.9 ± 6.0	d	12.0 ± 5.8	c	0.0	c	12.0 ± 5.4	d	2.8 ± 1.9	C
213	57.4 ± 6.0	b	46.3 ± 5.3	b	57.4 ± 6.0	b	44.4 ± 5.7	b	42.6 ± 6.7	B
225	11.1 ± 5.6	d	8.6 ± 4.1	c	7.4 ± 4.2	c	6.8 ± 3.9	d	5.6 ± 4.1	C
Average	18.7 ± 2.3		17.0 ± 2.1		10.9 ± 2.2		15.3 ± 1.9		11.2 ± 2.2	

*Averages followed by the same letter do not differ according to the Scott-Knott average test; † Standard error was not added due to the low number of repetitions.

from the natural orientation (Taiz & Zeiger 2018).

The same pattern of sprout development over time observed in this study was also reported by Nascimento et al. (2018), who noted that horizontally positioned branches can have a longer lifespan but lower productivity. According to the same authors, horizontally oriented branches of *I. paraguariensis* can remain active for approximately 300 days. This same behavior was also observed by Silva et al. (2023) in *Drimys brasiliensis* Miers. In contrast, Wendling et al. (2013) reported that vertically oriented branches of *I. paraguariensis* can provide higher and faster sprout production but have a shorter lifespan. These findings indicate that the storage direction can directly influence sprout production and lifespan.

Despite substantial differences in sprouting variables between the two disposition senses, no branch mortality was observed. Additionally, no signs of degradation or senescence, such as excessive drying or changes in coloration, were detected.

3. Exploratory analyses of individuals and epicormic material

Given the detection of significant differences in cutting survival according to the individual, selection of individual may be crucial for this method of propagation. For *I. paraguariensis*, survival appeared to be closely associated with individuals, with variations of more than 50% observed among materials from the same population (Nascimento et al. 2022). The authors

TABLE 5: Survival, callus formation, rooting, sprouting and original leaves permanency of *Moquiniastrum polymorphum* cuttings according to the source of material, in the municipality of Lages, Santa Catarina state, Brazil

Material source	Survival (%)	Callus formation (%)	Rooting (%)	Sprouting (%)	Original leaves (%)
Canopy	18.7 ± 5.1†	17.0 ± 4.7	10.9 ± 5.3	15.3 ± 4.0	11.2 ± 3.6
Branches	25.8 ± 5.4	17.5 ± 5.2	11.2 ± 4.7	12.2 ± 2.8	16.3 ± 4.5
Average	22.2 ± 3.7	17.3 ± 2.8	11.0 ± 3.4	13.8 ± 2.3	13.8 ± 2.4

† Standard error was not added due to the low number of repetitions.

TABLE 6: Survival, callus formation, rooting, sprouting and original leaves permanency of *Moquiniastrum polymorphum* canopy cuttings according to different individuals in two rooting environments, in the municipality of Lages, Santa Catarina state, Brazil.

	Individual						
	3	**7**	**50**	**64**	**80**	**190**	**Average***
Environment	Survival (%)						
MT[1]	20.4 ± 3.4†	5.6 ± 3.8	5.6 ± 2.5	7.7 ± 2.3	7.4 ± 3.7	9.3 ± 3.4	8.6 ± 1.5
GWIN[2]	9.3 ± 3.4	13.0 ± 3.4	0.0	0.0	1.9 ± 1.9	7.4 ± 4.7	5.2 ± 1.3
Average*	14.8 ± 2.8 a	9.3 ± 2.7 a	2.8 ± 1.5 b	1.9 ± 1.2 b	4.6 ± 2.1 b	8.3 ± 2.8 a	6.9 ± 0.9
Environment	Callus formation (%)						
MT	18.5 ± 2.3	5.6 ± 3.8	3.7 ± 2.5	3.7 ± 2.3	7.4 ± 3.7	9.3 ± 3.4	8.0 ± 1.4
GWIN	9.3 ± 3.4	13.0 ± 3.4	0.0	0.0	1.9 ± 1.9	7.4 ± 4.7	5.2 ± 1.4
Average*	13.9 ± 2.4 a	9.3 ± 2.7 a	1.9 ± 1.2 b	1.9 ± 1.2 b	4.6 ± 2.1 b	8.3 ± 2.8 a	6.6 ± 0.8
Environment	Rooting (%)						
MT[1]	3.7 ± 2.3	0.0	3.7 ± 2.3	0.0	0.0	7.4 ± 2.3	2.5 ± 0.8
GWIN[2]	5.6 ± 3.8	0.0	0.0	0.0	1.9 ± 1.9	5.6 ± 3.8	2.2 ± 1.0
Average*	4.6 ± 2.1 a	0.0 b	1.9 ± 1.2 b	0.0 b	0.9 ± 0.9 b	6.5 ± 2.1 a	2.3 ± 0.5
Environment	Sprouting (%)						
MT[1]	7.4 ± 3.7	3.7 ± 2.3	5.6 ± 2.5	3.7 ± 2.3	0.0	7.4 ± 3.7	4.6 ± 1.1
GWIN[2]	5.6 ± 2.5	11.1 ± 2.9	0.0	0.0	1.9 ± 1.9	7.4 ± 4.7	4.3 ± 1.2
Average*	6.5 ± 2.1 a	7.4 ± 2.1 a	2.8 ± 1.5 b	1.9 ± 1.2 b	0.9 ± 0.9 b	7.4 ± 2.8 a	4.5 ± 0.7
Environment	Original leaves (%)						
MT[1]	18.5 ± 4.7	3.7 ± 2.3	3.7 ± 2.3	0.0	7.4 ± 3.7	1.9 ± 1.9	5.9 ± 1.5 a
GWIN[2]	5.6 ± 3.8	1.9 ± 1.9	0.0	0.0	0.0	0.0	1.2 ± 0.8 b
Average*	12.0 ± 3.5 a	2.8 ± 1.5 b	1.9 ± 1.2 b	0.0 b	3.7 ± 2.1 b	0.9 ± 0.9 b	3.5 ± 0.7

[1] MT: Mini Tunnel; GWIN: [2] Greenhouse With Intermittent Nebulization
*Averages followed by the same letter do not differ according to the Scott-Knott average test; †Standard error was not added due to the low number of repetitions.

also suggest that each individual, in addition to the physiological conditions of the mother trees, requires specific environmental conditions for rooting in order to enhance survival. Similar conditions may also apply to *M. polymorphum*, as variations in survival exceeding 75% were observed among individuals. It is possible that the rooting environmental conditions were optimal for individual 152, explaining its overall superior development.

Furthermore, survival rates can be improved for *I. paraguariensis* and *D. brasiliensis* cuttings produced from epicormic sprouts, reaching levels higher than 75% and 68%, respectively (Stuepp et al. 2017; Silva et al. 2023). However, in the case of *M. polymorphum*, cuttings produced from epicormic sprouts from detached branches did not exhibit improved survival rates. Although detached branches are efficient in producing epicormic sprouts, their vigor, i.e., ontogenetic age, is still influenced by their collection height (Hartmann et al. 2014). Since the *M. polymorphum* branches were collected at heights above 1.5 m, the ontogenetic factor may have played a significant role, and the epicormic sprouts produced could be considered mature.

In this context, studies by Wendling et al. (2013), Stuepp et al. (2017), and Nascimento et al. (2018) have mentioned that the presence of callus is common in mature propagules of *I. paraguariensis* that have not undergone any rejuvenation technique. However, high maturity does not necessarily mean an inability to root. Callus formation indicates the initiation of cell division and differentiation (Hartmann et al. 2014), and under optimal conditions, there is a high likelihood of rooting the propagule (Stuepp et al. 2017; Nascimento et al. 2018). This may also apply to *M. polymorphum* cuttings, as most of the rooted material exhibited callus formation regardless of individuals or material origin.

According to Nogueira et al. (2017) and Belniaki et al. (2018), the presence of leaves in cuttings of plant species promotes greater root development, with more roots and longer lengths. This relationship can be explained by the production of phenolic compounds, such as auxin, by the aerial part of the plant (Vignolo et al. 2014). Therefore, for some species, the presence of leaves is a crucial factor for successful rooting during the cutting process (Nogueira et al. 2017). The best-rooted *M. polymorphum* individuals exhibited high percentages

of new sprouts, original leaves, or both, corroborating the existing literature.

The differences observed among individuals in the rooting of cuttings indicate that this process may not be exclusively inherent to the species. Fachinello et al. (2005) mention that the ability of a cutting to form roots can be greatly influenced by the clone, due to its correlation with endogenous concentrations of essential substances in root induction, which vary with the origin of the genetic material. Wendling et al. (2017) highlight that, for the *Araucaria angustifolia* (Bertol.) Kuntze, the rooting process of cuttings is linked to the genotype, as the rooting capacity of the cuttings is impacted by the provenance of the genetic material. In *M. polymorphum*, the rooting potential of cuttings also showed a high discrepancy among the studied individuals. Despite the absence of previous studies on the vegetative propagation of this specific species, it can be inferred that the observed variations are primarily of genetic nature. Since the individuals are derived from the same population, it is likely that the differences observed are a result of individual genetic variations. In other words, different individuals within the population may exhibit distinct responses in terms of their rooting capacity through vegetative propagation (Franzon et al. 2010).

4. Cutting according to adventitious rooting environments and individuals

As the rooting environments did not make significant differences for most of the variables, it is reasonable to assume that the previously discussed conditions in this paper remain the most relevant factors. Alternatively, the lack of significant differences suggests that further research is necessary to improve the analysis of rooting in *M. polymorphum* cuttings.

However, it should be noted that the retention of original leaves was nearly five times higher in the MT environment compared to the GWIN environment, although it remained below 6% in both environments. It is possible that the higher and more stable relative humidity provided by the MT environment reduced stress levels for the original leaves, resulting in reduced senescence. Furthermore, Brondani et al. (2008) mention that the presence of leaves in cuttings is primarily determined by the genetics of the mother plant, but the conditions of the rooting environment can either promote or hinder leaf retention.

The relationship between survival and rooting rates in different rooting environments may appear perplexing for certain individuals. For instance, when examining individuals 3 and 80, it is evident that although survival was considerably higher in the MT environment, rooting was lower or did not occur in this particular setting. However, this pattern was not consistently observed for individuals 190, which displayed similar survival and rooting ratios between both environments. A similar phenomenon was also noted by Nascimento et al. (2020; 2022) in their study on *I. paraguariensis* cuttings. The authors observed that, in general, survival rates were approximately 20% higher in the MT environment, while rooting rates were nearly two times more successful in the GWIN environment. However, this pattern did not hold true for all individuals.

Furthermore, the authors suggested that for *I. paraguariensis*, high humidity levels favored higher survival rates but hindered rooting, while the opposite could also be true. This relationship appears to be applicable to *M. polymorphum* cuttings as well. The higher humidity provided by the MT environment may have contributed to maintaining survival rates, while the lower humidity in the GWIN environment may have stimulated rooting. Further analyses in different rooting environments under singular climatic conditions are necessary to assess this hypothesis.

Conclusions

In addressing the questions raised in the introduction, the following key findings should be emphasised:

(1) There are distinct responses in terms of vegetative rescue and propagation among different individuals of *M. polymorphum* within the same population;

(2) No significant differences were found in the rooting of cuttings between canopy and epicormic material; and

(3) Specific rooting environments do not have a significant impact on the rooting of cuttings.

The vegetative propagation of *M. polymorphum* is feasible using canopy material, which simplifies the process of vegetative rescue. Simple rooting environments with minimal control of climatic conditions can be utilised for successful rooting of the cuttings.

Considering the lack of existing studies on vegetative rescue and propagation of *M. polymorphum,* it is recommended that further research be conducted in this area, exploring new variables, and conducting more comprehensive analyses to enhance our understanding of these processes. New tests could consider more populations, alternative techniques for material reinvigoration, and different rooting environments.

Competing interests

The authors declare they have no competing interests.

Authors' contributions

ACSS established the hypothesis, performed data collection, evaluations and analyses, besides writing results and discussion. BN conducted a review of the subject and performed the writing of methodology, results, discussion and translated the manuscript. BJSC performed material collection and processing. GNS performed material collection and processing. MCN, LMO, AM and MOP perfomed a review of the manuscript and supervised its general development, besides approving the final version.

Acknowledgements and Funding

We are thankful to the Post Graduation Program of Vegetal Production (PPGEF) and the Laboratory of Forest Propagation and Improvement (LAPROMEF) of the University of Santa Catarina State (UDESC). We also thankful for the scholarships provided by Support Fund for the Maintenance and Development of Higher Education (FUMDES/UNIEDU) and the Foundation of Amparo to Research and Innovation of Santa Catarina (FAPESC).

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Southern Forests: a Journal of Forest Science

ISSN: 2070-2620 (Print) 2070-2639 (Online) Journal homepage: www.tandfonline.com/journals/tsfs20

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To cite this article: Patrícia Fukushima de Souza, Marcio Carlos Navroski, Mariane Oliveira Pereira, Marcos Felipe Nicoletti & Ramon Silveira de Andrade (2024) Growth, vegetative rescue and vegetative propagation in *Eucalyptus* species and hybrids, Southern Forests: a Journal of Forest Science, 86:1, 65-73, DOI: 10.2989/20702620.2023.2284753

To link to this article: https://doi.org/10.2989/20702620.2023.2284753

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Southern Forests 2024, 86(1): 65–73
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SOUTHERN FORESTS
ISSN 2070-2620 EISSN 2070-2639
https://doi.org/10.2989/20702620.2023.2284753

Growth, vegetative rescue and vegetative propagation in *Eucalyptus* species and hybrids

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The objective of the study was the evaluation of survival and productivity indicators in different species/hybrids of *Eucalyptus* in the adult stage, as well as the vegetative rescue of adult trees and cuttings, testing for species/hybrids, concentration of growth regulator and substrates in rooting. In the evaluation of species/hybrids, height (m), diameter at breast height (DBH, cm) and individual volume (m^3 per individual) were measured, and survival was evaluated. In the vegetative rescue treatment, the number of shoots produced at 90, 120 and 210 days was evaluated. In the cutting experiments, the species that produced rescue shoots were tested, in addition to indole-3-butyric acid (IBA) doses (0, 1 500, 3 000 and 4 500 mg L^{-1}) and four substrates for rooting cuttings. The experiments of IBA doses and substrates were carried out using *Eucalyptus saligna* plants. In the cutting experiments, after 120 days of cultivation, the following variables were evaluated: callus formation (%), survival (%), rooting (%), number and length of roots. The best results regarding survival and quantitative variables of the species/hybrids were obtained for *E. dunnii, E. dunnii* × *E. grandis* and *E. pellita*. In the vegetative rescue, *E. dunnii* × *E. grandis* and *E. pellita* had the highest formation of shoots and the girdling vegetative rescue method had the best results in terms of generated shoots. *E. saligna* had better results regarding the variables related to the rooting of cuttings. The substrate S3 and the use of 1 500 mg L^{-1} of IBA provided the highest survival rate and better variables related to the rooting of the cuttings.

Keywords: adaptation, clonal forestry, growth regulator, invigoration, rooting cuttings

Introduction

Faced with the growing demand for forest-based products, there has been an expansion in areas with planted forests in Brazil, which currently corresponds to around 9.55 million hectares (IBÁ 2021). *Eucalyptus* are among the most popular species (7.47 million hectares). They are distributed across different regions of the country due to their adaptability to different climate and soil conditions, in addition to their rapid growth (Fernandes et al. 2018).

The average annual increment of eucalyptus is affected by climatic, genetic and management factors (Binkley et al. 2017; Elli et al. 2017). However, climate is the one factor that foresters have little or no control over. Thus, concern has been raised about the impacts of climate change and its uncertainties on eucalyptus productivity, and new possibilities for species and hybrids should increasingly be tested (Booth 2013). The choice of appropriate species, hybrid and origin is essential for the success of a eucalyptus breeding programme (Silva et al. 2021). In addition, it determines the most adaptable or most stable genotypes among different environments (Araújo et al. 2019).

Genetic improvement programmes carried out through techniques such as cloning have contributed to the success of forest enterprises in Brazil. Through these techniques, forest species are selected over time, obtaining more productive genotypes, better adapted to different edaphoclimatic conditions and with greater resistance to biotic and abiotic stresses (Studart-Guimarães et al. 2003). As a result, there is a progressive increase in clonal forest plantations in the most varied environmental conditions of the Brazilian territory (IBÁ 2021).

In the genetic improvement of forest species, the greatest warranty of superiority is obtained in mature trees. However, as maturation increases the potential for vegetative propagation decreases, especially in the rooting of cuttings, requiring the use of youth reversion techniques, such as vegetative rescue. The rescue of adult plants can be accomplished by inducing the development of epicormic and adventitious shoots. Epicormic buds develop from dormant axillary buds after the death, removal or breakage of branches along the trunk, increasing the level of incident light in that region (Bisognin et al. 2018). Furthermore, its induction may also result from cambium cell morphogenesis, which gives rise to new shoots (Rast et al. 1988). Adventitious shoots originate in locations other than those that would form buds and branches in the natural course of plant development and have relatively greater growth vigour (Wendling et al. 2014). The formation of adventitious shoots in adult plants is induced by burning or girdling the base of the trunk or by thinning the tree (Almeida et al. 2007; Wendling et al. 2013; Stuepp et al. 2016).

Adventitious rooting is a complex multifactorial process which leads to the formation of new roots at the base of the cuttings, configuring the establishment of a complete and autonomous plant. Many factors influence rooting and

seedling production, amongst others, the species/clone of origin, the use of growth regulators, the substrate used, the propagation environment, in addition to the time of collection and the type of propagule (Costa et al. 2013).

Plant phytoregulators, or growth regulators, are essential for adventitious rooting as they are involved in the induction of lateral and adventitious roots. Depending on the species and/or clones the application of a growth regulator can promote root initiation. Among the auxins is indole-3-butyric acid (IBA), characterised by being a photostable substance, with localised action and lower sensitivity to biological degradation, which generates more effective results as an inducer of root formation (Taiz and Zeiger 2017).

Selecting a suitable substrate is important for the rooting of cuttings. A proper rooting medium for establishing cuttings requires sufficient moisture and good aeration. So, a good quality rooting medium is a prerequisite for getting healthy plants. A good growing medium provides sufficient support for the plant, serves as a nutrient and water reservoir, allows diffusion of oxygen to the roots and allows for gas exchange between the roots and the atmosphere (Rajkumar et al. 2016).

Therefore the aim of this study was to assess survival and productivity indicators in various *Eucalyptus* species/hybrids during the mature phase, along with exploring vegetative rescue and propagation of mature trees. This involved investigating different species/hybrids, growth regulator concentrations and substrates in the rooting of cuttings.

Material and methods

Experimental area and species/hybrids evaluation

The population of *Eucalyptus* spp. in which the study was carried out is in Rio Negrinho, SC, Brazil (26°06′41″ S, 49°48′19″ W). The municipality has an altitude close to 790 m a.s.l. and its soils are predominantly from the Haplic Dystrophic Cambisoil and Dystrophic Regolitic Neosoil classes. These soils have the following characteristics according to soil analysis: pH: 4.28; SMP Index: 4.20; ca^{+2} (cmolc dm^{-3}): 0.88; mg^{+2} (cmolc dm^{-3}): 0.76; $A1^{+2}$ (cmolc dm^{-3}): 4.64; P Mehlich^{+2} (mg dm^{-3}): 0.72; K^{+2} (mg dm^{-3}): 73.80; orgnaic matter (%): 3.45; effective cation exchange capacity (CEC; cmolc dm^{-3}): 6.46; CEC pH 7.0 (cmolc dm^{-3}): 36.32; base saturation (%): 5.00; Al saturation (%): 71.84; and clay (g kg^{-1}): 18.50.

The North Plateau region of Santa Catarina, where the test was carried out, is considered as Cfb climate (temperate, humid subtropical climate, with regular rainfall throughout the year) according to the Köppen classification (Alvares et al. 2013). The average annual temperature of the municipality is 16.8 °C, with an annual average minimum temperature of 13 °C and a maximum of 22.8 °C, and average total annual rainfall of 1 720 mm (Wrege et al. 2012).

The different species/hybrids were planted in December 1999, spaced 3 × 2 m apart, using seedlings of seminal origin. The following pure species are present in the experimental area: *E. saligna*, *E. dunnii*, *E. pellita* and *E. tereticorni*. The hybrids comprised the following crossings: *E. viminallis* × *E. saligna*, *E. dunnii* × *E. grandis*, E. *robusta* × *E. grandis* and *E. urophylla* × *E. grandis*.

The hybrids used are separate full siblings produced through controlled pollination known as artificially induced protogyny, which aims to intercross certain *Eucalyptus* species in an easy and secure manner. This methodology was developed by Assis et al. (1993), who produced the hybrids used in this study. Confirmation that the crossbred species produced hybrids was carried out morphologically, using descriptors refined by Assis et al. (1993), who are leading experts in *Eucalyptus* hybridisation worldwide.

The experiment was set up in a randomised block design with three replications and linear plots. Each planting line comprised one species or hybrid with 40 trees per line. In places where individuals died, seedlings of *E. dunnii* were replanted, due to the difficulty in finding seedlings of the same genetic material, and these were cut before starting the evaluation of the experiment (except for the treatment consisting of *E. dunnii* due to the difficulty in separating the ones from the original planting and the replanted ones). It was not possible to obtain other information related to pre and post-planting, such as soil preparation, fertilisation, ant control, weed competition control and other activities carried out.

In characterising the species/hybrids the individuals were evaluated for survival (%), height (m), diameter at breast height (DBH; cm) and individual volume (m^3) 17 years after planting (evaluation in 2016). DBH was measured in all individuals using a graduated scale; 30% of the individuals' total heights were measured using the Trupulse 360 laser hypsometer. To estimate the remaining height 12 mathematical models were adjusted by regression, according to previous work published by Souza et al. (2016) using this same data, and according to results in the graphical analysis of residuals, adjusted coefficient of determination (0.7456) and standard error of the estimate in percentage (14.62%). The equation that provided the best estimates for the models studied was one obtained by the AD 1 model:

$$ln\ h = \beta_0 + \beta_1 dap + \beta_2 dap^2 + \varepsilon_i \quad (1)$$

The individual volume was obtained by selecting 20 sample trees by species/origin, covering all diameter classes. As this is an experimental area whose objective is to select superior material, the use of destructive methods to obtain the individual volume was considered impracticable. Therefore cubage was performed using the Smalian method, using the Criterion RD1000 dendrometer. To estimate the individual volume 12 models were tested, according to Frigotto et al. (2020) in the same experimental area. According to results in the graphical analysis of residuals, standard error of the estimate (Syx) and adjusted coefficient of determination (adjusted R^2), the model that generated estimates with the best fit was the Stoate model. The adjusted equation was:

$$v = 0.08357 - 0.0002 \times dap^2 + 0.0000404\ (dap^2 \times h) - 0.0028 \times h + \varepsilon_i \quad (2)$$

in which v = volume; dap = diameter at breast height; h = total height; and ε_i = absolute error. The equation had adjusted R^2 of 0.9696 and Syx of 15.1%.

To classify the species and hybrids, considering the real potential of wood production, the DBH (cm) × survival (%) variable was created according to the methodology proposed by Dobner Jr (2013). This result generates a value to express

the growth rate penalised by the relative survival of species/hybrids.

Vegetative rescue

In the vegetative rescue two experiments were carried out, one using the different species/hybrids and the other to evaluate two methods of rescue (girdling and semi-girdling).

The experiment based on species and hybrids used the following species/hybrids: *E. dunnii*, *E. pellita, E. saligna, E. dunnii* × *E. grandis, E. viminalis* × *E. saligna* and *E. robusta* × *E. grandis*. Species/hybrids with at least 10 individuals were selected. After selecting the trees, girdling was applied as a treatment to rescue vegetative material, approximately 30 cm from the ground.

Girdling was performed by sectioning two transverse lines on the trunk of each selected tree with the aid of a chainsaw, cutting only the depth of the bark. Then, with the aid of a machete, approximately 2 cm wide sections of bark ring between the sectioned lines were removed, removing 100% of the bark. The experimental design used was completely randomised with 10 repetitions of a tree each.

In the experiment on forms of vegetative rescue, the *E. saligna* species was used (because it had a greater number of homogeneous individuals). Median trees were selected based on DBH. After the selection of the trees, the treatments for the rescue of vegetative material (semi-girdling or girdling) were applied. Girdling and semi-girdling were performed in the same way, and in semi- girdling 50% of the trunk circumference was removed. The experimental design used for the rescue forms was completely randomised with 10 repetitions of one tree each.

Both experiments were installed in September 2016, with three evaluations of shoots carried out respectively 90 (December 2016), 120 (January 2017), and 210 (April 2017) days after the application of girdling and semi-girdling treatments. The experiments had the number of shoots per tree and the percentage of trees sprouted as the analysed variable.

Vegetative propagation

Three independent experiments were carried out involving vegetative propagation, one of them in function of the species/hybrids, and the others in relation to growth regulator tests and substrates in the rooting of cuttings. Table 1 shows the vegetative propagation experiments carried out, addressing the species/hybrids, growth regulator and substrate used.

In all experiments, the shoots collected from the vegetative rescue were transported in styrofoam boxes with ice on the bottom, to the Forest Nursery on the UDESC–CAV campus (Lages/SC/Brazil), a trip that took approximately five hours. The cuttings were approximately 7 ± 2 cm in length, with leaves reduced to 50% of their total area, making a bevel cut in the lower portion and a straight cut in the upper part of the cutting. Afterwards, the basal region was immersed for 30 seconds in a hydroalcoholic solution (water : alcohol, 1 : 1, v/v) containing indolbutyric acid (IBA; depending on the treatment). Subsequently, the cuttings were placed to root in 110 cm^3 tubes containing substrate according to the experiment/treatment denoted as S1, S2, S3 and S4 (Table 1) and 5 g L^{-1} of controlled release fertiliser (14–14–14). The trays containing the tubes were arranged in a mini tunnel (structure presenting 8 m long × 1.20 m wide × 0.9 m high). The temperature inside the mini tunnel generally ranged between 20 °C and 32 °C, with relative humidity of the air remaining above 80%. Irrigation was performed by micro-sprinkler for 5 min × 5 times daily.

In the three vegetative propagation experiments, a completely randomised experimental design was employed. Within each separate experiment, 8 replications of 10 cuttings each were used. After 120 days of cultivation, the following variables were assessed: callus formation (%), survival (%), rooting (%), number and length of roots.

Statistical analysis

After confirming data normality through the Kolmogorov–Smirnov test and homogeneity through the Bartlett test, analysis of variance (ANOVA) at a 5% significance level was performed. It is worth noting that the data used were balanced. When required, data were transformed using the function $(x + 0.5)^{\wedge 0.5}$. Means were compared using the Scott–Knott *t*- test, with a significance level of 5%.

Results

Species/hybrids evaluation

Significant differences ($p < 0.05$) were found in all evaluated growth characteristics (height, diameter and volume) used for species/hybrid selection. The highest survival rate was in *E. dunnii* (72.5%), followed by *E. pellita* (27.5%), *E. dunnii* × *E. grandis* (26.2%) and *E. saligna* (25.0%). *E. urophylla* × *grandis* had few surviving individuals and *E. tereticornis* had no individuals in the area (Table 2).

In the height variable, the hybrid with the best result was *E. urophylla* × *E. grandis* but it did not differ statistically from the species *E. pellita*, *E. saligna*, and the hybrids *E. viminalis* × *E. saligna* and *E. robusta* × *E. grandis*. The highest DBH value found was in the hybrid *E. viminalis* × *E. saligna* (47.7 cm) but there was no statistical difference with *E. dunnii* and *E. dunnii* × *E. grandis*. Regarding individual volume *E. viminalis* × *E. saligna* had the highest average (2.95 m^3), not differing from *E. dunnii* and *E. dunnii* × *E. grandis*. Considering the rank of the actual wood production potential and using the DBH × survival variable, *E. dunnii* was first in the ranking, followed by *E. dunnii* × *E. grandis* and *E. pellita*. *E. tereticornis*, which did not present any living individuals, was placed last.

Vegetative rescue

The percentage of sprouted trees and the number of sprouts showed statistical difference ($p < 0.05$) depending on the species in all collection periods. In the evaluation at 90 days, *E. dunnii* × *E. grandis* and *E. pellita* had the most trees with sprouts (90%), *E. dunnii* × *E. grandis* had all the trees sprouted in the other evaluations and *E. pellita* maintained 90% (Figure 1a). *E. saligna* and *E. viminalis* × *E. saligna* also had a significant number of sprouted trees (80% in all collections). *E. dunnii* had an increase in the number of trees sprouted over time, starting with 50% at 90 days and increasing to 70% in the evaluation at 210 days. *E. robusta* × *E. grandis* had the lowest number of sprouted trees, obtaining 20% in the three evaluations.

Table 1: Vegetative propagation experiments carried out, addressing the species/hybrids, growth regulator and substrate used

Experiment	Species/hybrids used	Growth regulator (IBA)	Substrate
	Treatments		
Species/hybrids	*E. dunnii, E. pellita, E. saligna* and hybrids *E. dunnii* × *E. grandis, E. viminalis* × *E. saligna* and *E. robusta* × *E. grandis*	3 000 mg L^{-1}	Commercial (pine bark, vermiculite, peat and charcoal) + vermiculite (1/1 v/v)
		Treatments	
IBA concentrations	* *E. saligna*	− 0 mg L^{-1} − 1 500 mg L^{-1} − 3 000 mg L^{-1} − 4 500 mg L^{-1}	Commercial (pine bark, vermiculite, peat and charcoal) + vermiculite (1/1 v/v)
			Treatments **
Commercial substrates in rooting	* *E. saligna*	3 000 mg L^{-1} ***	S1. Pine bark, vermiculite, peat and charcoal S2. Composted pine bark, carbonised pine bark, coconut fibre, vermiculite, limestone and NPK S3. Composted pine bark, coconut fibre, carbonised rice husk, expanded vermiculite and charcoal S4. Canadian peat, rice husks and vermiculite

* Species/hybrids with shoots in sufficient quantity to carry out the experiments

** Basic characteristics of the substrates according to the manufacturer:
S1: water retention capacity (WRC) 50%, electrical conductivity (EC) −0.35 mS cm^{-1}, pH 6.5 and density of 285 kg m^{-3};
S2: water holding capacity (WHC) 60%, EC 0.3 mS cm^{-1}, pH 6.0 and density 240 kg m^{-3};
S3: WHC 90%, EC 0.5 mS cm^{-1}, pH 6.0 and density 310 kg m^{-3} and S4: WHC 55%, EC 0.4 mS cm^{-1}, pH 5.5 and density 155 kg m^{-3}

*** Due to the experiment being conducted simultaneously with the assessment of IBA dosages, a dosage of 3 000 mg L^{-1} was used, which is consistent with dosages used in other Eucalyptus species for vegetative rescue, particularly in the study by Frigotto et al. (2020) conducted in close proximity to our research

Table 2: Survival (%); height = *H* (m); diameter at breast height = DBH (cm); and *v* = individual volume (m^3) in different *Eucalyptus* species/hybrids at 17 years of age

Species/hybrid	Survival (%)*	H (m)	DBH (cm)	v (m^3)	Ranking
E. dunnii	72.5 [a]	32.5 [b]	44.7 [a]	2.31 [a]	1
E. pellita	27.5 [b]	34.9 [a]	38.0 [b]	1.88 [b]	3
E. saligna	25.0 [b]	35.3 [a]	34.2 [b]	1.54 [b]	4
E. tereticornis	0.0 [d]	–**	–	–	8
E. viminalis × *E. saligna*	10.0 [c]	36.6 [a]	47.7 [a]	2.95 [a]	5
E. urophylla × *grandis*	0.7 [d]	37.7 [a]	37.9 [b]	2.01 [b]	7
E. dunnii × *E. grandis*	26.2 [b]	31.2 [b]	45.3 [a]	2.64 [a]	2
E. robusta × *E. grandis*	10.4 [c]	34.6 [a]	40.2 [b]	2.05 [b]	6

* Averages followed by the same letters in the column do not differ by the Scott-Knott test at the 5% significance level

** There were no survivors

For the number of shoots, *E. dunnii* × *E. grandis* had the highest at 90 and 210 days (Figure 1b). At 90 days it was already possible to verify a high number of shoots (8.0), in relation to the other species/hybrids, followed by *E. pellita. E. robusta* × *E. grandis* had practically no shoots in the 90-day collection, and the other species/hybrids had between 2 and 4 shoots per ringed tree. The low production of shoots was maintained in the other collections in *E. robusta* × *E. grandis. E. viminalis* × *E. saligna* had an increase in the number of shoots over time, reaching 8 shoots in the last collection. *E. dunnii, E. pellita* and *E. saligna* had some stability in production throughout the collections.

Regarding the experiment in which rescue methods were tested in *E. saligna*, the girdling method had the best results (Figure 2a). After 90 days it was possible to obtain 70% of the trees with sprouts using girdling, increasing to 80% in the other evaluations. For the semi-girdling treatment, 10% was obtained in the second and third evaluations. For the

number of shoots (Figure 2b), girdling provided an average of between four and six shoots per tree and semi-girdling provided less than one shoot per tree.

Vegetative propagation

For survival rate a significant effect of the species/hybrids was verified, with *E. saligna, E. saligna* × *viminalis* and *E. pellita* presenting the highest survival of cuttings, ranging from 40% to 23% (Table 3), statistically differing from the other species/hybrids whose survival was nil, except for *E. robusta grandis* which obtained 8%. The highest rooting of cuttings was obtained for *E. saligna*, which had 18%, followed by *E. pellita* and *E. saligna* × *viminalis*, which obtained 10%. The other species/hybrids had no or low percentage of rooted cuttings. In the *E. saligna* × *viminalis* hybrid and in *E. saligna*, the appearance of approximately 10% of calluses was observed. The highest number and length of roots also went to *E. saligna* followed by the hybrid *E. saligna* × *viminalis*.

Regarding the experiment in which the influence of IBA was tested (performed only on *E. saligna*) it was possible to observe that the dosage of 1 500 mg L^{-1} provided greater survival rate, rooting and callus formation, and (although without difference with the other doses) a greater number and length of roots. The use of a dose equal to or greater than 3 000 mg L^{-1} of IBA caused a decrease in survival rate and variables related to rooting.

Using different substrates in the cuttings, which was also performed only with *E. saligna*, substrate S3 provided greater survival rate, rooting and callus formation than the other substrates. For rooting, S3 obtained a value greater than 20% and the use of other substrates provided rooting lower than 15%. The number and length of roots did not vary depending on the substrates used.

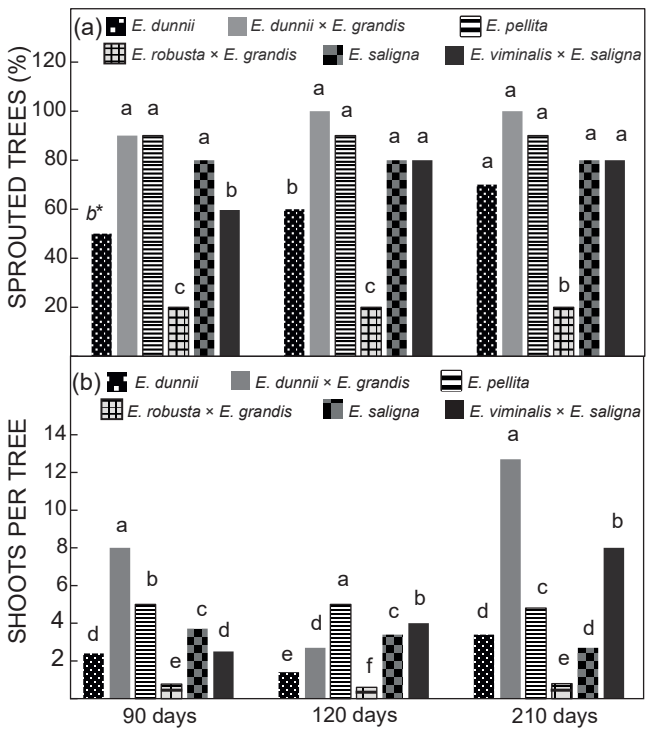


Figure 1: Percentage of sprouted trees (a) and number of sprouts (b) per individual originating from the rescue of selected *Eucalyptus* spp. by stem girdling at 90, 120 and 210 days. Averages followed by the same letters do not differ according to the Scott−Knott test at the 5% significance level

Discussion

Species/hybrids evaluation

Among the species/hybrids tested in the experiment, *E. dunnii* had the highest survival rate (>70%), much higher than the other species which had results below 30%. One of the factors responsible for the greater survival of *E. dunnii* was the replanting in the area. As mentioned in the methodology, the entire experiment was replanted with *E. dunnii* due to the lack of seedlings. *E. dunnii* used in the replanting was thinned out before the evaluation except for the lines in which it was not possible to identify whether the tree was from the original planting or the replanting. Regardless of this, the species is recognised for its good adaptation to the southern region of Brazil, being one of the most planted, mainly due to its greater adaptation to frost. Frigotto et al. (2020) also observed a higher survival rate for *E. dunnii* (83%). Studies carried out in several countries indicate that *E. dunnii* tolerates negative temperatures

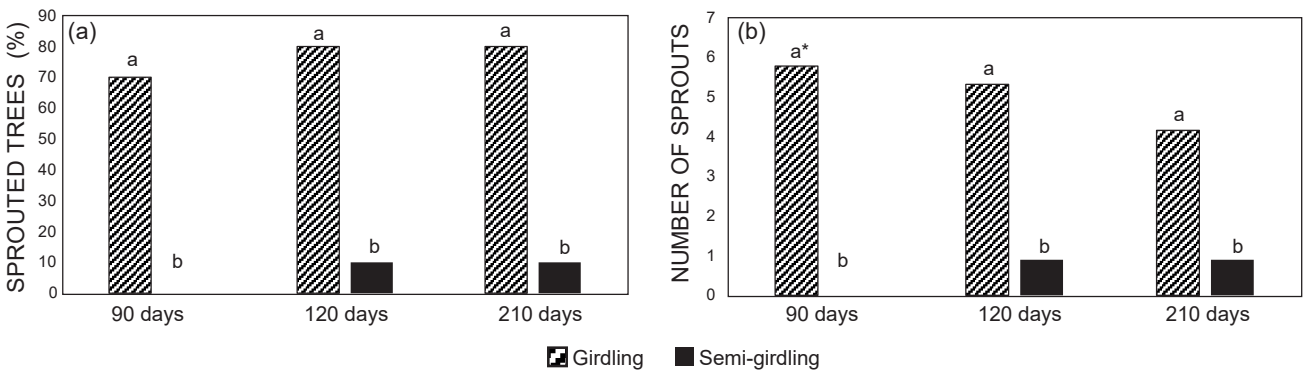


Figure 2: Percentage of sprouted trees (a) and number of sprouts (b) per individual originating from rescue treatments of *Eucalyptus saligna* at 90, 120 and 210 days. * Averages followed by the same letters do not differ from each other using the *t*-test at the 5% significance level

Table 3: Survival = S (%); rooting = R (%); callus (%); number of roots = NR; and root length = RL (cm) in cuttings of different *Eucalyptus* species/hybrids submitted to IBA experiments and substrates

Exp.	Treatments	S (%)	R (%)	Callus (%)	NR	RL (cm)
Species/hybrid	*E. dunnii* × *grandis*	0[b]	0[c]	0[b]	0[c]	0[d]
	E. robusta × *grandis*	8[b]	3[c]	0[b]	0.3[c]	2.1[c]
	E. saligna × *viminalis*	28[a]	10[b]	10[a]	1.2[b]	5.3[b]
	E. dunnii	0[b]	0[c]	0[b]	0[c]	0[d]
	E. pellita	23[a]	10[b]	0[b]	0.9[b]	2.6[c]
	E. saligna	40[a]	18[a]	13[a]	2.1[a]	8.4[a]
IBA (mg L^{-1})	0 mg L^{-1}	35[b]	15[b]	10[b]	0.9[b]	8.1[ns]
	1 500 mg L^{-1}	58[a]	25[a]	12[b]	2.3[a]	10.4
	3 000 mg L^{-1}	28[b]	11[b]	20[a]	1.6[a]	9.3
	4 500 mg L^{-1}	20[b]	9[b]	15[b]	1.5[a]	9.6
Substrates	S1	20[c]	8[b]	15[b]	1.0[ns]	7.7[ns]
	S2	42[b]	14[b]	36[a]	1.3	9.8
	S3	56[a]	22[a]	45[a]	1.9	9.9
	S4	21[c]	9[b]	17[b]	1.2	9.5

Averages followed by the same letters do not differ from each other using the Scott–Knott test at the 5% significance level
ns = not significant

and can be planted in regions with absolute minimum temperatures above −11 °C, as observed in China (Arnold et al. 2004).

Other species or hybrids deserve to be highlighted in terms of survival, such as the hybrid *E. dunnii* × *E. grandis*, *E. pellita* and *E. saligna*, with survival rates close to 25%. The hybrid *E. dunnii* × *E. grandis* possibly had a greater survival rate due to the presence of *E. dunnii* in the composition of the hybrid, conferring greater capacity to adapt to the cold, since *E. grandis* is a species with better adaptation to tropical climate (Silva et al. 2019), as seen when crossed with *E. urophylla* (also tropical), which had less than 1% survival rate. *E. saligna* also had prominence in survival rate (25%). *E. saligna* is indicated for regions with up to eight annual frosts, however, the absolute minimum temperature that it supported in an experiment carried out in China was up to −10 °C (Arnold et al. 2004), which may explain the survival obtained in our study.

Likewise, *E. pellita* had good survival numbers (when compared to the other treatments) being a tropical species. In the study site, this species was classified by Flores et al. (2016) as having low climatic aptitude, since it requires an average annual temperature between 22 °C and 27 °C and annual precipitation between 1 110 and 2 800 mm, with low frost tolerance. Frost mortality is only one of the factors that explain survival. Factors such as drought, weed competition, pests and diseases may also be responsible for mortality. Unfortunately, the lack of a silvicultural history hampered this analysis. One of the factors in considering other climatic or silvicultural conditions to be important was the low survival rate of the hybrid *E. viminalis* × *E. saligna* (10%), since *E. viminalis* is one of the species most tolerant of cold, and the total mortality of *E. tereticornis*. *E. tereticornis* is a species widely distributed in Australia, with a preference for a tropical climate, but it can occur at 2 000 m altitude in Papua New Guinea (Brooker and Kleinig 2004). In the study region, the appearance of the species on the edges of fences and small plantations is common, which may indicate a certain adaptation of the species.

A survival performance greater than 75% would be desirable so that there would be no significant damage in the establishment of forest populations, so much so that this value can be used as a reference for indicating species (Fernandes et al. 2018). In our study, only *E. dunnii* came close to this value. However, since ours was an experiment using hybrids or species with lower initial potential for the region, mainly because they have a mostly tropical distribution, the results can serve as a basis for new tests to verify the real possibility of implantation after a long cycle (17 years).

The climate question represented by parameters such as minimum or maximum temperature, precipitation and humidity are the main points to be observed in the establishment of new species or genetic materials, with the analogy of the place of origin of the material and the place of planting fundamental for the possibility of adaptation (Santibáñez et al. 2015). As the first two years after planting are the most sensitive to climatic problems such as frost, high temperatures and lack of rain, in several locations or in alternate years would help avoid indicating or rejecting a species or genetic material by seasonality.

The winter in southern Brazil is generally quite severe, limiting the cultivation of several *Eucalyptus* species. The choice of appropriate genetic material is essential, as the desirable growth characteristics, the ability of the species to tolerate cold and the ability to regrow must be observed. In addition to genetic characteristics, the adoption of a set of complementary silvicultural measures is recommended. For best results, planting should be carried out from September to October; and soil preparation techniques, fertilisation and silvicultural treatments must be adequate to favour initial growth and reduce the period of susceptibility to frost, since in late plantings, fertilisation can decrease plant resistance to cold (EMBRAPA 1988).

Regarding productivity variables, *E. dunnii* deserves to be highlighted again. This species had one of the highest

individual volume and DBH values, but one of the lowest average heights. Despite this, the species was placed first in the classification proposed by Dobner Jr (2013). The hybrid between *E. dunnii* and *E. grandis* also had one of the highest DBH and individual volume averages, taking second place in the ranking. Despite the low survival rate in the hybrid *E. viminalis* × *E. saligna*, this one had the highest DBH and volume. This result indicates a high volumetric potential, requiring, however, further studies on adaptation.

Vegetative rescue

In forestry improvement programmes, adoption of cloning is an efficient way to accelerate forest improvement.Several eucalyptus species are propagated by cuttings, and they stand out as the most planted species in the world (Dobner Jr and Huss 2019). One way to increase the possibility of propagation by cuttings would be to try and reverse maturation as a function of age, especially in adult individuals. Thus, rescue methods can be employed, such as girdling and variations, cutting, use of fire at the base or pruned branches. Therefore in our study, we evaluated the non-destructive girdling method and the semi-girdling variation (50%).

After 90 days, it was already possible to observe that shoots had formed, indicating that the tested species/hybrids have rescue potential even in older stands, as in our study (17 years old). However, in this collection a few shoots were still obtained, possibly due to the thickness of the bark verified at the ring line, which initially may have interfered with the emergence rate of the shoots. With the passage of time and the alteration of the endogenous levels of the chemical components involved, among them the growth regulators (auxin/cytokinin) (Fachinello et al. 2005), it was possible to verify the induction of sprouting in the selected trees. The highest number of sprouts was obtained after 210 days, which can be explained by the natural response of the plants to sprouting. In addition, since the sprouts were collected at the end of the summer , it may have been influenced by the greater availability of light and higher temperature.

Testing the different species and hybrids, the best number of sprouted trees was obtained in *E. dunnii* × *E. grandis* and *E. pellita*, with values greater than 90% of sprouted trees. Also, in general, they were responsible for the greater formation of shoots, with *E. dunnii* × *E. grandis* standing out by, for example, reaching more than 12 shoots per tree in the collection 210 days after girdling. This result is much higher than that found in experimental planting in a nearby area by Frigotto (2016), in which values close to two shoots per tree were found for *E. dunnii* and *E. grandis*. This superior result in our study may be linked to the positive effect of hybridisation between both species.

E. pellita, despite still being little known and planted in Brazil, has potential as seen in the growth and adaptation; it also had good potential for regrowth. This species had approximately 5 sprouts and a sprouting percentage of 90% in all collections. The sprouting potential was confirmed in the study by Higa and Sturion (1991), in which *E. pellita* had 94% of trees sprouted after 10 years of planting. The hybrid *E. robusta* × *E. grandis* had the lowest possibility of rescuing by girdling, with only 20% of the trees sprouting, generating less than 1 sprout per tree. *E. robusta* generally has a high ability to regenerate as it occurs naturally in swamps, edge gaps and close to coastal rivers, where it is subjected to flooding (Boland et al. 2006), but when hybridised with *E. grandis*, it did not have this characteristic.

In the experiment comparing girdling with semi-girdling, girdling had a much higher efficiency than semi-girdling. The girdling technique more efficiently increases the concentration of substances that promote sprouting, such as cytokinin, after complete removal of the bark ring, accompanied by a reduction in the auxin concentration at this point, causing an imbalance between auxin and cytokinin (Pereira et al. 2015). This change in concentration of growth regulators leads to apical meristem activity, causing elongation of dormant buds below girdling (Zimmermann and Brown 1975). Superior girdling results in relation to semi-girdling were also obtained in *Toona ciliata* var. *australis* (Pereira et al. 2015), *Ilex paraguariensis* (Nascimento et al. 2018) and *Cedrela fissilis* (Santos Junior et al. 2021).

The shoots produced below the girdling generally have the greatest competence for rooting of cuttings. This is due to the youthfulness and high vigour of the buds induced at the base of the plant and it justifies the use of these less developed shoots to rescue adult plants of most tree species. The greater youthfulness of these shoots is due to the presence of lateral buds at the base of the plant, which are more like the tissue formed during seed germination and, thus, favour the formation of new tissues, such as adventitious roots (Hartmann et al. 2011).

Although the stem semi-girdling technique induced a smaller number of shoots, this technique is of great importance in species where the effects of girdling on the survival of individuals are not known. However, there was no record of death of trees submitted to girdling and semi-girdling treatments, signifying that the species supports the removal of a partial and total ring in the bark without harming the survival of the trees. Girdling and semi-girdling are also important techniques for endangered species where clear-cutting is prohibited and for genotypes where regrowth may not occur after clear-cutting (Pereira et al. 2015).

Vegetative propagation

In vegetative propagation by cuttings, when comparing the different species/hybrids, *E. saligna* and the hybrid *E. saligna* × *viminalis* were superior, in that the survival rate was close to or superior to 30% and rooting superior to 10%, mainly for *E. saligna* which had 18% rooting. These low survival and rooting values are typical when using adult material, as in our study. However, the use of girdling might have made it possible to obtain rooting, since the vegetative rescue of selected individuals is necessary to partially reverse the physiological maturation of adult trees, rooting cuttings that will be the beginning of the multiplication and cloning programme of the species (Pires et al. 2011). Definitions of adult material rescue strategies are essential for genetic improvement programmes for *Eucalyptus* species, as there is greater assurance of superiority when selecting adult trees. After establishing this material in clonal gardens, the use of techniques such as mini-cuttings begins, in which the rooting potential is higher.

This greater rooting observed in *E. saligna* and its hybrid is related to the natural potential that the species has in relation

to the other tested species. According to Corrêa et al. (2005), the greatest potential of species considered easier to root, such as *E. saligna*, lies in the greater availability of sugars and auxins in the mother plant, as opposed to recalcitrant species such as *E. glubulus*, for example, which was tested in their study. In our study, *E. dunnii* and its hybrid with *E. grandis* were the only species/hybrids that had total absence of rooted cuttings. This result is due to the known recalcitrance of adventitious rooting by *E. dunnii* (Spassin et al. 2016), especially in adult individuals.

Regarding the use of IBA, the use of 1 500 mg L^{-1} provided the highest rate of survival and rooted cuttings, in addition to improving root quality and reducing callus formation. It is important to highlight that in this experiment, *E. saligna* was used, which has a greater possibility of rooting. In these species, the use of exogenous growth regulators can help rooting. In recalcitrant species, not even the use of exogenous auxin can help in rooting, as discussed by Corrêa et al. (2005).

According to Hartmann et al. (2011), increasing the concentration of auxin applied to cuttings stimulates the induction of roots up to a maximum point, after which any increase in the level of auxin becomes inhibitory, as observed in our study. IBA enhances the induction of adventitious roots in vegetative propagules but, depending on the management style and the genetic material, the concentrations should be adjusted to obtain better rooting rates (Corrêa and Fett Neto 2004).

Regarding substrates used in vegetative propagation, S3 (composed of composted pine bark, coconut fibre, carbonised rice husks, expanded vermiculite and charcoal) had the best survival rate (56%) and rooting (22%) results. Among the substrates used, this is one had the highest water holding capacity, which may explain the result obtained. It is essential to select substrates that retain enough water to prevent desiccation of the base of the cutting and have pore space to facilitate the supply of oxygen for root initiation and development (Yamamoto et al. 2013).

In cuttings, using substrates that have greater porosity is best due to the higher humidity in the rooting environment. However, there is the problem of lack of aggregation of the roots when only a very porous substrate is used, and it may be beneficial to add, for example, decomposed pine bark (Caldeira et al. 2011). Despite not having the lowest density among the substrates tested in our study, S3 presents components indicated for the composition of substrates for cuttings, such as coconut fibre, carbonised rice husks and vermiculite (Kratz et al. 2012). The results found in this study corroborate those of Silva et al. (2012), in which the use of coconut fibre and carbonised rice husk had the best results for *E. urophylla* × *E. grandis*.

Conclusions

The species/hybrids with the best results in terms of survival rate and quantitative variables were *E. dunnii*, *E. dunnii* × *E. grandis* and *E. pellita*.

In vegetative rescue, *E. dunnii* × *E. grandis* and *E. pellita* had the greatest potential for shoot formation. The girdling vegetative rescue method resulted in the highest number of shoots and the highest number of sprouted trees, being recommended as a vegetative rescue method in adult trees without the need for clear-cutting.

E. saligna had better results regarding variables related to rooting of the cuttings. Regarding the use of IBA, the concentration of 1 500 mg L^{-1} provided the highest survival rate and rooting of *E. saligna* cuttings (tested species). Substrate S3, comprising pine bark, coconut fibre, carbonised rice husk, expanded vermiculite and charcoal, is the most suitable for rooting *E. saligna* cuttings (tested species).

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ISSN: 1696-8352

Pinecone failure, hydrogel, fertilizer and volume of containers in the production of araucaria seedlings

Falha de pinha, hidrogel, fertilizantes e volume de recipientes na produção de mudas de araucária

Falla de la piñona, hidrogel, fertilizante y volumen de contenedores en la producción de plántulas de araucária

DOI: 10.55905/oelv22n3-199

Originals received: 02/23/2024
Acceptance for publication: 03/15/2024

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ISSN: 1696-8352

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ABSTRACT
The objective of this work was the evaluation of initial growth and quality of araucaria seedlings under the influence of alterations to different proprieties of the substrate. Four different experiments were installed, comparing volume of tubes, concentrations of controlled release fertilizer (FLC), proportion of crushed pinecone failures (substrate characteristics were also evaluated) and the effect of hydrogel concentrations in seedlings derived from different mother trees. The evaluations were carried out 10 months after sowing, varying according to the experiment, and were comprised of height, diameter, height/diameter ratio (h/d), survival rate and germination speed index. The seedlings developed inside the larger tubes (280 cm^3) averaged higher in height, diameter and h/d ratio. The 6 g L^{-1} dose of fertilizer generated higher averages for all analyzed variables. The addition of pinecone failure to the substrate improved some physical attributes such as density, total porosity and aeration space, however it worsened the attributes related to water availability in the substrate. As for the growth of seedlings, the addition of pinecone failure did not change the height, diameter and h/d ratio. The use of hydrogel (3 g L^{-1} and 6 g L^{-1}) generated better results for height. It is recommended the use of 180 cm^3 tubes and 6 g L^{-1} of the contloed release fertilizer. Pinecone can be used in the substrate, as well as hydrogel as a additional resource.

Keywords: *Araucaria angustifolia*, quality seedlings, substrate improvement.

RESUMO
O objetivo do trabalho foi avaliar o crescimento inicial e qualidade de mudas de araucária sob influência de alterações em diversas propriedades do substrato. Foram instalados 4 experimentos separadamente, onde foram comparados tubetes de diferentes volumes, concentrações de fertilizante de liberação controlada (FLC), utilização de proporções de falha de pinha moída (avaliou-se também as caracteristicas do substrato) e o efeito de concentrações de hidrogel em mudas com sementes oriundas de matrizes de araucária. As avaliações foram realizadas aproximadamente 10 meses após a semeadura, variando de acordo com o experimento, e incluíram altura, diâmetro, relação h/d, sobrevivência, e índice de velocidade de germinação. As mudas produzidas em tubetes de maior volume

ISSN: 1696-8352

(280 cm^3) obtiveram maiores médias de altura, diâmetro e relação h/d. A dosagem de 6 g L^{-1} de FLC propiciou as maiores médias para todas as variáveis analisadas. A adição de falha de pinha ao substrato melhorou alguns atributos fisicos como densidade, porosidade total e espaço de aeração, contudo piorou os atributos relacionados a disponibilidade de água no substrato. Quanto ao crescimento das mudas, a adição da falha de pinha não alterou as médias de altura, diâmetro e relação h/d. O uso do hidrogel (3 g L^{-1} e 6 g L^{-1}) apresentou melhores resultados para altura. Recomenda-se o uso de tubetes de 180 cm^3 e adubação com 6 g L^{-1} de FLC. Pode ser feito o uso da falha de pinha como substrato e do hidrogel como recurso adicional na adição ao substrato.

Palavras-chave: *Araucaria angustifolia*, mudas de qualidade, melhoria de substratos.

RESUMEN

El objetivo de este trabajo fue evaluar el crecimiento inicial y la calidad de plántulas de araucaria bajo la influencia de alteraciones de diferentes propiedades del sustrato. Se instalaron cuatro experimentos diferentes, comparando el volumen de los tubos, las concentraciones de fertilizante de liberación controlada (FLC), la proporción de piñas trituradas fallidas (también se evaluaron las características del sustrato) y el efecto de las concentraciones de hidrogel en plántulas derivadas de diferentes árboles madre. Las evaluaciones se realizaron a los 10 meses después de la siembra, variando según el experimento, y estuvieron comprendidas por altura, diámetro, relación altura/diámetro (h/d), tasa de supervivencia e índice de velocidad de germinación. Las plántulas desarrolladas dentro de los tubos más grandes (280 cm3) promediaron mayor altura, diámetro y relación h/d. La dosis de fertilizante de 6 g L-1 generó promedios más altos para todas las variables analizadas. La adición de piña al sustrato mejoró algunos atributos físicos como densidad, porosidad total y espacio de aireación, sin embargo empeoró los atributos relacionados con la disponibilidad de agua en el sustrato. En cuanto al crecimiento de las plántulas, la adición de piña fallida no cambió la altura, el diámetro y la relación h/d. El uso de hidrogel (3 g L-1 y 6 g L-1) generó mejores resultados para la altura. Se recomienda el uso de tubos de 180 cm^3 y 6 g L-1 de fertilizante de liberación controlada. Como sustrato se puede utilizar piña, así como hidrogel como recurso adicional.

Palabras clave: *Araucaria angustifolia*, plántulas de calidad, mejora del sustrato.

1 INTRODUCTION

One of the biggest obstacles in the production of seedlings from native tree species is the high cost of production of high- performing individuals, and that is mainly due to the lack of knowledge about the production demands, such as, substrate, fertilizing and container to utilize. These factors have to cater to each species, due to the differing

ISSN: 1696-8352

demands. In this sense, various aspects can be managed in the plant nursery, seeking the production of seedlings adapted to establishing and growth in the field (BERNARDI *et al*., 2012).

Fertilization is one of the most important phases in a tree species seedling production program. The substrate to be used has to contain the necessary nutrients for the growth of the seedlings and to make up for the loss by leaching. Thus, the volume of the container, in association with the physiochemical characteristics of the substrate, the frequency and intensity of the irrigation determine the length of permanence of the nutrient in the system (JOSÉ *et al*., 2009). Among the fertilizers utilized are the ones with controlled liberation, which can diminish the labor, time and energy expenses (NAVROSKI *et al*., 2016). The nutrients are encapsulated by special resins, and are released through porous structures, taking more time to reach the plant's root system. This characteristic can guarantee the maintenance of a synchrony between the release of nutrients over time and the nutritional needs of plants, thus favoring their development (DINALLI *et al*., 2012).

The use of substrates is also a fundamental part in the production of tree seedlings, since it is in this environment that the sowing of seeds and/or rooting of cuttings occurs, with the proper support to the plants, the supply of water, nutrients and oxygen to the root system (RIBEIRO et al., 2018). An alternative in mixtures for substrates may be the pinecone failure, found in the female strobili of *Araucaria angustifolia* (Bertol.) Kuntze, a species from the Mixed Ombrophilous Forest naturally occurring mainly in the South region (AVILA *et al*., 2016). According to Vieira-Silva and Reis (2009) the pinecone has four components: seeds (pinion), central axis, sterile or unfertilized scales (failures) and fertile scales not fertilized or aborted (cut pine nuts), and between 50 - 60% of the pinecone consists of "failures".

In addition to the sizing of containers, fertilization, and formulation of the substrate, the use of additives, such as hydrogels or water-retaining polymers, appears as an alternative to obtain greater efficiency in the use of water and nutrients, in the production of seedlings in nurseries. The polymer has been commercialized with the purpose that, when incorporated into the substrate, it allows for greater retention of water

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and fertilizers, which can be released in a slower way to the plants due to the absorption - release cycles (NAVROSKI *et al*., 2016).

The objective is to verify whether the araucaria needs a larger container for seedling production and a controlled release fertilizer dosage that maximizes growth responses. In addition, some alternatives such as pinecone failures in the incorporation to the substrate and whether the hydrogel can assist in seedling quality, resulting in a reduced use of water and nutrients. In this context, it was sought to test the influence of container volume, controlled release fertilizer dosage, addition of pinecone failure and hydrogel to the substrate in the production of *Araucaria angutifolia* seedlings.

2 MATERIALS AND METHODS

2.1 SEED ORIGINS AND SOWING

The seeds were harvested from mother trees located in the municipality of São José do Cerrito (SC, Brazil). Each experiment was set up using seeds (pine nuts) obtained from the same mother, except the experiment in which the mother was a source of variation. Sowing was carried out in June 2016 directly in the tubes, with each seed deposited at an angle of ± 45°, in order to facilitate the germination, especially regarding the radicle emission. The experiments were installed in a shade house (nylon shade cover with 30-35% of light blocking) at the Forest Nursery of the University of the State of Santa Catarina in the municipality of Lages, Santa Catarina, at an altitude of approximately 900 meters, in the approximate coordinates of 27 ° 47'33 "S; 50 ° 18'40" O. According to the Köppen classification, the climate of Lages is predominantly of the Cfb type, mesothermal, humid subtropical, with cool summers, without defined dry seasons and with the occurrence of severe frosts. This type of climate is common in the Southern Brazilian Plateau. The annual average temperature is 12 to 19 °C; the minimum average is 5 to 8 °C, the maximum average is 22 to 31 °C and the absolute minimum is -12 °C (ALVARES *et al*., 2013). The annual precipitation is around 1,300 to 2,400 mm. The irrigation in the shade house was carried out with micro sprinklers, with four daily irrigations (9:00 am; 12:00 pm; 3:00 pm; 6:00 pm) of approximately 8 daily mm of water.

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2.2 PINECONE FAILURE ADDED TO SUBSTRATE

Four treatments were tested divided into different proportions of *Araucaria angustifolia* pinecone failure (0, 20, 40, and 60%) added to the commercial substrate. The commercial substrate for plants, according to the manufacturer, is composed of peat, expanded vermiculite, pine bark and vegetal charcoal.

The physical and chemical properties of the substrates with their respective mixtures of pinecone failure were analyzed in the Substrates Laboratory of the Department of Horticulture and Forestry, Federal University of Rio Grande do Sul, in accordance with the 17ª normative instruction of the Ministry of Agriculture, Livestock and Food Supply (MAPA, 2007) as well as according to Fermino (2003).

Among the physical properties related to the density and moisture of the substrate we have considered the wet density – WD (kg m^{-3}); the dry density – DD (kg m^{-3}); and the current humidity – CH (%). Pressure funnels of 0, -10, -50 and -100 *hPa* were used for determining both the aeration space and the available water (FERMINO, 2003). Therefore it was possible to calculate the total porosity (TP); aeration space (AS); readily available water (RAW); water buffering capacity (WBC); available water (AW); soil water retention (SWR 100); and water holding capacity (WHC) in different pressures.

As far as chemical properties are concerned, the electric conductivity and the pH were analyzed through a conductivimeter and a potentiometer (pHmeter), respectively. A dilution of 1:5 (v/v) with deionized water was applied in both cases. Also, the pH, electric conductivity and total soluble salts (TSS) of washed sand samples containing pinecone failure were analyzed so as to serve as parameter. Both the pH and the electric conductivity of samples were determined by the same means as the pH and the electric conductivity of substrates. Yet the total soluble salts (TSS) of samples were determined through calculation, by considering the EC (mS cm^{-1}) as well as the density of the material, in suspension sand: deionized water ratio of 1:10 (m/v), expressed as KCl content (RÖBER; SCHALLER, 1985).

For the analysis of the substrate, fertilization was not used. For the production of seedlings, 6 g L^{-1} of controlled release fertilizer (19-6-10) were added with a release period between 5 to 6 months. The pinecone failures came from natural forests in the city

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of São Joaquim - SC. These were spread and dried in a natural environment, then crushed in an industrial hammer mill, resulting in a material like sawdust. The container used was a conical tube with a circular section with 280 cm³ containing six longitudinal and equidistant internal friezes.

2.3 CONTAINER VOLUME

The type of container used was a conical tube with a circular section, with the following volumes: 50, 110, 180 and 280 cm³, containing four longitudinal and equidistant internal friezes in the 50 cm³ container, six friezes in the 110 cm³, and eight in 180 and 280 cm³. The substrate used was commercial based on peat, expanded vermiculite, pine bark and vegetal charcoal (characteristics described in the previous item). To the substrate, 6 g L^{-1} of controlled-release fertilizer (19-6-10) were added with a release period between 5 to 6 months.

2.4 CONTROLLED-RELEASE FERTILIZER DOSES

Controlled-release fertilizer doses were added in the NPK 19-6-10 formulation, characterized by slow release, along with the commercial substrate composed by peat, expanded vermiculite, pine bark and vegetal charcoal (described previously) inserted in 280 cm³ tubes. The controlled-release fertilizer doses were: 0, 2, 4, 6, 8 and 10 g L^{-1}.

2.5 ADDITION OF HYDROGEL TO THE SUBSTRATE

The experimental was conducted in a bifactorial scheme, were the tested factors were different hydrogel concentrations mixed with the substrate (0 g L^{-1}, 3 g L^{-1}, and 6 g L^{-1}), and the mother tree in which the seeds were collected, named: A10-9, A21-10, A23-3, and A10-6. The mothers used in this study had a minimum distance of 300 meters from each other. The commercial polymer corresponded to a mixed product of acrylamide copolymer (C_3H_5NO) and potassium acrylate ($K_2S_2O_8$) used to absorb and retain large amounts of water and nutrients, with the following characteristics: white powder insoluble in water, with particles of size ranging from 0.3 to 1.0 mm, anionic, with 10% humidity, density of 0.8 g cm^{-3} and usable pH index of 5 to 9, which can make up to 95%

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of the stored solution available to the plant (5% retained at high tension), capable of absorbing up to 300 times its mass in water and 100 times its volume, being compatible with most of the inputs used, but with affected retention capacity and variable life of one to five years (according to granulometry). Sowing was carried out in tubes of 280 cm^3 using the commercial substrate based on peat, expanded vermiculite, pine bark and vegetal charcoal (characteristics described in the previous item) and addition of 6 g L^{-1} of controlled release fertilizer (19- 6-10) with a release period of 5 to 6 months. In order to provide a homogeneous mixture and ensure a good distribution of the hydrogel and fertilizer to the substrate, a five liter portion of substrate was used for each mixture. After mixing the polymer and the fertilizer, the tubes were filled with substrate according to each treatment. It was sought to fill the tubes in approximately 80% of the maximum possible volume to avoid drainage of the substrate after hydration with the hydrogel due to the expansion of the particles of the hydride retainer during its hydration.

2.6 DESIGN, VARIABLES AND STATISTICAL ANALYSIS

All experiments were installed in a completely randomized design with ten replications, each plot consisting of nine seedlings. The variables were evaluated 280 days after the installation of the experiment, where, height - h (cm), shoot diameter - dc (mm) and h/d ratio. Height was measured using a graduated ruler (in cm) and the diameter of the collection with the aid of an electronic caliper (0.01 mm). In the experiment pinecone failure added to substrate was also evaluated the germination percentage (%) and germination speed index (GSI).

The treatment variances were tested for homogeneity by the Bartlett test. When there was homogeneity of variances, the data was subjected to analysis of variance and when there was a significant difference by the F test, the Tukey test and/or polynomial regression was applied at the 5% probability level. In the pinecone failure added to substrate experiment, we opted for the averages test due to the use of only 4 treatments. The maximum technical efficiency (MTE) of the controlled-release fertilizer for each evaluated characteristic was determined from the calculation of the partial derivatives of the equations adjusted by the regression analysis.

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3 RESULTS AND DISCUSSION

It is observed that with the exception of buffering water (WBC), all the other physical and chemical attributes evaluated showed a significant effect due to the addition of pinecone failure to the substrate (Table 1).

The pH of treatments with the addition of pinecone failure decreased compared to the commercial substrate. The pH of treatments with pinecone failure was close to 5.5, while the commercial substrate obtained 6.7. However, the pH found for all treatments is practically within the limit considered adequate, which according to Kratz *et al*. (2013), for forest species, varies between 5.5 and 6.5.

Table 1 - Analysis of the commercial substrate as a function of the addition of pinecone failure used for the production of *Araucaria angustifolia* seedlings.

Attributes analyzed	Pinecone failure ((%)			
	0	20	40	60
pH	6.7 a*	5.5 b	5.4 b	5.6 b
EC (mS cm^{-1})	0.27 b	1.11 a	1.02 a	0.93 a
WD (kg m^{-3})	222.1 c	329.8 b	485.8 a	453 a
DD (kg m^{-3})	188.2 b	241.9 a	200.9 ab	180.6 b
CH (%)	15.2 b	54.3 a	58.6 a	60.2 a
TP (%)	78.9 b	84.1 a	86.9 a	88.3 a
AS (%)	19.2 b	42.1 a	46.0 a	45.7 a
AW (%)	22.1 a	11.6 b	13.9 b	12.7 b
RAW (%)	20.4 a	7.9 b	10.3 b	9.82 b
WBC (%)	1.8 a	3.7 a	3.6 a	2.9 a
AW (%)	37.5 a	30.3 b	28.1 c	28.5 c

Were - pH= potential of hydrogen; EC= electric conductivity; WD=wet density; DD= dry density; CH= current humidity; TP= total porosity; AS- aeration space; AW= available water; RAW= easily available water; WBC=water buffering capacity; AW= remaining water.

* Means followed by the same letter on the line, do not differ by Tukey's test at the level of 5% probability of error. Source: Authors.

Electrical conductivity (EC) also varied with the addition of pinecone failure, increasing the values. The measurement of EC is important in plant cultivation, since it is a limiting factor of water availability and effects on the absorption of nutrients by plants (COSTA *et al*., 2001). In general, for forest species, the electrical conductivity must be between 1.5 to 3.0 mS cm-1 (KRATZ *et al*., 2013). Values above this limit may reflect toxic effects, generating problems of osmotic pressure, making nutrient absorption

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unfeasible (SANTANA *et al*., 2003). The results found for the substrate are well below this range, however, as the analysis was performed only with the fertilization present in the commercial substrate, with the added fertilization the values tend to increase depending on the fertilizer dose used (NAVROSKI *et al*., 2015).

The wet density (WD) was higher when 40 and 60% pinecone failure was used, and lower only when using the commercial substrate. The dry density (DD) was higher with the addition of 20% of pinecone failure, with lower values in the absence of the failure and with the use of 60%. The dry density (DD) value, considered ideal for substrate used in containers, is between 400-500 kg m^{-3} (SCHMITZ *et al*., 2002). Both the commercial substrate and the treatments with the addition of pinecone failure showed DD below this range, although there was a significant increase in density, with the addition of 20% failure. The observed DD values for 20 and 40% of failure are in the range of 200-400 kg m^{-3}, considered acceptable for cultivation in containers up to 15 cm high (KÄMPF, 2000). The low density characteristic of these materials can cause problems in plant fixation and tipping, if the cultivation is done in tall containers like tubes (JANSEN *et al*., 1989). Thus, regarding the density values, it can be considered that the pinecone failure is beneficial for the cultivation of araucaria seedlings, however the density is related to porosity, an important parameter for the adequate growth of plants.

The addition of pinecone failure, at any dosage, provided significant increases for current humidity (CH), total porosity (TP) and aeration space (AS). All treatments showed adequate TP values according to the values indicated by Kratz *et al*. (2013), above 75%. According to De Boodt and Verdonck (1972), a substrate considered ideal must have a total porosity of at least 85%. Thus, treatments with the addition of pineapple failure have the best characteristics for this attribute. As for AS (%), which is the difference between TP and the volume of water retained at a tension of 10 hPa, all treatments are outside of what is considered ideal (30%) by Schmitz *et al*. (2002). Treatments with pinecone failure showed very high values, which can result in water deficiency in plants, especially in the case of infrequent irrigation, but can be used as substrate conditioners (SCHMITZ *et al*., 2002). On the other hand, the use of the

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commercial substrate without adding the fault had AS below the ideal range, which can hinder gas exchange in the roots and hinder the development of the plant.

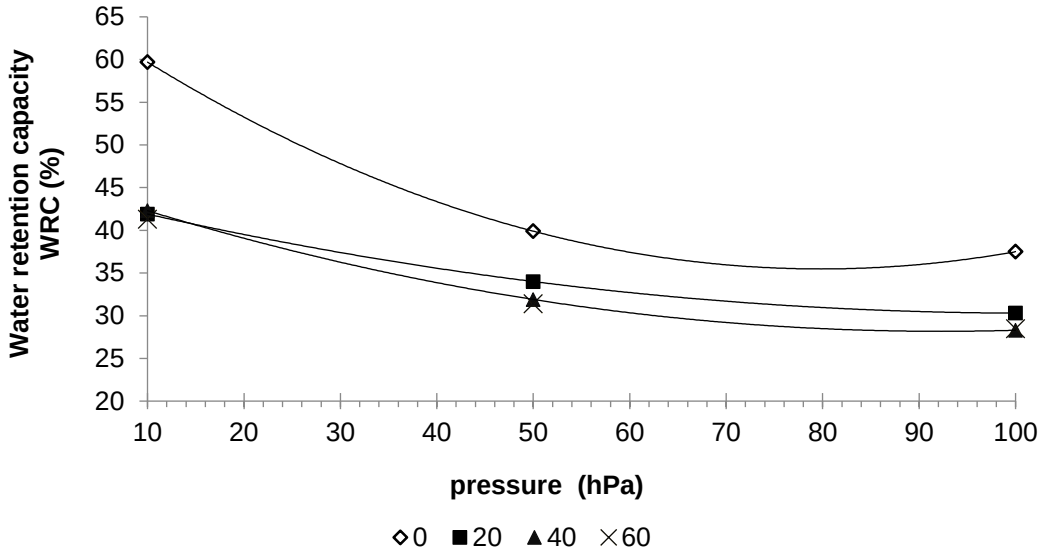
Regarding the available water (AW), easily available water (RAW) and remaining water (AW), the addition of the pinecone failure caused a significant reduction in attributes, showing that the addition of the failure can harm the issue of water availability for the plants. According to Abad *et al*. (1993), in optimal conditions, the ideal substrate should have between 24 and 40% of AW and between 20 and 30% of RAW for plants. For these two attributes, the addition of pinecone failure, even with the addition of 20%, caused a drastic reduction in water availability, keeping limits below those considered ideal for plants.

The remaining water (AW), on the other hand, the ideal value is in the range of 25 to 30% (ZORZETO *et al*., 2014), which places the failed treatments within the ideal range. However, the AW is the volume of water retained in the substrate after applying the tension of 100 hPa corresponds to the volume of water not available to the plant (DE BOODT; VERDONCK, 1972). Increasing the availability of water in the substrate, mainly at lower tensions, reduces the amount of water used for irrigation, or the frequency of irrigation. Thus, the addition of pinecone failure would require adjustments in irrigation to avoid water deficit problems in the plants.

For water retention capacity (WRC), in all evaluated suction columns, there was a decrease in retention with the addition of pinecone failure to the substrate (Figure 1). On average, there was a 30% decrease in WRC with the addition of the failure. As discussed above, adding pinecone failure can increase the frequency or amount of water in irrigation. Substrates with less water retention capacity require more water to be applied to each irrigation, or to increase its frequency (NAVROSKI *et al*., 2016).

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Figure 1 - Characteristic curve of water retention capacity – WRC - under suction of 10, 50 and 100 cm of water column of the commercial substrate as a function of the addition of pinecone failure used for the production of *Araucaria angustifolia* seedlings.



Source: Authors.

Regarding the variables analyzed as a function of the amount of pinecone failure added to the substrate, there was no difference between treatments for germination (%), height (h), shoot diameter (dc) and h/d ratio (Table 2). There was only difference for the GSI (p-value=0.01), in which 40% and 60% of failure had the lowest averages. The average germination obtained was 90.7%. These results show that the pinecone failure can be used in the composition of the substrate for the production of araucaria seedlings, without decreasing the quality. The species had no difference in growth, even using up to 60% of pinecone failure. Pinecone failure is a product that is usually discarded after the harvest of pine nuts. It has the advantage of being used on the property itself to produce the substrate and only drying and grinding is necessary.

For the production of substrates for plants, components available in the region must be used, and they must mainly have good physical characteristics, such as porosity, water retention, among others. New products should be researched as alternatives to the formulation of substrates, such as the use of agro-industrial, forestry and urban residues for the production of seedlings, as large volumes of these products are generated,

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representing an environmental problem if they do not have an adequate final destination (KRATZ *et al*., 2013; CHEMETOVA *et al*., 2019).

Table 2 - Germination (%), GSI (germination speed index), height (cm), shoot diameter (mm) and h/d ratio (height/shoot diameter) of *Araucaria angustifolia* according to pinecone failure added to the commercial substrate, container volume and hydrogel added to substrate.

Pinecone failure percentages					
Pinecone failure (%)	Germination (%)	GSI	Height (cm)	Shoot diameter (cm)	h/d Ratio
0	89 a*	0.80 a	23.4 a	3.5 a	6.7 a
20	89 a	0.82 a	24.9 a	3.7 a	6.8 a
40	94 a	0.72 b	23.5 a	3.5 a	6.8 a
60	91 a	0.76 b	22.8 a	3.4 a	6.8 a
Average	90.7	0.77	23.6	3.5	6.8
CV** (%)	2.25	4.9	14.6	14.2	5.6

Container volume			
Container volume	Height (cm)	Shoot diameter (cm)	h/d ratio
55 cm^3	20.4 b*	3.4 a	6.1 a
110 cm^3	21.3 b	3.4 a	6.3 a
180 cm^3	24.6 a	3.5 a	6.5 a
280 cm^3	23.7 a	3.7 a	6.7 a
Average	22.5	3.5	6.4
CV** (%)	8.6	7.3	8.1

Hydrogel added to substrate and mother trees					
			Height (cm)		
Hydrogel (g L^{-1}) / Mother	A10-9	A21-10	A23-3	A10-6	Average
0	32.9	31.0	27.5	27.8	29.8 b
3	33.7	34.5	28.1	33.8	32.5 a
6	34.7	33.4	26.4	31.0	31.4 a
Average	33.8 a	32.9 a	27.3 b	30.9 ab	
			Diameter (mm)		
0	4.2	4.1	4.0	3.7	4.0
3	3.8	3.9	3.9	4.0	3.9
6	3.8	4.0	3.6	3.7	3.8
Average	3.9	4.0	3.8	3.8	
			h/d ratio		
0	7.8	7.7	6.8	7.6	7.5 b

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3	9.2	8.7	7.3	8.4	8.4 a
6	9.1	8.4	7.4	8.5	8.4 a
Average	8.7 a	8.3 b	7.2 c	8.2 b	

* Averages followed by the same letter do not differ from each other by the Tukey averages test (P<0,05); ** Coefficient of variation for each variable. Source: Authors.

According to Jacinto *et al*. (2014), the content of extractives in hot water, toluene and lignin are higher in pinecone failure than for wood such as pine. These findings could result in probable toxicity to the seedlings, however, there were no symptoms of deficiency or excess of nutrients, even in the highest proportion of failure tested (60%) after 280 days of cultivation. There are no studies in the literature involving the use or addition of pinecone failure in substrates for seedling production by seeds.

In relation to the experiment of volume of the containers, the growth in height of the araucaria seedlings was higher in the 180 cm³ (24.6 cm) and 280 cm³ (23.7 cm) tubes, 280 days after sowing (Table 1). Smaller tubes (55 cm³ and 110 cm³) did not favor the development in height of the seedlings (20.4 cm and 21.3 cm respectively), as those produced in larger tubes. In shoot diameter and h/d ratio, there were no significant differences between the different tube sizes. Although there was no difference in diameter, it is recommended to use tubes of 180 cm³, due to the bigger height of the seedlings. In addition, if it is necessary to maintain the seedlings for a period longer than 200 days in the nursery, damage to the quality of the seedlings may occur. Similar results were obtained in *Parapiptadenia rigida* (Benth.) Brenan seedlings, which had considerably better height development in 300 cm³ tubes 110 and 50 cm³ tubes 170 days after emergence (FERRAZ; ENGEL, 2011).

As for the addition of the hydrogel to the substrate, there was no interaction between the hydrogel and mother factors for any of the variables. For the sources of variation hydrogel and mother, there was significance for the variables height and h/d ratio (Table 1). It was observed that the bigger height of the seedlings was obtained using a dose of 3 or 6 g L^{-1} of the polymer. The use of 3 g L^{-1} provided an increase of 10% in the height of the seedlings in relation to the control group. Depending on the mother, a difference of 20% was obtained between the best (A10-9) and the lowest average (A23-3). As for shoot diameter, which had no difference between the factors, the general

ISSN: 1696-8352

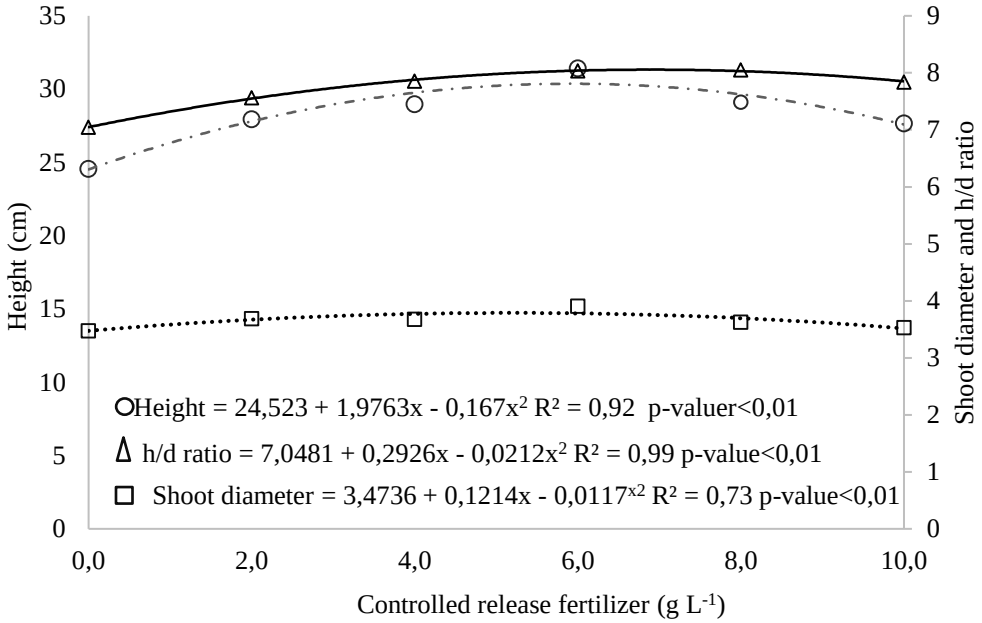
average was 3.9 mm. Similar to height, the hydrogel dosage and mother had an effect on the h/d ratio. The use of hydrogel (3 or 6 g L^{-1}) provided the highest averages of h/d ratio, differing from the absence of the polymer. As for the mothers, A10-9 had the highest average, and A23-3 the lowest. It was observed that the bigger height of the seedlings was obtained using a dose of 3 or 6 g L^{-1} of the polymer.

Probably, the increase in water retention with the hydrogel dose contributed to the water retention in the substrate and provided better growth, such as Navroski *et al*. (2015), where the hydrogel dose of 3 g L^{-1} added to the cultivation substrate provided greater growth in *Eucalyptus dunnii* seedlings, and also in *Aspidosperma parvifolium* seedlings (MENEGATTI *et al*., 2017). This ability of plants to use the hydrogel water is attributed to the contact surface of the roots with the hydrated hydrogel granules. Analyzing the growth results of araucaria seedlings as a function of controlled release fertilizer doses, there was significance for quadratic regression (Figure 2) for height, stem diameter and h/d ratio. Also obtaining adjusted R values, considered high for height (0.92) and for h/d ratio (0.99) and average for shoot diameter (0.73) as a function of controlled release fertilizer doses. The height and diameter variables were more responsive to the 6 g L^{-1} dosage of the controlled release fertilizer. At this dosage, the highest mean height (31.4 cm) was approximately 7 cm greater than the non-application of the fertilizer (24.6 cm). The doses of 2 g L^{-1} and 10 g L^{-1} promoted a very similar development for height (27.9 cm and 27.7 cm).

The maximum technical efficiency (MTE) of controlled release fertilizer for seedling height was 5.9 g L^{-1}. The diameter and h/d ratio proportionally followed the development in height of the seedlings with the fertilizer. The largest diameter (3.9 cm) and the highest h/d ratio (8.0) were also obtained at a dose of 6 g L^{-1}. The MTE for the collection diameter was 6.9 g L^{-1} and for the h/d ratio it was 5.2 g L^{-1}. A similar response to the present study was also verified for araucaria by Rossa *et al*. (2011), in which doses between 6 and 9 g L^{-1} had greater growth in height, diameter and dry mass.

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Figure 2 - Height (cm), shoot diameter (cm) and height/shoot diameter ratio (h/d) as a function of doses of controlled release fertilizer in the growth of *Araucaria angustifolia* seedlings.



Source: Authors.

Similar responses to the present study were also obtained in other tree species, as in the study by Moraes Neto *et al*. (2003), in which these authors verified an increase in height with the application of controlled release fertilizer in the production of seedlings of *Guazuma ulmifolia*, *Peltophorum dubium*, *Eucalyptus grandis*, *Calycophyllum spruceanum* and *Pinus caribaea* var. *caribaea*, but with different amplitudes. For these species, grown in 50 cm³ tubes, the doses between 4.3 and 6.4 g L^{-1} of controlled release fertilizer (14-14-14) presented the highest values in height, after 125 days. In contrast, a lower dose (2.93 g L^{-1}) of controlled release fertilizer (19-06-10) was necessary to obtain a bigger height in *Pinus taeda* seedlings (WILSEN NETO; BOTREL, 2009).

The plants in 0 g L^{-1} treatment 200 days after sowing developed chlorotic characteristics, possibly due to the low fertility of the standard substrate and high demands of the species, corroborating with Rossa *et al*. (2011). The most visible effect is the yellowing or chlorosis of the leaves, due to the decrease in chlorophyll synthesis, often associated with the lack of N in araucaria plants. Regarding the h/d ratio, all treatments obtained averages within the limits between 5.4 and 8.1 (SILVA *et al*., 2019), indicating

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a greater capacity of survival of the plants when transferred to the field in permanent planting.

4 CONCLUSION

The addition of pinecone failure to the substrate improved some physical attributes such as density, total porosity and aeration space, however it worsened the attributes related to water availability in the substrate. As for the growth of seedlings, the addition of pinecone failure did not change the height, diameter and h/d ratio.

Seedlings produced in 180 cm^3 tubes had greater growth than those in smaller containers, and resulted in growth equivalent to that of seedlings produced in the 280 cm^3 containers.

Fertilizer doses influenced the growth of the seedlings. All variables had better values with the use of 6 g L^{-1}. To save resources and time, it is not necessary to add higher concentrations of fertilizer in the production of seedlings in tubes.

The use of hydrogel in a regular irrigation regime can favor the height development of the seedlings.

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REVISTA CONTRIBUCIONES A LAS CIENCIAS SOCIALES

Sistema de gestão Lean: estudo de caso aplicado no setor florestal

Lean management system: study case applied in the forest sector

Sistema de gestión Lean: estudio de caso aplicado en el sector forestal

DOI: 10.55905/revconv.17n.12-017

Originals received: 09/30/2024
Acceptance for publication: 10/21/2024

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RESUMO
Este estudo objetivou analisar a eficiência da implementação de um sistema de gestão com base na filosofia Lean no setor de pátio de madeiras de uma empresa de base florestal comparando diferentes períodos de implementação através dos indicadores-chave de performance. O projeto foi desenvolvido em uma empresa de base florestal, localizada na região dos Campos Gerais, no estado do Paraná/BR. A metodologia de implementação das práticas de gestão Lean foi feita em

ciclos semanais de melhoria contínua, divididos em 12 semanas, contando com a capacitação dos gestores das áreas, construção das ferramentas e aplicação nas respectivas áreas. A eficiência do Sistema de Gestão Lean (SGL) foi definida pelos KPI's - Key Performance Indicator (Indicador-Chave de Performance) que a área estabeleceu de acordo com a estratégia da mesma. A média geral do indicador Tempo de Pátio teve uma queda, saindo de 0,622 h no período de implementação, para uma média de 0,597 h após a segunda reavaliação. Juntamente com o indicador de produtividade, que no período de implementação atingiu uma média geral de 197,05 t.h-1, para uma média de 221,43 t.h-1 após a segunda reavaliação. A correta aplicação das práticas e ferramentas Lean tendem a refletir em aumentos de performance dos indicadores-chave.

Palavras-chave: gestão florestal, pátio de madeira, melhoria contínua, otimização de produção.

ABSTRACT

This study aimed to analyze the efficiency of a management system implementation based on the Lean philosophy in the wood yard sector of a forest-based company, comparing different implementation periods through key performance indicators. The project was developed in a forest-based company, located in the Campos Gerais region, in the state of Paraná/BR. The implementation of the Lean management practices methodology occurred in weekly cycles of continuous improvement, divided into 12 weeks, with the training of area managers, construction of tools and application in the respective areas. The efficiency of the Lean Management System (LMS) was assessed by the KPI's (Key Performance Indicator) which the area established according to its strategy for itself. The overall average of the Yard Time indicator dropped from 0.622 h in the implementation period to an average of 0.597 h after the second reassessment. Along with the productivity indicator, which in the implementation period reached an overall average of 197.05 $t.h^{-1}$, for an average of 221.43 $t.h^{-1}$ after the second reassessment. The correct application of Lean practices and tools tend to reflect in performance increases in key indicators.

Keywords: forest management, lumber yard, continuous improvement, production optimization.

RESUMEN

Este estudio tuvo como objetivo analizar la eficiencia de la implementación de un sistema de gestión basado en la filosofía Lean en el sector de patios de madera de una empresa de base forestal, comparando diferentes períodos de implementación a través de los indicadores clave de desempeño. El proyecto se desarrolló en una empresa de base forestal ubicada en la región de Campos Gerais, en el estado de Paraná, Brasil. La metodología de implementación de las prácticas de gestión Lean se llevó a cabo en ciclos semanales de mejora continua, divididos en 12 semanas, incluyendo la capacitación de los gestores de las áreas, la construcción de las herramientas y la aplicación en las respectivas áreas. La eficiencia del Sistema de Gestión Lean (SGL) se definió por los KPI's - Key Performance Indicators (Indicadores Clave de Desempeño) que el área estableció de acuerdo con su estrategia. El promedio general del indicador Tiempo de Patio disminuyó, pasando de 0,622 h en el período de implementación a un promedio de 0,597 h después de la segunda reevaluación. Junto con el indicador de productividad, que en el período de implementación alcanzó un promedio general de 197,05 $t.h^{-1}$, pasó a un promedio de 221,43 $t.h^{-1}$ después de la segunda reevaluación. La correcta aplicación de las prácticas y herramientas Lean tiende a reflejarse en aumentos de desempeño de los indicadores clave.

Palabras clave: gestión forestal, patio de madera, mejora continua, optimización de la producción.

1 INTRODUÇÃO

O sistema de gestão de uma empresa reflete diretamente em sua missão, visão e objetivos, bem como no sucesso para atingir as metas estabelecidas para que haja a sustentabilidade a longo prazo. Independentemente do tamanho, as empresas contam com líderes cuja função é guiar suas respectivas equipes, realizando a gestão das pessoas, conflitos, resultados e desenvolvimento de competências, e cada qual possui diferentes características pessoais para realização de tais tarefas.

O desafio de buscar níveis crescentes de produtividade e qualidade não implica apenas na modernização das tecnologias de processo. A introdução de novas metodologias voltadas à melhoria de processos como o Lean Manufacturing (Manufatura Enxuta) tem motivado mudanças no sistema físico de produção, mas, para se alcançar os resultados superiores vislumbrados, é imperativo que as pessoas desenvolvam novas competências para trazer novos conhecimentos, habilidades e atitudes. Isso requer o desenvolvimento das capacidades pessoais de trabalho em equipe, criatividade, inovação e iniciativa para que a organização possa se adaptar às constantes mudanças no ambiente de negócios, absorver novas tecnologias e sustentar o processo de melhoria contínua dos processos de negócio (Fleury; Fleury, 2003; Ubeda; Santos, 2008).

O sistema Lean surgiu da necessidade das empresas japonesas do setor automobilístico, em especial a Toyota Motor Company, desenvolverem métodos diferentes de fabricar veículos em relação aos utilizados pela indústria americana. O destaque era o sistema de produção em massa da Ford Company e General Motors, pois perceberam que não conseguiriam competir com base nos mesmos conceitos e, disso, resultou um novo modelo de sistema de produção conhecido como Sistema de Produção Enxuto ou Sistema Toyota de Produção (Lean Manufacturing/Lean Production).

A abordagem Lean Manufacturing faz parte de um movimento associado a um termo muito utilizado pelas empresas, que é a ‘melhoria contínua’. Caffyn (1999) conceitua melhoria contínua como um amplo processo concentrado na inovação incremental que envolve toda a

organização. Por constituir-se num conceito simples, de fácil entendimento e de baixo nível de investimento, a melhoria contínua tem se consagrado como uma das formas mais eficientes de aumentar a competitividade de uma empresa (Bessant *et al*., 1994).

Pressionada pelo acirramento da concorrência, nos últimos tempos as empresas conquistaram notáveis ganhos em produtividade e qualidade, mas sabem que não podem arrefecer seus esforços de melhoria contínua e que é preciso melhorá-las ainda mais. Neste esforço de aumento da competitividade, em empresas líderes, a função manufatura tem se sobressaído das demais áreas da organização pela expressiva melhoria de seu desempenho operacional, e pelo seu pioneirismo na introdução de novas abordagens de gestão (Silva *et al.*, 2011).

No setor florestal, as abordagens focadas em qualidade e melhoria contínua aconteceram de maneira mais lenta quando comparadas a outros setores. Grandes players do setor trabalham atualmente na estruturação e continuidade dos trabalhos realizados com este foco. Por isso, há a necessidade de entender os possíveis impactos relacionados a implementação de sistemas de gestão com base em melhoria contínua em empresas florestais, demonstrando o seu valor quando há uma mudança cultural.

Estes possíveis impactos na mudança da forma de fazer gestão e nos resultados operacionais devem ser medidos. A medição de desempenho pode ajudar a detectar o que está acontecendo com o desempenho da empresa, quais as razões prováveis que configuram a situação atual e quais podem vir a ser as ações a serem tomadas (Bond, 1999). De acordo com Hronec (1994), medidas de desempenho são os “sinais vitais” da organização. Elas informam às pessoas o que estão fazendo, como elas estão se saindo e se elas estão agindo como parte do todo. Elas comunicam o que é importante para toda a organização: a estratégia da gerência de primeiro escalão para os demais níveis, resultados dos processos, desde os níveis inferiores até o primeiro escalão, e controle e melhoria dentro do processo.

Neste contexto, a combinação da filosofia de Lean Manufacturing junto com ferramentas e processos pertencentes a este sistema tendem a proporcionar uma produção cada vez mais competitiva em um cenário de constantes mudanças e inovações tecnológicas. É necessário analisar e entender a influência que um novo sistema de gestão com foco em ferramentas Lean pode trazer para os indicadores de performance das operações de uma empresa.

A relação entre a aderência do sistema de gestão focado em melhoria contínua após sua

implementação, com a evolução da eficiência dos processos e resultados operacionais das empresas e os possíveis impactos positivos na sua cadeira produtiva, se torna essencial para a estratégia e sustentabilidade do negócio, além de considerar se as ferramentas e a filosofia de gestão do novo sistema adotado trazem os resultados esperados.

Este estudo objetivou analisar a eficiência da implementação de um sistema de gestão com base na filosofia Lean no setor de pátio de madeiras de uma empresa de base florestal comparando diferentes períodos de implementação através dos indicadores-chave de performance.

2 METODOLOGIA

2.1 LOCAL DE REALIZAÇÃO DO ESTUDO

O projeto foi desenvolvido em uma empresa de base florestal, que atua nos segmentos de celulose, papel-cartão, caixas de papelão e sacos industriais localizada na região dos Campos Gerais, no estado do Paraná/BR. A implementação piloto do Sistema de Gestão Lean (SGL) deu início no pátio de madeira da empresa.

2.1.1 Pátio de madeira

As operações que envolvem o pátio de madeira têm relação com a gestão de estoque e abastecimento das linhas de produção fabril. A operação de abastecimento das linhas de produção ocorre 24 horas por dia, divididas em 3 turnos.

O processo tem início com a chegada dos caminhões de transporte de madeira na balança, localizada na portaria da empresa, após há a retirada das cintas de amarração da carga e direcionamento dos caminhões para as pilhas de estoque interno no pátio ou diretamente nas linhas de abastecimento fabril onde ocorre a descarga. Ao finalizar a descarga, deve ser realizada a limpeza da carroceria e saída do caminhão do pátio.

A área de pátio conta com 80 colaboradores, distribuídos nas diferentes funções para a atividade, sendo as principais: operadores de escavadeiras para carregamento/descarregamento das linhas de produção (consumo direto) e pilhas de madeira (estoque interno) e motoristas de

caminhões de baldeio interno.

2.1.2 Equipe de implementação

Para a implementação do SGL nas áreas, houve a estruturação de uma equipe base, contendo os seguintes participantes:

Mentores estratégicos: responsáveis por direcionar os agentes de mudança nas áreas de atuação, oferecendo o suporte técnico e engajando os gestores das unidades florestais na metodologia.

Agentes de mudança: responsáveis por capacitar e implantar as ferramentas Lean na rotina das áreas em questão, sendo os especialistas da metodologia e mentores dos líderes Lean das áreas.

Líderes Lean: responsáveis por internalizar a metodologia na rotina da área onde atuam e conduzir um sistema de gestão Lean sustentável, buscando melhorias no processo diariamente, tendo uma dedicação exclusiva Lean.

Gestores das áreas: responsáveis por garantir o engajamento e as condições necessárias para a implementação e sustentabilidade do Lean na gestão da rotina. Fazem parte da gestão da área gerentes, coordenadores, supervisores e líderes operacionais.

2.2 PROCESSO DE IMPLEMENTAÇÃO DO SISTEMA DE GESTÃO LEAN

A metodologia de implementação das práticas de gestão foi realizada em ciclos semanais de melhoria contínua, divididos ao longo de 12 semanas.

A capacitação ao longo das semanas passou por um processo contínuo de desenvolvimento, em que o conhecimento e implementação de ferramentas de gestão da metodologia foram repassados para a gestão das áreas até atingir um estado contínuo, conforme estratégia definida para as 12 semanas.

As primeiras 4 semanas são focadas em capacitação técnica, coordenados pelos mentores estratégicos e agentes de mudança. Essa capacitação é voltada para os líderes Lean e equipe de gestão das áreas, para dar se o início do processo de implementação de ferramentas e rotinas. Nas semanas 4 a 8, há a identificação de obstáculos (como por exemplo espaço físico, horário de troca

de turno, dentre outros) e a continuidade de implementação das ferramentas Lean, com orientações constantes para o líder Lean e gestores das áreas.

Nas semanas 8 a 12, há uma transição de responsabilidades que ocorre de forma gradual, onde o líder Lean e os gestores das áreas assumem as rotinas e ferramentas, seguindo para o final do processo de implementação e início de estado contínuo do SGL na área.

2.3 EFICIÊNCIA DO SISTEMA DE GESTÃO LEAN

A eficiência do SGL foi definida pelos KPI's - Key Performance Indicator (Indicador-Chave de Performance) que a área estabeleceu de acordo com a estratégia da mesma.

A fim de acompanhar a eficiência do pátio de madeira, definiram-se dois principais indicadores pela gestão da área, os quais serão pormenorizadamente explanados a seguir.

2.3.1 Tempo de pátio

Para determinação do tempo de permanência dos caminhões de transporte de madeira da frota dentro do pátio, considerou-se o tempo desde a passagem do caminhão carregado pela balança na entrada da unidade fabril até a sua saída após o descarregamento da madeira e pesagem vazio na balança, conforme se expõe na equação (1).

$$TMP=HPS-HPC \qquad (1)$$

onde:

> TMP (tempo médio de pátio) corresponde ao período em que os caminhões ficam no pátio para o descarregamento da madeira, HPC (hora de pesagem na chegada), é o horário de chegada e pesagem dos caminhões carregados na balança, HPS (hora de pesagem na saída), é o horário da pesagem dos caminhões vazios na balança.

2.3.2 Produtividade

A Produtividade média foi dada em toneladas por horas, na equação (2). Para tanto

considerou-se o volume de madeira em que as máquinas escavadeiras de rodas descarregavam nas linhas de produção da fábrica em determinada unidade de tempo, para este caso, horas.

$$P=T/H \qquad (2)$$

onde:

P é produção em toneladas pelas horas de efetivo trabalho t.h-1; T corresponde a quantidade total descarregada e H são as horas de efetivo trabalho dedicadas para o descarregamento da madeira.

2.4 COLETA DE DADOS

Os dados coletados para o presente estudo foram obtidos através do sistema de gestão de informação interno que a empresa utiliza para gestão operacional e análise de indicadores, além de medições in loco realizadas pela equipe de implementação.

Para os indicadores do Pátio de Madeira (tempo de pátio e produtividade), foram considerados os dados obtidos por meio do apontamento de produção das máquinas, coletados diariamente, durante os três turnos de produção. Esse período englobou o início da implementação do SGL na área até o período pós implementação.

2.5 ANÁLISES REALIZADAS

Para a análise dos indicadores do Pátio de Madeira (tempo de pátio e produtividade), utilizou-se o software Sisvar para realização do teste de Tukey a 5% de erro para obtenção das médias e identificação de diferenças estatísticas entre os dados classificados.

O software Minitab foi utilizado para gerar gráficos Boxplot, e para estes indicadores com o objetivo de avaliar as medidas estatísticas do conjunto de dados, como variabilidade, média, e outliers, facilitando a visualização da evolução entre turnos e entre períodos de implementação do SGL.

Os demais dados foram analisados utilizando estatística descritiva e gráficos de barras e de linhas, para comparar visualmente valores entre categorias e mostrar tendências ao longo de determinados períodos.

3 RESULTADOS E DISCUSSÃO

Os indicadores chave das operações que envolvem as atividades no pátio de madeira da empresa refletem a eficiência final das operações logísticas da área florestal em interface com o início do processo industrial.

3.1 TEMPO DE PÁTIO

Os resultados obtidos considerando o tempo de pátio nos 3 turnos de atuação, em 3 etapas diferentes (durante a implementação do SGL, após a primeira avaliação de aderência e após a reavaliação de aderência do processo) apresentou interação entre os fatores (Tabela 1).

Tabela 1 – Tempo de pátio (h) em função dos turnos de avaliação e etapas de implementação do Lean.

Etapa	**Tempo de Pátio (h)**			**Média**
	Turno 1	**Turno 2**	**Turno 3**	
Implementação *Lean*	0,569 aA*	0,643 bB	0,652 bB	0,622
Pós *Lean* avaliação 1	0,589 aA	0,613 abAB	0,629 abB	0,610
Pós *Lean* reavaliação	0,599 aA	0,578 aA	0,615 aA	0,597
Média	0,583	0,619	0,636	

* Médias com letras iguais maiúsculas na linha e minúsculas na coluna não diferem entre si pelo teste de *Tukey* a 5% de erro.

Fonte: O autor (2022)

Observou-se um comportamento distinto no turno 1 em relação aos turnos 2 e 3. No compasso em que o turno 1 diferiu significativamente dos turnos 2 e 3 durante o processo de implementação do SGL, com o menor tempo de pátio (0,569 h), não apresentou evoluções com o passar do tempo no período pós implementação do SGL.

O turno 1 apresentou médias que não diferiram estatisticamente entre os turnos 2 e 3 após a última avaliação, indicando que este indicador se manteve em patamares positivos desde o início. Os demais turnos demonstraram evolução quando comparadas as médias da implementação do *Lean* com as medições pertencentes ao Pós *Lean* reavaliação, não diferindo estatisticamente da avaliação intermediária (Pós *Lean* reavaliação).

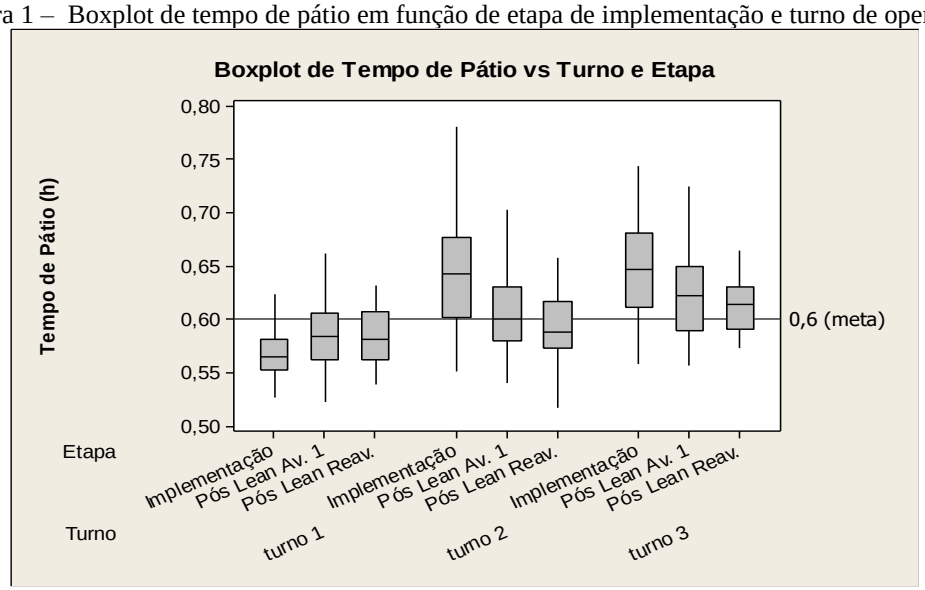
O turno 2 obteve uma média de 0,643h de tempo de pátio durante a implementação do SGL, reduzindo esta média para 0,613h no momento de realização da primeira avaliação de aderência (final do processo de implementação) em diante, e atingindo uma média de 0,578h no período pós reavaliação de aderência.

O turno 3 apresentou a média de tempo de pátio mais elevada durante o período de implementação do SGL no pátio de madeira (0,652h) e, assim como o turno 2, apresentou diminuições gradativas na média de tempo de permanência dos caminhões no pátio com o passar do tempo, atingindo o valor de 0,615h no período pós reavaliação do SGL.

Observou-se através de análise comparativa (Figura 1) que 50% dos tempos de pátio observados no turno 2 estavam entre 0,601h e 0,677h durante a implementação do SGL, ao passo que na reavaliação pós SGL, 50% dos tempos estavam entre 0,572h e 0,616h.

O comportamento foi semelhante no turno 3, onde 50% dos tempos estavam entre 0,611h e 0,681h no período de implementação do SGL e os mesmos 50% dos tempos estavam entre 0,591h e 0,630h pós reavaliação do SGL, indicando potencial redução para este indicador.

Figura 1 – Boxplot de tempo de pátio em função de etapa de implementação e turno de operação.



Fonte: O autor (2022)

No geral, houve uma redução na média global de tempo de permanência dos caminhões dentro do pátio, sendo um resultado bastante positivo para a empresa tendo em vista a eficiência do transporte da frota responsável por abastecer a unidade com matéria-prima.

Durante a implementação do SGL, o tempo de pátio encontrou-se nos patamares de 0,622h de média, enquanto no período pós reavaliação da aderência dos processos *Lean* na área, a média geral atingiu patamares de 0,597h, dentro da meta estipulada para operação que era de 0,600h.

A análise dos dados no estudo sobre a implementação do Sistema de Gestão Lean (SGL) no setor florestal destaca importantes ganhos de eficiência, especialmente no pátio de madeiras. Segundo Bond (1999), o uso de indicadores de desempenho (KPI's) é fundamental para identificar os pontos críticos de melhoria em qualquer processo. No caso deste estudo, a diminuição do tempo de pátio e o aumento da produtividade reforçam a importância de práticas de melhoria contínua.

Conforme Caffyn (1999), a filosofia Lean é uma estratégia voltada para incrementos contínuos de inovação, o que, neste estudo, foi refletido na redução do tempo de pátio de 0,622 h para 0,597 h, e no aumento da produtividade de 197,05 $t.h^{-1}$ para 221,43 $t.h^{-1}$ após a segunda reavaliação do processo Lean. Isso está em consonância com a ideia de Fleury e Fleury (2003), que afirmam que o desenvolvimento de competências é crucial para o sucesso da implementação de novos sistemas de gestão, como foi observado aqui, onde a capacitação dos gestores e operadores resultou em melhorias significativas de desempenho.

3.2 PRODUTIVIDADE

Os resultados obtidos de produtividade apresentaram diferença significativa referentes as etapas de implementação do SGL na área de Pátio de Madeira (Tabela 2).

Esse indicador de produtividade de abastecimento nas linhas de produção fabril auxilia a gestão da área de pátio de madeira a medir o desempenho de sua operação, turnos e operadores.

Tabela 2 – Produtividade (t.h-1) em função das etapas de implementação do Lean.

Período	**Produtividade (t.h-1)**
Implementação *Lean*	197,05 a*
Pós *Lean* avaliação 1	215,20 b
Pós *Lean* reavaliação	221,43 b

* Médias com letras iguais não diferem entre si pelo teste de *Tukey* a 5% de erro.
Fonte: O autor (2022)

Observou-se que houve uma evolução no KPI Produtividade desde o início da implementação do SGL até o período pós avaliações de aderência dos processos. Durante a implementação, obteve-se uma média de 197.05 $t.h^{-1}$ no geral, considerando os 3 turnos. Após o SGL implementado na área, obteve-se uma média de 221.43 $t.h^{-1}$, resultado este superior a meta estipulada para a operação, que era de 210.00 $t.h^{-1}$.

A distinção da visualização da produtividade entre turnos, contribui para a gestão da área identificar onde necessita focalizar os esforços de melhoria para evolução dos indicadores de performance. Os resultados obtidos de produtividade apresentaram diferença significativa entre os diferentes turnos de operação na área de Pátio de Madeira (Tabela 3).

Pode-se identificar que o turno 2 apresentou menores médias de produtividade (199.18 $t.h^{-1}$), não atingindo a meta estipulada para a operação (210.00 $t.h^{-1}$) e necessita de utilização da ferramenta para resolução de problemas para identificação e organização das tratativas com foco na evolução do indicador. Os turnos 3 (212.65 $t.h^{-1}$) e 1 (216.72 $t.h^{-1}$) apresentaram médias superiores a meta estipulada para a operação e podem servir como benchmarking interno para o turno 2 no objetivo de evolução deste KPI.

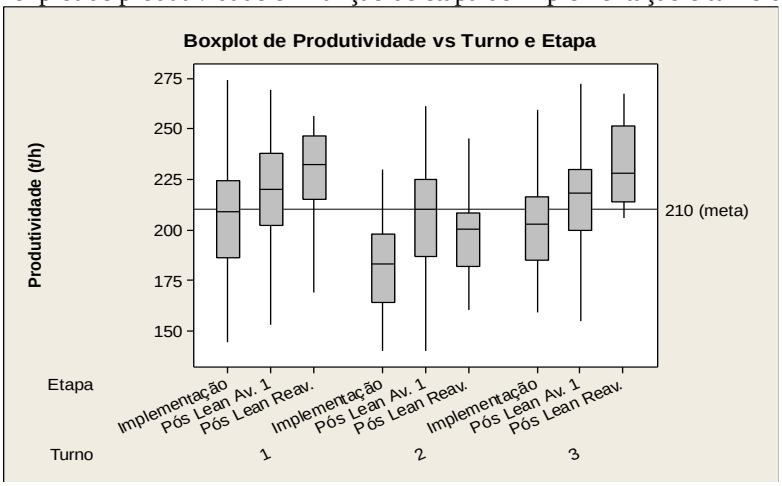
Tabela 3 – Produtividade (t.h-1) em função dos turnos de operação no pátio de madeira.

Turno	**Produtividade ($t.h^{-1}$)**
2	199.18 a*
3	212.65 b
1	216.72 b

* Médias com letras iguais não diferem entre si pelo teste de Tukey a 5% de erro.
Fonte: O autor (2022)

Observou-se por meio de análise comparativa (Figura 2), que 50% dos dados de produtividade observados no turno 1 estavam entre 186 $t.h^{-1}$ e 224 $t.h^{-1}$ durante a implementação do SGL, enquanto na reavaliação pós SGL, 50% dos dados de produtividade estavam entre 215 $t.h^{-1}$ e 246 $t.h^{-1}$. Houve comportamento semelhante no turno 3, no qual 50% dos dados de produtividade estavam entre 185 $t.h^{-1}$ e 216 $t.h^{-1}$ no período de implementação do SGL e os mesmos 50% dos dados de produtividade estavam entre 213 $t.h^{-1}$ e 251 $t.h^{-1}$ pós reavaliação do SGL, indicando um potencial aumento de produtividade nestes turnos.

Figura 2 – Boxplot de produtividade em função de etapa de implementação e turno de operação.



Fonte: O autor (2022)

O estudo também evidencia a aplicação de práticas de benchmarking interno, identificando diferenças de desempenho entre os turnos e utilizando os resultados como referência para melhorias nos turnos com menor eficiência. Silva *et al*. (2011) destacam que a introdução de metodologias como o Lean Manufacturing tem um impacto direto na produtividade, o que foi comprovado pelos dados apresentados.

O impacto da redução da variabilidade no tempo de operação, como destacado no estudo, é consistente com as análises de Shah e Ward (2007), que argumentam que a implementação de práticas Lean está diretamente associada à melhoria dos processos, reduzindo ineficiências e criando sistemas mais robustos e previsíveis. Os autores destacam que a melhoria contínua, um dos pilares da filosofia Lean, é uma das principais razões pelas quais as empresas conseguem ganhos consistentes em indicadores operacionais.

Por outro lado, Netland (2016) salienta que a aplicação de sistemas de gestão Lean em diferentes contextos, como o florestal, pode enfrentar desafios relacionados à adaptação cultural e operacional. No presente estudo, a necessidade de capacitação dos gestores e a adaptação gradual dos turnos refletem esse ponto. O autor também ressalta que, para que o Lean seja eficaz em longo prazo, ele deve ser incorporado à cultura organizacional, o que também é discutido pelos autores deste estudo.

Em geral, os resultados apresentados corroboram a visão de Hines, Holweg e Rich (2004) sobre os efeitos de médio e longo prazo da implementação do Lean, destacando que as melhorias em indicadores de produtividade são sustentáveis quando há um acompanhamento contínuo e uma reavaliação periódica dos processos. A evolução dos indicadores no estudo, tanto no tempo

de pátio quanto na produtividade, sugere que a introdução das práticas Lean criou uma base sólida para a manutenção de altos níveis de desempenho operacional.

4 CONCLUSÕES

Iniciativas de mudanças culturais de gestão dentro de empresas proporcionam evolução na mentalidade das pessoas e uma cultura de melhoria contínua necessária para adaptação e sobrevivência em um mercado cada vez mais dinâmico e competitivo. Entretanto, toda mudança passa por processos de adaptabilidade, aprendizados e acompanhamento para que o novo modelo de gestão se estabeleça e crie raízes em todas as áreas e colaboradores de uma empresa.

Apesar de um processo de implementação de um novo modelo de gestão necessitar de tempo para que atinja a maturidade e os resultados esperados, observou-se uma tendência de melhoria nos indicadores de performance das áreas mesmo no curto prazo.

Indicadores apontaram melhorias quando comparados períodos antes da implementação *Lean* e após a implementação no âmbito geral, como é o caso na produtividade no pátio de madeiras. Melhorias também puderam ser observadas em um nível maior de detalhes, como na diferença entre turnos no tempo de permanência dos caminhões de madeira pátio, sendo uma ferramenta poderosa de gestão tendo em vista que as boas práticas do melhor turno podem ser multiplicadas nos demais, tendo potencial de maximizar ainda mais os resultados de performance.

Por tratar-se de uma filosofia nova de trabalho e modelo de gestão, as ferramentas também devem seguir um processo de melhoria contínua, realizando as adequações necessárias para que haja efetividade de acordo com a realidade das áreas e da empresa. O acompanhamento por meio da avaliação de aderência mostra-se essencial para que isso aconteça e o engajamento da alta liderança será um diferencial na perpetuidade do sistema adotado.

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