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Original Research

Seedling Growth Characteristics of *Celtis australis* L. Genotypes on Different Substrates Under a Limited Fertilization

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Abstract

This study was carried out to determine the effect of different growing media on growth characteristics of *Celtis australis* genotypes native to the Serik district of the Antalya province. For this purpose, seeds of five genotypes (GT1, GT2, GT3, GT4 and GT5) were sown in pots filled with 4 different growing media in January. In the experiment, four different substrates were prepared by mixing loamy soil (LS), sand (S), well fermented manure (M), peat (P), perlite (PER) and spent mushroom compost (SMC) in different formulations and ratios. During the study, a limited and fixed fertilization program was used. Results indicated that there were significant differences in the growth characteristics with respect to both the genotype and the growing medium. The best results for the growth characteristics of *C. australis* seedlings were seen in the growing media of SMC+S and LS+M+S. Different genotypes also showed significant variation which might provide opportunities to breeders and nursery owners to introduce alternative new forms to the industry.

Keywords: growing medium, growth, *Celtis australis*, Mediterranean hackberry, nursery production

Introduction

Mediterranean hackberry (*Celtis australis*), is a deciduous, round crown tree from the Cannabaceae (formerly Ulmacea) family; it can grow up to 20-25 m high and is native to North-eastern Africa, Southern Europe, Western Transcaucasia and Turkey [1-5]. In addition to its elegant crown structure that provides a large shade, the tree has a high drought tolerance and is resistant to parasites [1, 2, 4, 6]. Thus it has a high design potential in urban green spaces. *C. australis* is also suitable as an alternative to deciduous species such as sweetgum, incense and ash tree.

In addition to ornamental purposes, *C. australis* is a multipurpose tree species which is largely utilized for fodder, fuelwood, fruit, medicine and timber [7, 8]. *C. australis* is the subject of many different studies all around the world most of which are related to the germination and emergence characteristics of the seeds of the species [1, 2, 6, 9-14]. Data regarding

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the vegetative propagation using cuttings of *C. australis* have also been published [15-18]. Many studies have been conducted on the nutritional, physicochemical, antioxidative, antibacterial and antifungal properties of the edible fruits of *C. australis* [19-23]. There are also studies on the nutrient and phenolic content of the leaves of *C. australis* [24, 25]. However, there are only a limited number of studies about the effect of different growing media on the growth characteristics of the species. Cattivello et al. [26] investigated the effect of peat-based growing media at different degrees of decomposition (poorly, medium and well) on the growth characteristics of *C. australis* seedlings. The best results were obtained in a poorly decomposed medium supplemented with fertilizer.

Producing healthy seedlings of high physical quality is of great importance in the ornamental plant cultivation industry and thus the selection of the type of growing media is one of the most essential aspects of which to be aware [27]. In order to determine which of the available and sustainable substrate options to select for use in the industry must be determined by taking their economic, chemical and physical aspects into account [28, 29].

Even though *C. australis* was evaluated for many aspects, the very few studies were reported in terms of growth conditions and the effects of different media on it. Since the plant has a high potential in helping to achieve and maintain a self-sustainable landscape, determining the optimum growing media to adapt it to landscape design is of significant importance. This study aims to find out the growth characteristics of *C. australis* genotypes in different growing media.

Materials and Methods

Plant Material

In this study, 5 *C. australis* genotypes were randomly selected from the rural areas of the Serik District (Antalya, South Anatolia, Turkey). Fruits of the selected genotypes (GT1, GT2, GT3, GT4 and GT5) were harvested in November 2013. The non-standard seeds were discarded in the laboratory following their separation from the fruit flesh.

Sowing and Growing Conditions

The seeds were sown in 3-liter pots in January 2014. Four different substrates were prepared by mixing loamy soil (LS), sand (S), well fermented manure (M), peat (P), perlite (PER) and spent mushroom compost (SMC) in different formulations and ratios as 2LS:1M:1S, 2P:1PER, 2P:1S and 2SMC:1S by volume, respectively. The pots were placed in an open field under natural conditions. Four holes, equidistant to each other and about 1.25 cm in depth, were dug in each pot and a seed was sown in each hole. Following germination and seedling growth, the seedling in the best condition of the four in each pot was chosen and kept while the other three were taken away from the pots in June 2014. The pots were hand-watered as needed, and a fertilizer program was used during the growth. Starting from the 6th month following sowing, 15 ml of liquid fertilizer, which includes N at 100 mg L^{-1}, P at 50 mg L^{-1} and K at 150 mg L^{-1}, was applied to each pot every 2 weeks. The experiment was performed from January to October 2014. Monthly means of minimum/maximum temperatures were recorded as 4.85/15.68ºC, 4.13/16.51ºC, 6.15/18.30ºC, 8.52/21.03ºC, 12.58/24.23ºC, 17.47/31.06ºC, 20.20/32.70ºC, 21.55/34.76ºC, 17.92/29.93ºC and 13.46/25.94ºC, respectively.

Data Collection

The plant height (height from soil to top of plant) (cm), stem diameter (10 cm above the soil) (mm), number of leaves (normal sized leaf throughout the growing period) and number of branches (longer than 1 cm) were recorded for each pot [27]. The stem and root dry weights (g per plant) were determined after oven drying at 105ºC for 24 h.

Physiochemical Property Analysis of the Substrates

The physical characteristics of the growing media were determined based on the methods presented by Fonteno and Bilderback [30]. The electrical conductivity (EC) (dS m^{-1}) and pH were measured in a 1:10 water-soluble extract (w/v) [31]. The organic matter content of the prepared growing media was determined following dry combustion at 550ºC [32]. Nitrogen (%) in substrates was determined by the Kjeldahl method [33]. The water-soluble extractions of substrates (BS EN 13652:2001) were analyzed by using inductively coupled plasma spectrometry (Laben Laboratory, Antalya, TR).

Data Analysis

The experiments were employed in completely randomized designs with three 20-plant replications and the analyses of variance (ANOVA) were used for statistical analyses of the data relating to the characteristics considered in this study [34]. Differences among the treatments were compared by Duncan's multiple range test at $P \leq 0.05$. Pearson's correlation coefficient was used to measure relations between physical and chemical characteristics of substrates and the growth characteristics of genotypes [35]. The statistical analysis procedures were evaluated with using SPSS software for Windows v. 13.0 (SPSS Inc. Chicago, United States).

Table 1. Physical characteristics of substrates used in the experiment.

Substrate	Bulk density (g cm^{-3})	Container capacity (%)	Air space (%)	Total porosity (%)	Water holding capacity (%)
P+S	0.88 c[z]	40.91 a	46.89 b	87.80 b	164.67 b
SMC+S	1.09 b	40.57 a	37.46 c	78.03 c	89.00 c
P+PER	0.35 d	39.75 a	57.46 a	97.87 a	782.00 a
LS+M+S	1.33 a	36.01 b	35.57 c	71.58 d	80.33 c

P+S: Peat+sand (2:1 by volume), SMC+S: Spend mushroom compost+sand (2:1 by volume), P+PER: peat+perlite (2:1 by volume), LS+M+S: loamy soil+well fermented manure+sand (2:1:1 by volume).
[z]: In columns (characteristics) means followed by different letters are significantly different at the 5% level according to Duncan's multiple range test. Values are the means of three substrate samples.

Results and Discussion

The substrate was used to anchor the plants and to supply nutrients, water and oxygen to the plants [36]. As shown in Table 1, the physical characteristics of the studied substrates were affected by the characteristics of their components. The bulk density was highest for LS+M+S (1.33 g cm^{-3}) and lowest for P+PER (0.35 g cm^{-3}). The container capacity of the substrates was highest for P+S (40.91%) and lowest for LS+M+S (36.01%); there was no statistical difference among P+S, SMC+S and P+PER substrates. The greatest air space and water holding capacity was recorded for P+PER, followed by P+S, SMC+S and LS+M+S. The highest total porosity value among the substrates was determined for P+PER while the lowest was for SMC+S.

Chemical characteristics of the substrates could also affect plant growth. The pH of the studied substrates varied between 7.3-8.1 (Table 2). The SMC+S had a higher electrical conductivity (EC) (2.495 dS m^{-1}) than other substrates. It is reported that the addition of spent mushroom compost to a substrate results in higher EC values [37, 38]. This value was within the recommended threshold value of ≤10 dS m^{-1} for the initial salt content in nursery container substrates [39]. The organic matter of the substrates was highest for P+PER and lowest for P+S and SMC+S. The SMC+S substrate had greater concentrations of available N, P, K, Ca and Mg than P+PER, LMS+M+S and P+S (Table 2).

The ANOVA results showed that significant effects were induced by genotype, substrate and genotype×substrate interactions on the plant growth characteristics of *C. australis* (Table 3). As shown in Table 4, the type of substrate selected influenced the plant height, stem diameter, number of branches, number of leaves, stem dry-weight and root dry weight of *C. australis* genotypes. Plants grown in SMC+S had the greatest allover plant growth, while plants grown in P+PER and P+S substrates had the lowest.

The substrates used in nurseries as a base for growth are usually composed mostly of peat and perlite. However, these substrates require intensive use of chemical fertilizers due to the lack of available organic content [40]. Thus the limited use of fertilizer in this study resulted in lower plant growth characteristics for P+S and P+PER substrates compared to SMC+S and LS+M+S substrates which were relatively rich in nutrients [41, 42] to support plant growth.

Numerous studies have been conducted presenting the effects of spent mushroom compost on growth and yield of different plants such as vegetables [37, 38], ornamentals [43] and medicinal plants [44, 45]. While most species present with positive effects, some species are negatively affected due to the presence of spent mushroom compost in the substrate [45].

Table 2. Chemical characteristics of substrates used in the experiment.

Substrate	pH	EC (dS m^{-1})	Organic matter (%)	N (%)	P (mg L^{-1})	K (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})
P+S	8.1 a[z]	0.151 d	13.00 b	0.14 b	0.52 c	37.78 c	92.35 c	4.44 b
SMC+S	7.5 c	2.495 a	13.00 b	0.65 a	27.09 a	1327.50 a	791.00 a	120.90 a
P+PER	7.3 c	0.245 c	71.67 a	0.28 b	13.90 b	138.65 b	64.48 c	5.99 b
LS+M+S	7.8 b	0.450 b	8.33 b	0.23 b	5.22 c	115.75 b	166.20 b	21.42 b

P+S: Peat+sand (2:1 by volume), SMC+S: Spend mushroom compost+sand (2:1 by volume), P+PER: peat+perlite (2:1 by volume), LS+M+S: loamy soil+well fermented manure+sand (2:1:1 by volume).
[z]: In columns (characteristics) means followed by different letters are significantly different at the 5% level according to Duncan's multiple range test. Values are the means of three substrate samples.

Table 3. Analysis of variance (mean squares) for plant height (PH), stem diameter (SD), number of branches (NOB), number of leaves (NOL), stem dry weight (SDW) and root dry weight (RDW) of five *C. australis* genotypes (G) evaluated for four growing substrates (S) in one growing season.

Source of variation	df	Mean square					
		PH	SD	NOB	NOL	SDW	RDW
Genotype (GT)	4	137.941***	0.252 ns	33.093***	2337.008***	38.238*	121.379**
Substrate (S)	3	6589.416***	53.386***	92.849***	16424.458***	4779.809***	13640.504***
GT × S	12	24.608ns	0.133 ns	6.624***	505.512***	4.931ns	44.171ns
Error	40	16.906	0.180	0.337	33.383	10.699	29.675

ns, *, **, ***: Non significant, significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

The studies confirm that higher yields and early growth of plants could be achieved with substrate mixtures consisting of spent mushroom compost in various ratios than for the plants that were grown in peat [37, 41, 45]. Since the ratio of spent mushroom compost in the substrate may differ in order to obtain improved plant characteristics, it is also necessary to investigate the proper ratio of substrate components prior to use. Using a higher proportion of spent mushroom compost in an amount greater than 25% in a substrate mix is not recommended since it may cause low water capacity, high salinity and neutral pH [38]. Depending on the source of spent mushroom substrate, it could also be used as a biofertilizer because it does not only affect the growth but also affect the physiochemical properties of the plant [46]. The spent mushroom compost and its associated microflora can also be used in bioremediation of fungicides and pesticides due to its high content of extracellular ligninocellulolytic enzymes [47].

The growth characteristics of *C. australis* genotypes used in this study exhibited significant variation. For instance, the GT4 genotype had the lowest mean plant height while it had the highest mean number of branches and leaves (Table 4). The GT3 genotype had the lowest mean stem diameter and root dry weight, while the GT5 genotype had the highest mean stem diameter and stem dry weight (Table 4). The present variation among genotypes was unexpected. Even though the area of seed collection was limited, it has great potential, providing opportunities for breeders and nursery owners to introduce alternative new forms for

Table 4. Mean comparison for the effect of four growing substrates (S) on plant height (PH), stem diameter (SD), number of branches (NOB), number of leaves (NOL), stem dry weight (SDW) and root dry weight (RDW) of five *C. australis* genotypes (GT) in one growing season.

Treatments	Growth characteristic					
	PH (cm)	SD (mm)	NOB (branches plant^{-1})	NOL (leaves plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})
Substrate						
P+S	28.18 c	3.14 b	1.33 c	27.94 b	5.09 b	18.66 c
SMC+S	67.00 a	6.43 a	5.99 a	87.03 a	36.61 a	78.25 a
P+PER	27.89 c	2.97 b	1.43 c	27.67 b	4.51 b	19.27 c
LS+M+S	61.20 b	6.21 a	5.35 b	83.07 a	34.78 a	61.39 b
Genotype						
GT1	46.84 a	4.69 ab	2.92 b	52.88 b	19.58 b	43.02 ab
GT2	46.88 a	4.63 ab	2.99 b	50.72 b	21.17 ab	46.73 a
GT3	48.08 a	4.52 b	2.58 b	48.27 b	19.10 b	39.76 b
GT4	40.13 b	4.68 ab	6.48 a	81.19 a	18.48 b	47.80 a
GT5	48.40 a	4.92 a	2.65 b	49.07 b	22.90 a	44.66 a

P+S: Peat+sand (2:1 by volume), SMC+S: Spend mushroom compost+sand (2:1 by volume), P+PER: peat+perlite (2:1 by volume), LS+M+S: loamy soil+well fermented manure+sand (2:1:1 by volume).

y: In each column and treatment factor means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range tests.

landscape industry [35, 48, 49]. In a study by Kumar et al. [8], eleven genotypes of *C. australis* were selected and tested in a nursery environment to identify suitable seed sources for plantation programs. A significant difference was determined by provenance selection. This was similarly evaluated as having great potential to improve different characteristics of *C. australis* for higher growth and productivity aspects. In general, diversity in characteristics of a plant depending on genotype selection has great significance in meeting different needs. In addition to meeting afforestation needs such as controlling soil erosion, mitigating climate change, improving carbon stock and providing fuel wood, fodder, fruit and timber, it could also be beneficial in the case of meeting different plant characteristics needs in landscape design such as height and spread, branching habit, flowers, fruit, and foliage.

Different genotypes and substrates independently showed significant effects on the plant height, stem diameter, stem dry weight and root dry weight values (Table 5). The highest mean plant height (71.42 cm) was measured for plants grown in SMC+S from GT3 genotype and plants grown in P+S from GT4 genotype had the lowest mean plant height of 24.92 cm (Table 5). The mean stem diameter ranged from 2.71 mm – 6.1 mm. The highest value measured for GT4 genotype grown in SMC+S and the lowest value was measured for GT3 genotype grown in P+S substrate. The greatest mean number of branches and number of leaves were recorded for plants grown in LS+M+S from GT4 genotype. The mean stem dry weight was highest for the GT5 genotype grown in SMC+S substrate. Similarly, the highest mean root dry weight was measured for the GT2 genotype grown in the same substrate. The lowest stem and root dry weight

Table 5. Mean comparisons for interaction effects of genotype × substrate on plant height (PH), stem diameter (SD), number of branches (NOB), number of leaves (NOL), stem dry weight (SDW) and root dry weight (RDW) of five *C. australis* genotypes (GT) in one growing season.

Genotype	Substrate	Growth characteristic					
		PH (cm)	SD (mm)	NOB (branches plant^{-1})	NOL (leaves plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})
GT1	P+S	27.93 gh	3.10 bc	0.73 g	23.35 g	4.18 d	14.56 h
	SMC+S	66.64 abcd	6.26 a	5.55 b	81.97 bc	33.86 bc	76.77 abc
	P+PER	29.39 gh	3.08 bc	1.07 fg	26.03 g	4.53 d	20.59 gh
	LS+M+S	63.40 bcde	6.32 a	4.33 cd	80.18 bcd	35.75 abc	60.18 ef
GT2	P+S	29.48 gh	3.22 bc	1.51 efg	29.73 fg	5.84 d	21.64 gh
	SMC+S	69.01 abc	6.49 a	5.22 bc	85.23 b	38.44 ab	85.85 a
	P+PER	27.03 gh	2.80 c	1.32 efg	23.09 g	4.26 d	18.55 gh
	LS+M+S	62.01 cde	6.01 a	3.92 d	64.82 e	36.15 abc	60.87 def
GT3	P+S	25.38 gh	2.71 c	1.05 fg	24.30 g	3.88 d	14.58 h
	SMC+S	71.42 a	6.19 a	4.32 cd	70.20 de	35.12 abc	70.48 cd
	P+PER	28.95 gh	2.92 bc	1.00 fg	25.23 g	4.33 d	17.99 gh
	LS+M+S	66.58 abcd	6.27 a	3.97 d	73.35 bcd	33.08 bc	55.99 f
GT4	P+S	24.92 h	3.02 bc	1.93 ef	31.72 fg	3.79 d	17.42 gh
	SMC+S	57.44 ef	6.71 a	10.62 a	126.85 a	34.88 abc	83.00 ab
	P+PER	24.40 h	2.81 c	2.35 e	36.92 f	3.82 d	22.09 gh
	LS+M+S	53.77 f	6.18 a	11.02 a	129.27 a	31.44 c	68.68 cde
GT5	P+S	33.16 g	3.67 b	1.44 efg	30.59 fg	7.79 d	25.11 g
	SMC+S	70.48 ab	6.50 a	4.23 cd	70.88 de	40.72 a	75.14 bc
	P+PER	29.68 gh	3.26 bc	1.42 efg	27.07 fg	5.61 d	17.15 gh
	LS+M+S	60.26 def	6.26 a	3.50 d	67.73 e	37.48 abc	61.24 def

P+S: Peat+sand (2:1 by volume), SMC+S: Spend mushroom compost+sand (2:1 by volume), P+PER: peat+perlite (2:1 by volume), LS+M+S: loamy soil+well fermented manuresand (2:1:1 by volume).

[y]: In each column (growth characteristic) means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range tests.

Table 6. Correlations (r) matrix between physical and chemical characteristics of substrates and growth parameters of genotypes. *P* values of correlations are given in italics.

Substrate characteristic	Growth parameters					
	Plant height	Stem diameter	Number of branch	Number of leaves	Stem dry weight	Root dry weight
Bulk density	0.762	0.803	0.576	0.693	0.795	0.719
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Container capacity	-0.347	-0.396	-0.274	-0.334	-0.392	-0.255
	0.007	*0.002*	*0.034*	*0.009*	*0.002*	*0.050*
Air space	-0.834	-0.863	-0.632	-0.754	-0.864	-0.816
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Total porosity	-0.821	-0.859	-0.625	-0.747	-0.858	-0.784
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Water holding capacity	-0.636	-0.663	-0.479	-0.572	-0.656	-0.618
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
pH	-0.147	-0.094	-0.116	-0.098	-0.117	-0.190
	0.262	*0.477*	*0.377*	*0.455*	*0.372*	*0.145*
EC	0.709	0.669	0.544	0.598	0.675	0.794
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Organic matter	-0.572	-0.607	-0.432	-0.518	-0.594	-0.545
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
N	0.616	0.575	0.490	0.528	0.582	0.680
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
P	0.496	0.443	0.398	0.413	0.450	0.574
	<0.001	*<0.001*	*<0.001*	*0.001*	*<0.001*	*<0.001*
K	0.655	0.609	0.502	0.545	0.612	0.742
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Ca	0.717	0.682	0.554	0.609	0.686	0.794
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Mg	0.730	0.694	0.565	0.619	0.694	0.791
	<0.001	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

was measured in P+S substrate and belonged to the GT4 genotype for stem dry weight and the GT1 genotype for root dry weight, respectively (Table 5).

Correlation analyses revealed several significant positive and negative correlations between the physical and chemical characteristics of the substrates and the growth parameters of *C. australis* genotypes (Table 6). The plant growth characteristics were significantly and positively correlated with bulk density, EC, contents of N, P, K, Ca and Mg while negatively correlated with container capacity, air space, total porosity, water holding capacity, pH and organic matter (Table 6).

Due to the limited number of studies regarding the response of *Celtis* species to different growing substrates used in nurseries, it is challenging to assess the results of this study in the light of previous studies in terms of seedling growth characteristics. Huxley [50] stated that loamy soil which has good drainage is suitable for the growth of *C. australis*. Cattivello et al. [26] studied the effects of peat with different decomposition degrees on *C. australis* and reported that the best growth and development was seen in a poorly decomposed peat. Even though many nurseries prefer to use peat as a growing substrate, environmental concerns limit the extraction process in many countries which leads to an increase in the price [40, 51]. On those grounds, other organic materials have been investigated for their potential as an alternative to peat [40]. In such cases it is noted that studies, carried

out with spent mushroom compost among those, presents better growth characteristics [37, 41, 44, 45].

In this study, the best results as in growth characteristics for *C. australis* plants were recorded using on a mixture of SMC+S and LS+M+S growing substrates. Even though a growing medium formulated with spent mushroom substrate initially contains elevated and potentially phytotoxic concentrations of soluble salts, it could be successfully used on the growth of numerous woody nursery plants produced in containers [39, 41, 44, 45, 51]. The obtained data shows that using SMC-amended substrate resulted in better growth characteristics when growing *C. australis* in containers, since SMC has stimulatory effects on it as one of those woody nursery plants. The preference of a growing substrate consisting SMC+S or LS+M+S is advantageous in terms of cheapness and accessibility while SMC+S also has beneficial environmental aspects by providing the reuse of waste products. The use of waste materials in growing substrates provides environmental benefits by replacing soil and peat in substrates thus reducing the damage caused by these sources when used [40]. The use of waste materials also provides better economic benefits than the use of known materials. Chong et al. [52] reported that the mushroom compost could be successfully recycled in the nursery container culture to make successful recycling. Thus, the result of this study is especially important since the SMC+S and LS+M+S growing substrates were determined as the most suitable growing substrates.

Conclusions

Since natural landscaping is increasingly practiced due to its self-sustainability and nature rehabilitative properties, providing landscape designing with new native species has significant importance. In a 5-month period under a limited and fixed fertilization program, satisfying results were obtained in terms of growth characteristics in *C. australis* seedlings. Despite performing a limited fertilization program, the result of the study suggests that an environmentally friendly *C. australis* cultivation with a sustainable fertilizing program could be achieved. The variation seen among genotypes may allow breeders and nursery owners to introduce new forms of *C. australis* to the industry. Also, in order to put forward *C. australis* as a contemporary part of the landscape, factors such as public perception, the cultivation period in the nurseries, the growth rate and the costs of *C. australis* should be given priority in follow-up work.

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Conflict of Interest

The authors declare no conflict of interest.

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