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Growing *Dimorphandra wilsonii* in different substrates and levels of shading

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Abstract. *Dimorphandra wilsonii* Rizzini is a tree species endemic to transition area between the “Cerrado” and the Atlantic Forest in the central region of the State of Minas Gerais, Brazil. The aim of this study was to evaluate the influence of different substrate compositions and levels of shading on the growth of *Dimorphandra wilsonii*. Seedlings were grown in different substrates: subsoil from Paraopeba; subsoil; commercial substrate BIOPLANT®; cattle manure; soil from Paraopeba and cattle manure (1:1); subsoil and cattle manure (1:1); commercial substrate and cattle manure (1:1); as well as in different levels of shading, as follows: abundant sunlight; half shading (Sombrite® 50%); and full shading (Sombrite® 80%). This study used a completely randomized experimental design. Producing *D. wilsonii* seedlings using a substrate comprised of 50% cattle manure and 50% subsoil in a shaded greenhouse proved to be effective.

Keywords: forest production, endemism, Faveiro de Wilson

Introduction

The production of forest seedlings is considered one of the most important and essential steps for achieving success in the implementation and establishment of forest stands (Jacobs et al., 2015). Low cost production of high quality seedlings is essential for the successful development of programs related to the establishment, rehabilitation and regeneration of native forests (Grassnickel and MacDonald, 2018).

Dimorphandra wilsonii Rizzini (Fabaceae – Caesalpinioideae), popularly known as Faveiro de Wilson, it is a tree species endemic to a small area in the central region of the State of Minas Gerais, Brazil. According to Rizzini (1969), it was described in areas of transition between the Cerrado and the semi-deciduous forest biomes and is one of the many endemic species in this region that are currently threatened with extinction due to habitat degradation.

Currently, there are around three hundred identified adult individuals, all of which are strongly endangered by varying forms human activity, such as agriculture and continuous expansive urbanization (Fernandes & Rego, 2014; Muniz et al., 2020). It is currently included in the Official National List of Endangered Flora Species (BRASIL, 2014), in the Red List of Threatened Species (IUCN, 2006) in the critically endangered category, as well as the Red List of Threatened Species of Extinction of the Flora of Minas Gerais (Martins et al. 2018).

The lack of protocols and even basic information regarding the processes involved in the production of endemic species, such as *D. wilsonii*, is one of the obstacles to the propagation of these species. The study of the factors that promote fast germination and homogeneous development of seedlings results in the optimization of the process of production of high-quality seedlings, in a shorter

timespan as well as in conditions that are accessible at small, medium and large scale, making the attainment of suitable material for use in reforestation projects possible (Grossnickle et al. 2018).

The knowledge surrounding the factors involved in the production of seedlings in nurseries can be considerably diverse, as the aspects to consider range from those related to the substrate, up to environmental conditions, such as luminosity and humidity. A significant part of the growth and development processes of plants is directly or indirectly conditioned by quantity, quality, presence or absence of light (Chmura et al., 2017). Differences in luminosity may cause morphological and physiological changes in plants, these changes are often associated to the individual characteristics of each species in interaction with their respective environments (Miandrimanana et al. 2019, Rother et al., 2015).

Substrates can influence the seedlings development by both their physical and their chemical characteristics. Subsoil, sand, vermiculite, manure and compost are some examples of materials that are used as substrates which can be combined or used separately. Alternatively, there are also materials whose known properties make them potential substrates, such as sewage sludge, biosolids, waste compost and sugar cane bagasse (Batistti et al., 2020; Pernot et al., 2019; Pimentel et al, 2016).

This work examines the differences in the growth of *Dimorphandra wilsonii* Rizzini seedlings cultivated in different combinations of substrates and levels of shading, providing insight and contributing to the improvement in the production of seedlings in nurseries.

Methods

Seven substrate combinations were used: (1) subsoil from Paraopeba – SP; (2) subsoil – SS;- the soil was characterized as a red-yellow Latosol, and it had low levels of nutrients, especially of phosphorus ; (3) commercial substrate BIOPLANT - CS; (4) cattle manure - CM; (5) subsoil from Paraopeba + cattle manure (1:1) - SPCM; (6) subsoil + cattle manure (1:1) - SSCM; and (7) commercial substrate + cattle manure (1:1) – CSCM. The use of soil from the city of Paraopeba/MG was due to the the region having a greater population individual of this species.

Samples containing 2.5 L of each substrate were sent to the Laboratory of Biotechnology – Analysis of Substrates for Plants, in the Department of Horticulture at the Universidade Federal do Rio Grande do Sul (UFRGS) to carry out chemical and physical analyses (Table 1).

D. wilsonii seed lot was obtained by collecting material between August and October 2016 from 23 matrices, in the central region of the State of Minas Gerais. After gathering the material and manually processing the fruit for the extraction of seeds, these seeds were then triaged based on tegument integrity, shape regularity and size uniformity, small and malformed seeds were discarded.

As observed by Matheus et al. (2017) *D. wilsonii* is a neutral photoblastic species, presenting physical dormancy with a pattern of germination that occurs towards the beginning of the rainy season. In order to break the physical dormancy, the seeds went through mechanical scarification with the use of a grinder at the opposite side of the hypocotyl-radicle axis (Matheus et al, 2017). The sterilization procedure was carried out with 70% (v/v) alcohol in which the seeds were immersed for one minute, followed by immersion in 2.5% (v/v) sodium hypochlorite for 20 minutes, and then rinsing the seeds three times in distilled water. After being rinsed, the seeds were put in distilled water for two hours for pre-imbibition.

The seeds were germinated in Biochemical Oxygen Demand (B.O.D.) incubators, at a temperature of 25°C +/- 3°C and a photoperiod of 12 hours. After germination, seedlings with a true pair of leaves, were transplanted into 18 x 24 cm polyethylene containers, containing the different substrate compositions and taken to a greenhouse under micro sprinkler irrigation for 30 days. After the establishment period, the seedlings were then cultivated for 180 days in three different levels of shading: abundant sunlight, half shading (Sombrite® 50%) and full shading (Sombrite® 80%).

Irrigation occurred daily in the morning period.

The experimental design was completely randomized in split-plot scheme (wholeplot factor: shading level; subplot second factor: substrate and subplot third factor: time).

Seedling growth was evaluated at 45, 90, 135 and 180 days after the start of the experiment, by measuring the following morphological parameters: Seedling Height (H) in cm, Collar Diameter (CD) in mm, Dry Mass of Shoot (DMS) in g and Dry Mass of the Root (DMR) in g. Using the evaluated morphological parameters, it was possible to determine the Total Dry Mass (TDM) in g, by the sum of DMS and DMR; and the quality parameters of seedlings via the ratio between seedling height and collar diameter (H/D), dry mass of aerial portion and dry mass of the root DMS / DMR) and the Dickson Quality Index, using the following formula:

$$DQI = \frac{TDM(g)}{\frac{H(cm)}{CD(mm)} + \frac{DMS(g)}{DMR(g)}}$$

In order to meet statistical assumptions, the variables CD, H, DMR, DMS, DMS/DMR and DQI were transformed using $\sqrt{\square}$, while the data of DMS, H/D was transformed using $\left(\frac{1}{y+0,1}\right) + 1$. The data was analyzed using Analysis of Variance (ANOVA) and the significant interactions between factors of qualitative nature (substrate and shading) were compared using Tukey's range test, while significant interactions between factors of quantitative nature (time) were analyzed using quadratic linear regression curves. Statistical analyses were carried

out using the software R® “Agricolae package”, with a 95% confidence interval.

Results and discussion

This is the first work to evaluate the behavior of *Dimorphandra wilsonii* seedlings in different substrates and levels shading, providing important knowledge for the production of seedlings of this species in nurseries.

As expected, measurement values of collar diameter (CD), height (H), shoot dry mass (DMS), dry mass of the root (DMR) and total dry mass (TDM), variables which correlate with plant growth, tended to increase over time (Table 2). The high coefficients of variation in experiments of this nature, indicates that the initial seedlings development of this species has high variability, as values above 20% have been recorded (Pimentel Gomes 1990).

Generally, the seedlings produced in substrates containing cattle manure in their composition, i.e. CM, SSCM, SPCM and CSCM, showed higher H / D and DMS / DMR ratios when compared to the seedlings produced without the addition of manure in their substrate (Table 2).

The highest initial development in substrates containing cattle manure corresponds to what was verified by Fonseca et al. (2010), in which seedlings of *D. wilsonii* grown on the natural soil of the current area of occurrence of this species showed higher ratios of growth in response to fertilization with NPK, as well as liming. Many Cerrado species present a

low nutritional requirements for initial development, in adaptation of natural conditions, been responsive for a few amounts of manure auditions in substrate (Costa et al. 2019, Sanches et al. 2017).

Suitable growth and build up of biomass in seedlings with substrates containing cattle manure can be related not only to the nutritional content provided by the material, but also to its role in microbiological processes, aeration, water retention capacity, structuring and temperature regulation properties of the substrates. Trazzi et al. (2012) noted that the use of manure provided an improvement in the chemical and physical attributes of the substrates, increasing the total and available nutrients, as well as promoting an increase in cation exchange capacity, exchange bases and base saturation capacity, directly proportional to the increase of manure in the substrate.

The highest values for the growth parameters were observed in the treatment containing subsoil plus manure. In other hands, growth parameters values in the CS substrate were inferior compared to the other treatments, presenting a higher mortality of seedlings over time.

Considering the physical characteristics of the different substrates, the only characteristic significantly different in the CS substrate in relation to the other substrates is the current moisture. The higher moisture of the CS substrate is related to the higher percentage of total porosity and aeration space compared to the other substrates.

Table 1 – Average values of pH, electrical conductivity (EC), moisture density (MD), dry density (DD), current moisture (CM), total porosity (TP), aeration space (AS), readily available water (RAW), buffering water (BW), remaining water (RW), water retention capacity (WRC) at 10, 50 and 100 cm

Substrate		SP	SS	CS	CM	SPCM	SSCM	CSCM
pH	H ₂ O	5,15	5,07	5,84	6,96	6,92	6,94	5,68
EC	mS / cm ¹	0,01	0,02	0,76	0,96	0,47	0,43	0,76
MD	Kg / m ³	1212,86	1261,71	442,83	577,2	953,05	1053,16	539,81
DD	Kg / m ³	1045,05	1165,63	242,98	443,08	803,03	920,2	374,76
CM	%	13,84	7,61	45,13	23,24	15,74	12,62	30,58
TP	%	69,72	57,59	86,23	77,44	70,33	65,15	81,92
AS	%	8,13	7,96	36,26	16,35	7,99	10,46	23,46
RAW	%	7,89	14,4	17,66	26,66	18,18	21,53	26,63
BW	%	11,91	6,22	3,55	4,57	6,82	4,13	3,98
RW	%	41,78	29,01	28,76	29,86	37,33	29,03	27,85
WRC(10)	%	61,59	49,64	49,97	61,09	62,34	54,69	58,46
WRC(50)	%	53,69	35,23	32,31	34,43	44,16	33,16	31,83
WRC100)	%	41,78	29,01	28,76	29,86	37,33	29,03	27,85

Subsoil from Paraopeba – SP; (2) subsoil – SS; (3) commercial substrate BIOPLANT - CS; (4) cattle manure - CM; (5) subsoil from Paraopeba + cattle manure (1:1) - SPCM; (6) subsoil + cattle manure (1:1) - SSCM; and (7) commercial substrate + cattle manure (1:1) – CSCM.

Table 2 – *D. wilsonii* seedlings average values of diameter (CD), height (H), dry mass of shoots (DMS), dry mass of roots (DMR) and total dry mass (TDM), under different substrates in 45, 90, 135 and 180 days after transplanting.

Diameter (D)		Time(days)							
		45		90		135		180	
Substrate	SP	2,34	A	2,49	BC	2,82	B	3,16	CD
	SS	2,00	A	2,48	BC	2,93	B	3,29	BC
	CS	1,93	A	1,92	C	1,93	B	2,10	D
	CM	2,14	A	3,34	A	3,93	A	3,89	AB
	SPCM	2,16	A	2,88	AB	3,05	B	3,59	BC
	SSCM	2,28	A	3,13	A	3,73	A	4,32	A
	CSCM	2,09	A	2,86	AB	2,90	B	3,63	BC
Height (H)		Time (days)							
		45		90		135		180	
Substrate	SP	8,93	C	9,99	D	12,75	C	12,76	D
	SS	8,04	C	11,18	C	13,31	C	16,12	C
	CS	8,45	C	8,18	E	7,65	D	10,46	D
	CM	9,98	AB	15,83	A	20,23	A	19,48	B
	SPCM	10,29	A	14,01	B	13,91	C	15,25	C
	SSCM	9,98	AB	15,64	A	18,98	B	20,91	A
	CSCM	8,95	BC	13,25	B	13,85	C	16,28	C
Dry mass of shoots (DMS)		Time (days)							
		45		90		135		180	
Substrate	SP	0,37	A	0,47	E	0,87	D	1,14	D
	SS	0,26	C	0,52	D	1,00	C	1,25	D
	CS	0,15	E	0,10	F	0,11	F	0,22	E
	CM	0,34	B	1,66	A	2,66	A	2,47	B
	SPCM	0,39	A	0,99	B	1,38	B	1,44	D
	SSCM	0,41	A	1,33	A	2,21	A	3,06	A
	CSCM	0,21	BC	0,84	C	0,82	E	1,31	C
Dry mass of roots (DMR)		Time (days)							
		45		90		135		180	
Substrate	SP	0,30	A	0,47	AB	1,00	AB	1,55	B
	SS	0,18	AB	0,37	AB	0,73	BC	1,33	BC
	CS	0,07	B	0,07	B	0,07	D	0,18	E
	CM	0,11	B	0,50	AB	1,04	AB	1,23	C
	SPCM	0,17	AB	0,44	AB	0,68	C	1,04	CD
	SSCM	0,17	AB	0,61	A	1,26	A	2,04	A
	CSCM	0,10	B	0,40	AB	0,48	CD	0,91	D
Total of dry mass (TDM)		Time (days)							
		45		90		135		180	
Substrate	SP	0,67	A	0,94	C	1,87	B	2,68	C
	SS	0,44	BC	0,90	C	1,73	B	2,59	C
	CS	0,22	C	0,18	D	0,18	D	0,40	E
	CM	0,45	ABC	2,16	A	3,70	A	3,70	B
	SPCM	0,56	AB	1,44	B	2,06	B	2,48	CD
	SSCM	0,58	AB	1,94	A	3,47	A	5,10	A
	CSCM	0,31	C	1,24	BC	1,30	C	2,22	D

Average followed by the same letter in the column not differ by Tukey test ($P > 0,05$). Subsoil from Paraopeba – SP; (2) subsoil – SS; (3) commercial substrate BIOPLANT - CS; (4) cattle manure - CM; (5) subsoil from Paraopeba + cattle manure (1:1) - SPCM; (6) subsoil + cattle manure (1:1) - SSCM; and (7) commercial substrate + cattle manure (1:1) – CSCM.

This aspect reinforces what has been observed in practice regarding the production of seedlings of *D. wilsonii* under nursery conditions, where it is reported the occurrence of sudden death when exposed to excessive moisture in the substrate. A possible explanation for the mortality of seedlings under high moisture conditions may be associated with the occurrence of endophytic species

already reported in the seeds of this species (Matheus et al. 2017).

There was no etiolation in seedlings grown under shading (Table 3), which indicates that *D. wilsonii* is tolerant to low light exposure, according to the criteria proposed by Valladares et al., 2016. It is important to point out that the tolerance to shading may vary throughout the development of the plant,

with the possibility of different observable patterns of response between seedlings and established mature

plants in relation to this aspect (Valladares et al, 2016).

Table 3 - Height, H/D ratio and DMS/DMR ratio of *D. wilsonii* seedlings under different substrates and shading levels

Height		Shading levels								
		Sunlight			Half shading			Full shading		
Substrate	SP	11,49	Cd	A	11,23	Cd	A	10,61	C	A
	SS	11,89	Cd	Ab	13,53	Bc	A	10,95	C	B
	CS	8,34	D	A	9,16	D	A	8,29	C	A
	CM	15,88	A	A	16,90	A	A	16,36	Ab	A
	SPCM	13,20	Abc	A	12,67	Cd	A	14,02	B	A
	SSCM	15,80	Ab	A	15,90	Ab	A	17,43	A	A
	CSCM	12,93	Bcd	A	12,28	Cd	A	13,75	B	A
H/D		Shading levels								
		Sunlight			Half shading			Full shading		
Substrate	SP	4,38	A	A	4,14	B	A	3,93	D	A
	SS	4,53	A	A	4,71	Ab	A	4,34	Cd	A
	CS	4,47	A	A	4,83	Ab	A	4,19	Bcd	A
	CM	4,85	A	A	4,93	A	A	5,26	Ab	A
	SPCM	4,68	A	A	4,54	Ab	A	4,79	Abc	A
	SSCM	4,61	A	B	4,42	Ab	B	5,77	A	A
	CSCM	4,94	A	A	4,15	B	B	4,79	Abc	A
DMS/DMR		Shading levels								
		Sunlight			Half shading			Full shading		
Substrate	SP	0,90	D	A	1,11	C	A	0,99	D	A
	SS	1,29	D	A	1,37	C	A	1,67	C	A
	CS	2,26	Abc	A	1,70	Bc	A	2,14	Bc	A
	CM	2,69	A	B	3,04	A	Ab	3,49	A	A
	SPCM	2,00	Bc	A	2,19	B	A	2,43	B	A
	SSCM	1,95	C	B	2,21	B	Ab	2,57	B	A
	CSCM	2,56	Ab	A	1,99	B	B	2,07	Bc	Ab

Average followed by the upper same letter in the column and lower same letter in the line not differ by Tukey test ($P > 0,05$). Subsoil from Paraopeba – SP; (2) subsoil – SS; (3) commercial substrate BIOPLANT - CS; (4) cattle manure - CM; (5) subsoil from Paraopeba + cattle manure (1:1) - SPCM; (6) subsoil + cattle manure (1:1) - SSCM; and (7) commercial substrate + cattle manure (1:1) – CSCM

Seedlings produced under a 50% restriction of light showed higher CD values in average, while those under 80% of light restriction had the lowest average values. For all levels of shading tested, there was a trend of lower DMS / DMR ratios in relation to time, this trend of decreasing ratios was intensified when light restriction was increased. The substrates SSCM and CM provided a greater increase in the majority of the evaluated parameters considering the different levels of shading.

Seedlings cultivated in an environment with 80% light restriction showed the lowest buildup of DMR, similarly to the patterns observed in collar diameter and height of the seedlings, indicating that *D. wilsonii* has its growth negatively affected by conditions of greater light restriction, possibly related to the lower production of photoassimilates and their distribution between the aerial portion and the root system, which is corroborated by the data regarding CD, the growth in diameter relies on the processes that occur in the cambium, which are in part stimulated by photoassimilates (Rahman et al. 2016).

It is also worth noting that for all the measurement intervals, the DQI of the seedlings

cultivated in the substrates that were added with cattle manure (SPCM, SSCM, CSCM and CM) were, in general, higher than the DQIs of seedlings produced without addition of manure (SP, SS and CS) (Table 4).

It is noted that at 45 days after transplanting the seedlings produced in the substrates CM and SSCM and at 90 days after transplanting seedlings produced in the substrates SS, CM and SPCM, seedlings present lower DQI average values when cultivated in full sunlight. Conversely, at 180 days after transplanting, seedlings produced on SSCM and SPCM substrates showed lower DQI values when grown in an environment with 80% light restriction.

The behavior observed in *D. wilsonii* seedlings may indicate that the better vigor and balance of the biomass distribution was produced when seedlings growth for approximately 90 days in a shaded environment.

Table 4 - Dickson Quality Index (DQI) average of *D. wilsonii* seedlings under different substrates, shading levels on 45, 90, 135 and 180 days after transplanting.

Time	Substrate	Shading levels								
		Sunlight			Half shading			Full shading		
45	SP	1,27	B	A	1,54	Cd	A	1,46	C	A
	SS	2,01	Ab	A	1,40	D	A	1,88	Bc	A
	CS	2,56	A	A	2,17	Bcd	A	2,34	Bc	A
	CM	2,75	A	B	3,57	A	Ab	4,23	A	A
	SPCM	2,00	Ab	A	2,71	Ab	A	2,69	B	A
	SSCM	2,31	Ab	B	2,88	Ab	B	4,00	A	A
	CSCM	2,28	Ab	A	2,51	Abc	A	2,37	Bc	A
90	SP	1,23	C	A	1,32	C	A	1,31	D	A
	SS	1,28	Bc	B	1,86	Bc	Ab	2,08	Cd	A
	CS	2,49	Ab	A	1,19	C	A	2,76	Bc	A
	CM	3,49	A	B	4,13	A	Ab	4,84	A	A
	SPCM	2,42	Abc	B	2,49	B	Ab	3,36	B	A
	SSCM	2,37	Abc	A	2,91	Ab	A	3,08	Bc	A
	CSCM	3,15	A	A	2,40	B	A	3,15	Bc	A
135	SP	1,28	C	A	1,58	D	A	1,28	C	A
	SS	1,57	Bc	A	2,03	Cd	A	2,07	Abc	A
	CS	1,53	Bc	A	-	-	-	1,52	Bc	A
	CM	3,53	A	A	3,50	A	A	3,63	A	A
	SPCM	2,74	Ab	A	2,69	Abc	A	2,82	Ab	A
	SSCM	2,89	Ab	A	3,10	Ab	A	2,59	Ab	A
	CSCM	2,68	Ab	A	2,31	Bcd	A	1,92	Bc	A
180	SP	1,29	C	A	1,62	C	A	1,51	B	A
	SS	1,63	Bc	A	1,68	C	A	1,60	B	A
	CS	2,46	Abc	A	-	-	-	1,30	B	A
	CM	3,35	A	A	3,27	A	A	3,12	A	A
	SPCM	2,26	Abc	A	2,33	Abc	A	2,29	Ab	A
	SSCM	3,10	A	A	2,82	Ab	Ab	2,10	Ab	B
	CSCM	2,94	Ab	A	2,18	Bc	Ab	1,76	B	B

Average followed by the upper same letter in the column and lower same letter in the line not differ by Tukey test ($P > 0,05$). Subsoil from Paraopeba – SP; (2) subsoil – SS; (3) commercial substrate BIOPLANT - CS; (4) cattle manure - CM; (5) subsoil from Paraopeba + cattle manure (1:1) - SPCM; (6) subsoil + cattle manure (1:1) - SSCM; and (7) commercial substrate + cattle manure (1:1) – CSCM

Conclusion

1) for the production of *D. wilsonii* seedlings, the use of substrate comprising 50% cattle manure and 50% of subsoil is an efficient alternative;

2) there was no technical feasibility in the production of *D. wilsonii* seedlings in plastic bag containers with pure commercial substrate;

3) *D. wilsonii* seedlings showed better performance in the nursery when initially produced in a shaded environment.

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