



## RUBBER TREE MINI CLONAL GARDEN: ELECTRIC CONDUCTIVITY OF THE NUTRITIONAL SOLUTION IN THE PRODUCTION OF PROPAGULES

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### ABSTRACT

The rubber tree (*Hevea brasiliensis*) is an important forest species that yields natural rubber. Traditionally, rubber tree grafts are cultivated in clonal gardens on soil, which necessitates enormous acreage. However, this approach is not only inefficient in terms of productivity, but it also has nutritional and water control constraints. A tiny clonal garden with a balanced fertilizer treatment is an approach that has previously been employed for other species. To apply this same system to the rubber tree, the goal was to investigate the effects of applying macro and micronutrients via fertigation on the productivity of a mini rubber tree clonal garden, as well as to assess the efficacy of using green stems produced under these conditions in three grafting techniques. Five dosages of macro and micronutrients were administered to grafted rubber tree seedlings (clone 'RRIM 600' on 'GT1') before transplanting them into plastic pots. A totally randomized design was adopted, with five treatments and ten replications, each with two plants per pot, for a total of twenty plants per treatment. The shoot apex was cut after 105 days, and the green stems measuring more than 20 cm were harvested throughout the course of a year. The number of stems was highest in the months of January to March 2014, after 135 days, reaching 3 green stems per plant<sup>-1</sup> per month<sup>-1</sup>, and productivity increased up to an estimated electrical conductivity of 1.64 mS cm<sup>-1</sup>. Nutrition was found to boost the output of the rubber tree clonal mini garden as well as the survival of grafts after grafting. The tiny clonal garden is a potential method for getting more vegetative rubber tree propagules.

**Keywords:** Fertigation; *Hevea brasiliensis*; Vegetative propagation.

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## MINIJARDIM CLONAL DE SERINGUEIRA: CONDUTIVIDADE ELÉTRICA DA SOLUÇÃO NUTRITIVA NA PRODUÇÃO DE PROPÁGULOS

**RESUMO** – A seringueira (*Hevea brasiliensis*) é uma importante espécie florestal que produz borracha natural. Tradicionalmente, os enxertos de seringueira são produzidos em jardins clonais cultivados em solo, o que demanda grandes áreas. No entanto, este sistema, além de ser pouco eficiente em termos de produtividade, apresenta limitações no controle nutricional e hídrico. Uma alternativa já empregada para outras espécies é a adoção do minijardim clonal com aplicação balanceada de nutrientes. Com intuito de empregar esse mesmo sistema à seringueira, objetivou-se investigar os efeitos da aplicação de macro e micronutrientes via fertirrigação na produtividade de um minijardim clonal de seringueira e avaliar a eficiência de utilização de hastes verdes produzidas nessas condições em três métodos de enxertia. Para isso, foram aplicadas cinco doses de macro e micronutrientes em mudas enxertadas de seringueira (clone 'RRIM 600' sobre 'GT1') e transplantadas para vasos plásticos. Foi utilizado delineamento inteiramente casualizado composto por 5 tratamentos e 10 repetições, com duas plantas por vaso, totalizando 20 plantas por tratamento. Após 105 dias, o ápice caulinar foi removido e as hastes verdes, com comprimento superior a 20 cm, foram colhidas ao longo de um ano. O número de hastes, foi maior nos meses de janeiro a março de 2014, após 135 dias, alcançando 3 hastes verdes  $\text{planta}^{-1} \text{ mês}^{-1}$  e, constatou-se aumento na produtividade até a condutividade elétrica estimada de  $1,64 \text{ mS cm}^{-1}$ . Conclui-se que a nutrição aumentou a produtividade do minijardim clonal de seringueira e a sobrevivência dos enxertos após a enxertia. O minijardim clonal representa uma opção promissora para a obtenção de mais propágulos vegetativos de seringueira.

**Palavras-Chave:** Fertirrigação; *Hevea brasiliensis*; Propagação vegetativa.

### 1. INTRODUCTION

The rubber tree [*Hevea brasiliensis* (Willd. ex Adr. de Jussieu) Muell. Arg.] is a forest plant

that produces natural rubber with properties suitable for the manufacturing sector (Silva et al., 2020). With significant development potential, primarily in tropical parts of the world (Yang et al., 2019), the harvested area increased by 3.3 million hectares between 2010 and 2020, particularly in Southwest Asia and China (Warren-Thomas et al., 2023). According to estimates, the global area will double by 2050 (Fox and Castella, 2013). Rubber tree plantings in Brazil ranged from 135,000 to 182,006 hectares between 2011 and 2022 (IBGE, 2024).

Since the previous century, the fundamental constraint on the spread of rubber trees in Brazil has been a scarcity of seeds, buds, and grafted seedlings of sufficient quality and quantity to fulfil demand (Medrado, 1992; Borelli, 2016). With a productivity of 5 stems per plant per year (IAC, 2024), the traditional rubber tree clonal garden is commonly formed in full sun (Alvarenga, 2014; Priyadarshan, 2017; Nair, 2021), where grafted rubber tree seedlings are planted directly into the soil (IAC, 2024), with limited water and nutritional control.

The clonal mini garden has been utilized to propagate the principal forest species since the 1990's (Assis et al., 1992; Xavier and Comério, 1996; Higashi et al., 2000). Later, the mini garden was utilized to produce miniature pine cuttings (Xiang et al., 2003). Currently, new species have shown promise for using this technique to efficiently control the environmental, phytosanitary, and nutritional conditions of parent plants (Oliveira et al., 2019; Packialakshmi and Sudhagar, 2019; Santos et al., 2020; Lowe et al., 2022; Gazzana et al., 2024).

It is thought that replacing the traditional clonal garden with the mini rubber tree clonal garden would bring innovation to the industry, enhancing nursery efficiency via improved scheduling of propagule availability and output. However, nutrition and green stem formation in rubber tree under clonal mini garden circumstances remain unclear.

In this context, the goal was to test increasing doses of macro and micronutrients in a mini rubber tree clonal garden, analyse the effects of the electrical conductivity of the nutrient solution on green stem production, and assess the efficiency of rubber tree stems produced under these conditions in various grafting methods.

## 2. MATERIAL AND METHODS

### 2.1 Location of the study area and treatments

The experiment was carried out in a greenhouse at ESALQ/USP, municipality of Piracicaba, SP ( $22^{\circ}42'30''$  S e  $47^{\circ}38'30''$  W, at an elevation of 546 meters). The region has a Cwa climate, with 1230 mm of annual precipitation and an average temperature of  $21.6^{\circ}\text{C}$  (Alvares et al., 2013).

During the experiment, relative air temperature and humidity were measured using a thermo-hygrometer in the interior of a vegetable house. The average temperatures ranged between  $39.5^{\circ}\text{C}$  and  $18.7^{\circ}\text{C}$ . The relative humidity of the air varied between 38.1% and 92.8%. The photoperiod (N) in

Piracicaba/SP was calculated for each season of the year.

The experimental design was totally randomized, with five treatments (T1, T2, T3, T4, and T5) and ten replications (pots) of two plants each, for a total of twenty plants per treatment. The treatments involved increasing macro and micronutrient dosages (Table 1).

The nutrient solutions were balanced using the Hoagland and Arnon (1950) principle, considering the average nutrient concentrations in rubber plant leaf tissues described by Mendes et al. (2012) and Raij et al. (1997), and the relationship between nutrients was then calculated for each treatment. The electrical conductivity (EC) and pH of the treatments were adjusted to values ranging from 0.5 to  $2.5 \text{ mS cm}^{-1}$  and pH close to 6.2.

**Table 1.** Quantities of macro and micronutrients in 5 nutrient solutions for the fertigation of the mini clonal garden of rubber trees (*Hevea brasiliensis*)

**Tabela 1.** Quantidades de macro e micronutrientes em 5 soluções nutritivas para fertirrigação do minijardim clonal de seringueira (*Hevea brasiliensis*)

Nutrients <sup>(1)</sup>	1	2	3	4	5
	mg L <sup>-1</sup>				
N	0	50	100	200	400
P	0	3	6	12	25
K	0	19	37	75	149
Ca	0	20	40	80	160
Mg	0	4	9	17	34
S	0	4	8	15	31
B	0	0.07	0.14	0.29	0.57
Cu	0	0.02	0.04	0.08	0.17
Fe	0	0.15	0.30	0.60	1.20
Mn	0	0.20	0.39	0.78	1.56
Zn	0	0.05	0.09	0.18	0.36
<b>EC (mS cm<sup>-1</sup>)</b>	<b>0.5</b>	<b>1.0</b>	<b>1.5</b>	<b>2.0</b>	<b>2.5</b>

<sup>(1)</sup> Fertilizers used: Urea (45% N), monoammonium phosphate (MAP; 11% N; 60% P<sub>2</sub>O<sub>5</sub>), potassium nitrate (12% N; 43% K<sub>2</sub>O), calcium nitrate (15.5% N; 18.5% Ca), magnesium sulfate (12% S; 9% Mg), boric acid (17% B), copper sulfate (12% S; 25% Cu), iron sulfate (10.9% S; 14% Fe), manganese sulfate (21% S; 25% Mn), and zinc sulfate (11% S; 20% Zn).

<sup>(1)</sup> Fertilizantes utilizados: Ureia (45% N), fosfato monoamônico (MAP; 11% de N; 60% de P<sub>2</sub>O<sub>5</sub>), nitrato de potássio (12% de N; 43% de K<sub>2</sub>O), nitrato de cálcio (15,5% de N; 18,5% de Ca), sulfato de magnésio (12% de S; 9% de Mg), ácido bórico (17% de B), sulfato de cobre (12% de S; 25% de Cu), sulfato de ferro (10,9% de S; 14% de Fe), sulfato de manganês (21% de S; 25% de Mn) e sulfato de zinco (11% de S; 20% de Zn).

To begin the experiment, grafted rubber tree seedlings from the 'RRIM 600' clone were planted on seminal 'GT1' rootstocks grown in plastic bags filled with dirt and purchased from a commercial nursery. The seedlings were 18 months old and had two mature leaf stalks.

Before placing the mini clonal garden, the seedlings' root systems were cleaned with running water to eliminate dirt. Subsequently, the seedlings were transferred into 13 L plastic pots filled with washed, gritty sand. Two seedlings were transplanted into each pot, 12 cm apart and with opposing grafts. The pots were set on metal grids 90 cm above ground. During the seedlings' acclimation period, they were irrigated daily and fertilized every fifteen days with T3 fertilizer (Table 1).

The treatments occurred 75 days after transplantation, with nutritional solutions made in 100 L plastic buckets for each treatment (Table 1) and refilled every 20 days. Fertigation was performed twice a day with an autonomous irrigation system. 5/8" plastic hoses were attached to the 40W submersible pumps and placed in the middle of the metal grid. Other 5/16" hoses were joined to these hoses, dispersed according to treatment, and secured close to the seedlings with nylon clamps. Using a timer, the pumps were triggered automatically, delivering 500 mL of nutritional solution per pot daily.

After 105 days of treatment, the seedlings were clipped to 2 cm above the initial leaf release, with the apical bud removed to promote the sprouting of new stems.

## 2.2 Assessments

Productivity assessments in the mini clonal garden began 30 days after pruning, with green stems longer than 20 cm plucked and recorded.

The nutritional content of rubber tree leaves was measured at the conclusion of each season (spring, summer, autumn, and winter in 2014). In phenological stage D (Hallé, Oldeman and Tomlinson, 1978), newly mature leaves were taken that lacked petioles and had leaf blades. After collecting, the leaves were rinsed with deionized water, packed in paper bags, and dried in an air circulation oven at 65 °C for 72 hours. The leaves were then finely processed into a powder. The samples were then transferred to a laboratory for macro and micronutrient examination and assessment (Malavolta, Vitti, & Oliveira 1997).

From January to April 2015, three grafting methods (budding grafting [EB], full cleft grafting [GFC], and lateral cleft grafting [EFL]) were performed on eight-month-old seminal rubber tree rootstocks (clone 'GT1') grown in plastic containers (15x35cm) filled with composted pine bark and vermiculite, kept in a suspended nursery in full sun, and with an automatic irrigation system (water depth of 6 mm day<sup>-1</sup>). Before grafting, diameters were measured at a height of 5 cm from the substrate of the rootstocks using a digital caliper, and six diametric classes were then formed (Table 2). Based on this knowledge, the grafting procedure was employed accordingly.

**Table 2.** Number of grafted rubber tree (*Hevea brasiliensis*) plants per diameter class using the budding (EB), Saddle graft (GFC), and side-venner graft (EFL) methods.

**Tabela 2.** Quantidade de plantas enxertadas de seringueira (*Hevea brasiliensis*) por classe diamétrica nos métodos de enxertia por borbulhia (EB), garfagem em fenda cheia (GFC) e em fenda lateral (EFL).

Diameter classes	Methods of grafting		
	EB	GFC	GFL
< 5.6 mm	0	20	0
5.6-7.1 mm	0	51	20
7.2-8.7 mm	39	48	13
8.8 – 10.3 mm	101	10	3
10.4 – 11.9 mm	58	0	0
> 11.9 mm	87	1	0
<b>Total</b>	<b>285</b>	<b>130</b>	<b>36</b>

Grafts were formed from green stems grown in the mini clonal garden during phenological phases A, B1, B2, C, and D (Hallé, Oldeman and Tomlinson, 1978). Stems 20 to 25 cm long were utilized for cleft grafting, whereas stems 50 cm long were used for budding. Graft survival was monitored on a weekly basis following grafting.

### 2.3 Data analysis

The nutrient contents of rubber tree leaves were examined for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). Taking the assumptions into consideration, the data were analysed for variance and compared using the Tukey test with a 5% probability. The production data of green stems from the mini clonal garden were subjected to regression analysis, and the survival of the grafted seedlings was investigated using Fisher's

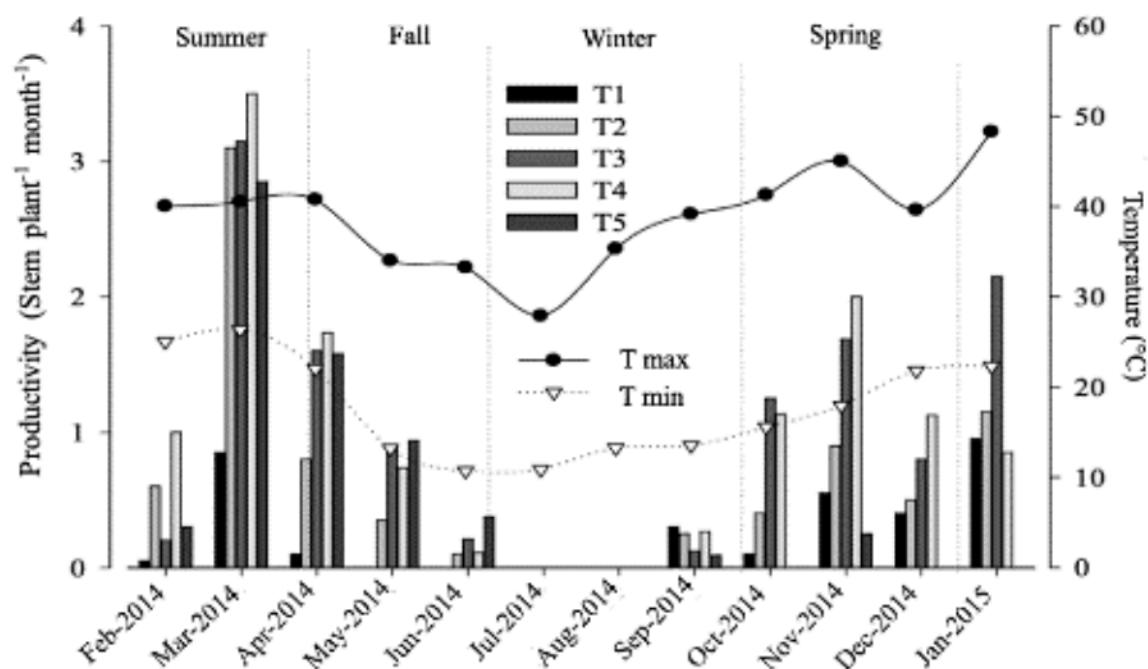
exact test. SAS Studio software was utilized for statistical analysis.

## 3. RESULTS

### 3.1 Productivity of the mini clonal garden

After a year of evaluation, the mini rubber tree clonal garden had the highest productivity (3 green stems plant<sup>-1</sup> per month<sup>-1</sup>) in the months of January and March 2014, which corresponded to summer in Brazil, with average temperatures of 32.5 °C and photoperiod of 12.67 h, when stem development was greatest. From October to December, with an average temperature of 28.9 °C and a photoperiod of 13.13 hours, the mini clonal garden had a maximum production of 2 stems plant<sup>-1</sup> per month<sup>-1</sup> (Figure 1).

Rubber tree stems were not gathered in July



**Figure 1.** Productivity of green stems of seedlings bud grafted in a mini clonal garden of rubber trees (*Hevea brasiliensis*) with 5 treatments of nutrient solutions across the 4 seasons of the year.

**Figura 1.** Produtividade de hastes verdes de mudas enxertadas em minijardim clonal de seringueira (*Hevea brasiliensis*) com 5 tratamentos de soluções nutritivas, nas 4 estações do ano.

or August due to slow development (Figure 1). During this time, the minimum temperature was 11.6 °C and the photoperiod was 11.23 hours, demonstrating that seasonality had a direct impact on the production of the mini rubber tree clonal garden.

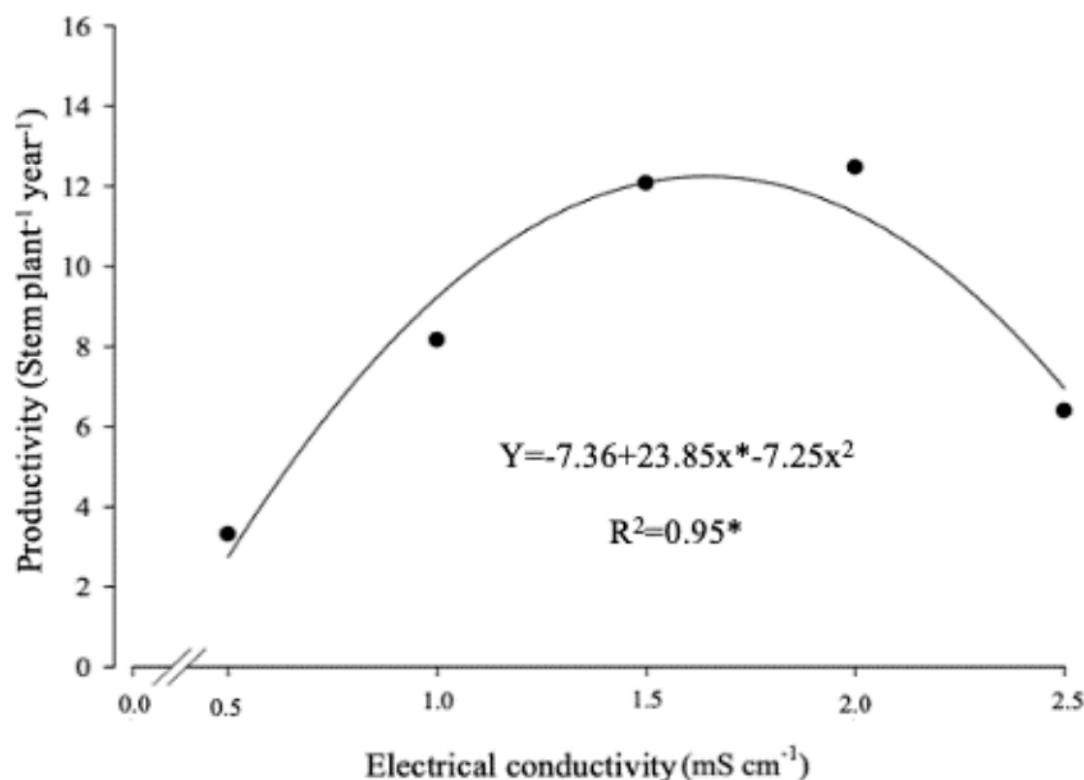
T4 generated the most stems per plant each month. While in the control treatment (T1), where the plants did not get fertigation, output was fewer than 3 stems plant<sup>-1</sup> month<sup>-1</sup>, in T3, production was four times greater in January 2015 (Figure 1).

A quadratic polynomial equation (Figure 2) demonstrated a substantial ( $p < 0.05$ ) correlation between green stem production and solution electrical conductivity. Using the equation, the electrical conductivity with the best productivity would be 1.64 mS cm<sup>-1</sup>.

At an electrical conductivity of 2.5 mS cm<sup>-1</sup>, certain nutrients accumulated excessively in the leaves, causing phytotoxicity and progressive seedling death. This treatment (T5) produced less stems at the start of the trial and eventually stopped, resulting in the death of the seedlings.

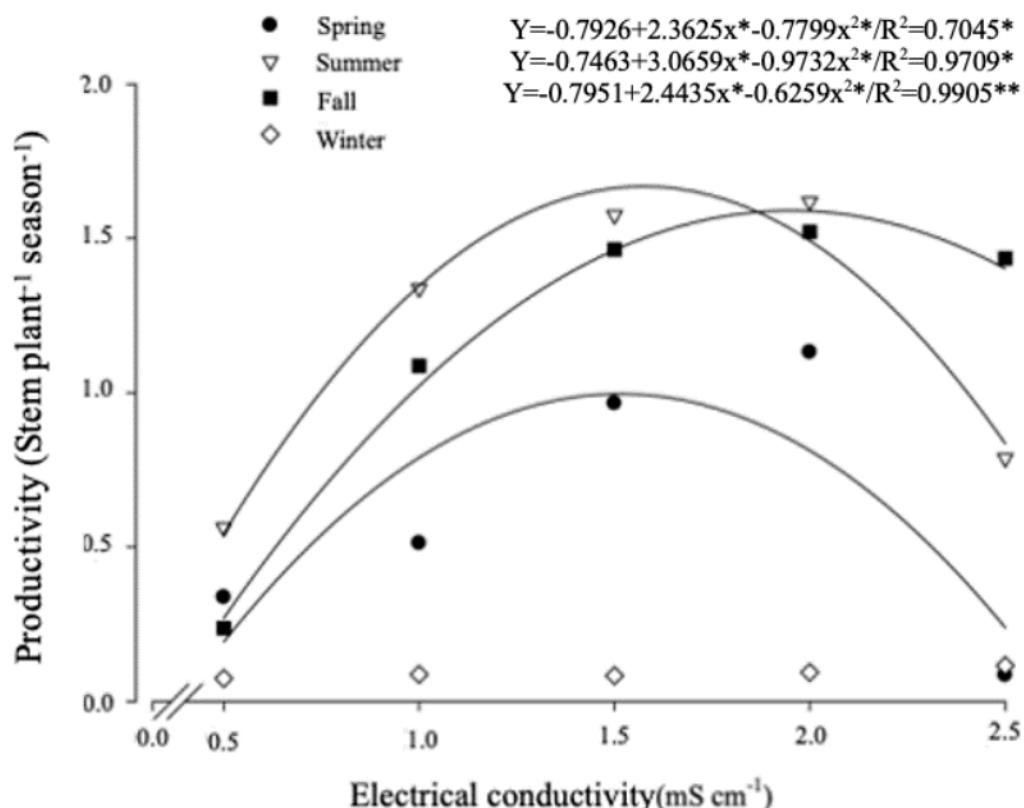
Analysing the reactions at different times of the year revealed the necessity for varied electrical conductivities in the nutrition solution (Figure 3). Mini garden productivity varied quadratically in spring, summer, and fall, peaking at electrical conductivities of 1.5, 1.6, and 2.0 mS cm<sup>-1</sup>, respectively. Winter productivity was linear, with minimal stem output due to vegetative rest.

### 3.2 Nutrient contents



**Figure 2.** Productivity of green stems of seedlings bud grafted in a mini clonal garden of rubber trees (*Hevea brasiliensis*) with 5 treatments of nutrient solutions as a function of electrical conductivity (EC).

**Figura 2.** Produtividade de hastes verdes de mudas enxertadas em minijardim clonal de seringueira (*Hevea brasiliensis*) com 5 tratamentos de soluções nutritivas e em função da condutividade elétrica (EC).



**Figure 3.** Productivity of green stems of seedlings bud grafted in a mini clonal garden of rubber trees (*Hevea brasiliensis*) with 5 treatments of nutrient solutions as a function of electrical conductivity (EC) across the 4 seasons of the year.

**Figura 3.** Produtividade de hastes verdes de mudas enxertadas em minijardim clonal de seringueira (*Hevea brasiliensis*) com 5 tratamentos de soluções nutritivas e em função da condutividade elétrica (EC) nas 4 estações do ano.

In addition to productivity disparities, the plants' leaves showed visible colour changes, suggesting nutritional insufficiency and/or toxicity (Figure 4). Plants that were not fertigated (T1) displayed chlorosis and yellowing of the leaves and stems, indicating nitrogen shortage. Plants fertilized with higher concentrated solutions (T5) also developed necrosis, probably owing to phytotoxicity.

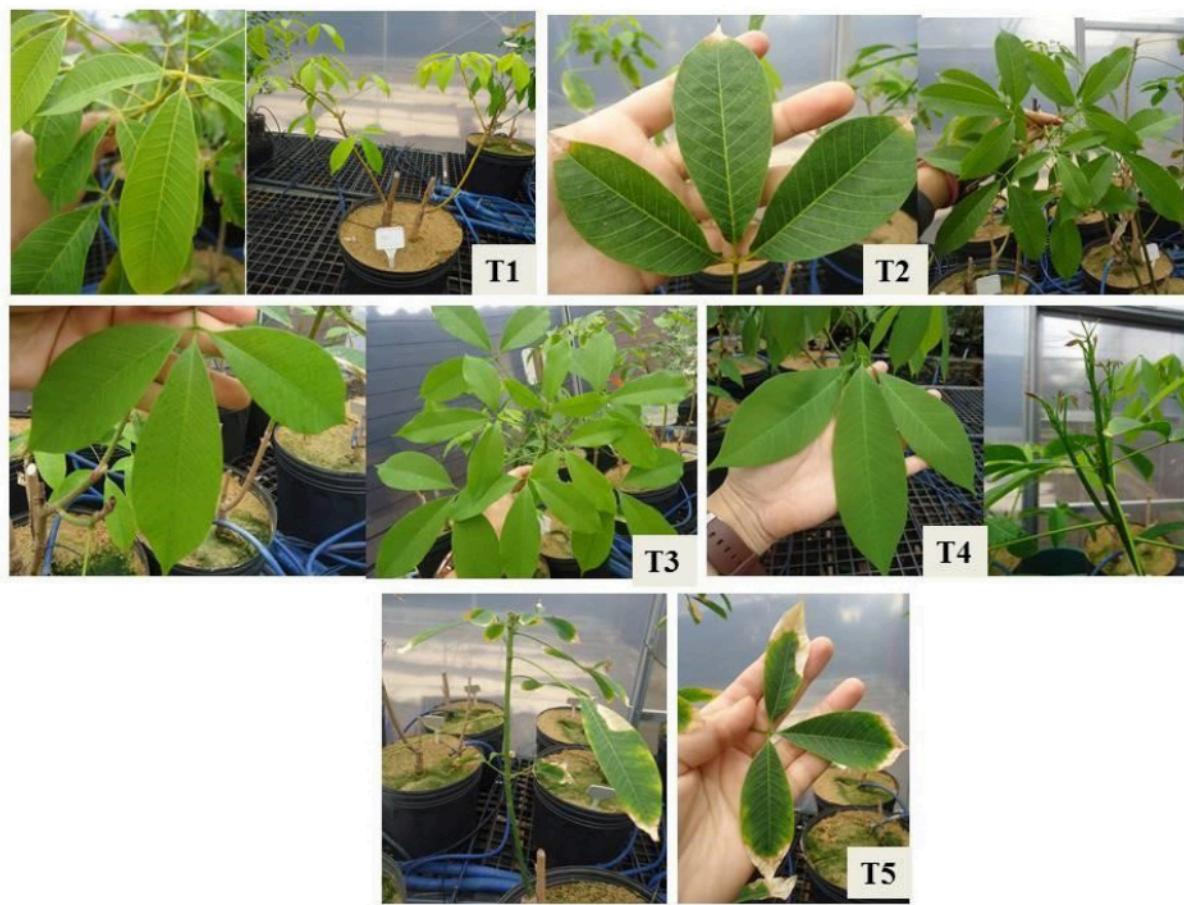
Rubber tree leaves had the following macronutrients in decreasing order of concentration: N, K, Ca, S, P, and Mg. Leaf Ca, S, and Mg levels vary between treatments and seasons (Table 3).

In autumn and winter, T1 had significantly greater Ca levels than the other treatments in terms of K and Mg levels (1.3 and 3.2 mg L⁻¹, respectively). T5 showed the statistically greatest amounts of S and Mn. Ca levels were greater in autumn than in other seasons of the

year, with no discernible variation between summer and winter. With regards to Mn, spring levels were greater than in other seasons of the year, with no change between summer and fall (Table 3).

There were substantial variations between treatments for N, P, Mg, and B. K, Fe, and Zn levels varied according to the seasons. K levels were greatest in the spring, Fe in the summer, and Zn in the autumn. T3 included the greatest concentrations of N, P, and Mg. The greatest amounts of B were found in T5, which might be attributed to the reported phytotoxicity. Cu was the sole nutrient that did not alter between treatment and season (Table 3).

The associations between leaf nitrogen and potassium concentration and rubber tree stem production were quadratic. Deriving the equation, the greatest productivity of 12.86 stems plant⁻¹ year⁻¹ was achieved when the N



**Figure 4.** Visual appearance of the leaves of rubber tree (*Hevea brasiliensis*) seedlings grafted in a mini clonal garden with 5 treatments of nutrient solutions.

**Figura 4.** Aspecto visual das folhas de mudas de seringueira (*Hevea brasiliensis*) enxertadas em minijardim clonal com 5 tratamentos de soluções nutritivas.

**Table 3.** Macro and micronutrient content in leaves of grafted rubber tree plants (*Hevea brasiliensis*) under fertigation irrigation with different nutrient concentrations in each season.

**Tabela 3.** Teores de macro e micronutrientes em folhas de plantas enxertadas de seringueira (*Hevea brasiliensis*) sob irrigação em fertirrigação com diferentes concentrações de nutrientes em cada estação do ano.

Treat- ments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
			g kg <sup>-1</sup>					mg kg <sup>-1</sup>			
Winter											
1	27.7	1.7	11.4	10.6	1.8	2.2	120	6.5	84.7	73	31
	c	bc	AB	a AB	ab	ab C	b		C	b B	AB
2	29.3	1.5	12.2	2.2	1.2	1.4	92	5.3	59.3	40	36
	c	c	AB	b AB	b	c C	b		C	ab B	AB
3	45.2	4.2	12	4.7	2	2.6	93	6.1	62.1	174	41
	a	a	AB	ab AB	a	ab C	b		C	ab B	AB
4	39.9	3.1	12.2	2.3	1.7	1.5	56	6.3	45.8	73	34
	b	a	AB	b AB	ab	bc C	b		C	ab B	AB

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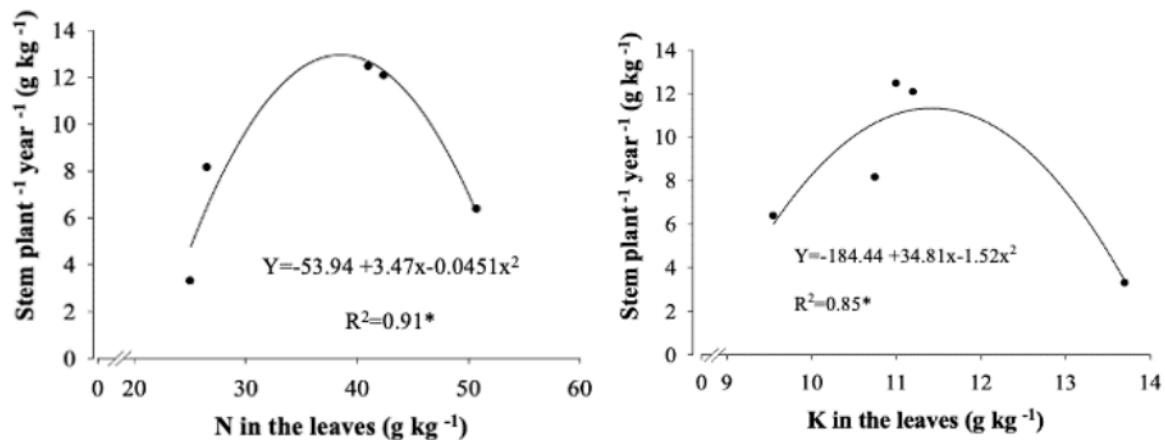
Treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	g kg <sup>-1</sup>						mg kg <sup>-1</sup>				
Winter											
5	49.1 a	2.6 ab	14.4 AB	5.4 ab AB	1.9 ab	2 a C	146 a	4.3	48.5 C	99 a B	28 AB
Spring											
1 c	30.1 bc	1.6 A	15.4 a B	4.9 ab	2.1 ab	2.5 ab AB	81 b	8.7	56.5 C	33 b A	24 B
2 c	29.8 c	1.3 c	12.4 A	2.2 b B	1.7 b	1.9 c AB	45 b	4.6	43.8 C	30 ab A	29 B
3 a	48.2 a	3.6 a	11.4 A	2.7 ab B	2.4 a	2.4 ab AB	36 b	7.2	51.6 C	46 ab A	31 B
4 b	40.3 a	2.6 a	13.6 A	2.1 b B	1.8 ab	2.1 bc AB	35 b	3.9	45.6 C	26 ab A	39 B
5	50.5 a	2 ab	10.8 A	6 ab B	2.7 ab	4.7 a AB	164 a	5.4	56.7 C	103 a A	29 B
Summer											
1 c	25.1 bc	2.1 B	12.0 a AB	4.5 ab	1.8 ab	2.4 ab BC	61 b	7.0	128.5 A	33 b AB	28 B
2 c	23.7 c	1.7 c	9.1 B	2.8 b AB	1.4 b	1.5 c BC	47 b	6.8	110.1 A	42 ab AB	20 B
3 a	38.2 a	2.5 a	11.0 B	4.1 ab AB	2.2 a	2.8 ab BC	42 b	7.3	137.8 A	86 ab AB	30 B
4 b	39.4 a	2.4 a	8.4 B	3.3 b AB	1.6 ab	1.7 bc BC	65 b	5.8	129.7 A	72 ab AB	22 B
5	52.6 a	2.6 ab	8.3 B	4.8 ab AB	1.3 ab	3.1 a BC	141 a	6.5	121.3 A	82 a AB	33 B
Fall											
1 c	17.2 bc	1.4 B	11.2 a A	8.5 ab	1.9 ab	3.4 ab A	74 b	6.8	102.0 B	38 b AB	41 A
2 c	23.3 c	1.6 c	9.5 B	5.8 b A	1.7 b	2.7 c A	106 b	6.7	86.6 B	73 ab AB	33 A
3 a	42.2 a	2.8 a	9.4 B	4.6 ab A	2.2 a	3.4 ab A	70 b	6.8	101.2 B	95 ab AB	70 A
4 b	44.4 a	3.0 a	9.2 B	5.0 b A	2.1 ab	3.0 bc A	127 b	6.6	86.6 B	81 ab AB	45 A
5 <sup>1</sup>	-	-	-	-	-	-	-	-	-	-	-

<sup>(1)</sup> In the fall, it was not possible to collect leaves from T5, as there were not enough plants for the collection.\*In the columns, means followed by the same lowercase and uppercase letter do not differ significantly by Tukey's test at the 5% probability level in treatments and seasons of the year.

<sup>(1)</sup> No outono, não foi possível fazer as coletas de folhas do T5, pois não havia plantas suficientes para a coleta.\*Nas colunas, médias seguidas pela mesma letra minúscula e maiúsculas não diferem significativamente pelo teste de Tukey ao nível de 5% de probabilidade nos tratamentos e estações do ano

content in the leaves was 38.47 g kg<sup>-1</sup>, which is within the suitable range (90% of maximum productivity) of 33-44 g kg<sup>-1</sup> of N. In K, the greatest productivity was 11.85 stems plant<sup>-1</sup>

year<sup>-1</sup>, which was achieved with K content in the leaf ranging from 10.6 to 12.4 g kg<sup>-1</sup> (Figure 5).



**Figure 5.** Relationship between the average levels of nitrogen (N) and potassium (K) in the leaves and the yield of green stems of seedlings grafted in a mini clonal garden of rubber trees (*Hevea brasiliensis*).

**Figura 5.** Relação entre os teores médios de N e K nas folhas com a produtividade de hastes verdes de mudas enxertadas em minijardim clonal de seringueira (*Hevea brasiliensis*).

### 3.3 Grafting

Budding grafting differed significantly between treatments ( $p=0.040$ ), with T1 and T4 having the best survival rates. However, T1 was less abundant, rendering it unsuitable for grafting, whereas T4 generated the greenest stems under rubber tree mini clonal garden conditions (Table 4).

Budding grafting at T5 did not occur. Survival did not differ between the whole cleft grafting procedures ( $p = 0.459$ ). In lateral cleft grafting, stems were only used in T3 and T4, however the stems did not survive, therefore this approach is not recommended for propagating rubber tree seedlings (Table 4).

**Table 4.** Number of grafted seedlings (NE) and percentage survival (PS) in three grafting methods on rubber tree rootstocks (*Hevea brasiliensis*) across 5 treatments with different nutrient solutions.

**Tabela 4.** Número de mudas enxertadas (NE) e percentual de sobrevivência (PS) em três métodos de enxertia nos porta-enxertos de seringueira (*Hevea brasiliensis*) em 5 tratamentos com diferentes soluções nutritivas.

Treatments	EB		GFC		GFL	
	NE	PS	NE	PS	NE	PS
1	8	50	12	8.3	-	-
2	15	13.3	42	12	-	-
3	35	34.3	7	29	17	0
4	73	43.8	20	25	8	0
5	4	0	1	0	-	-
<b>Total</b>	<b>135</b>	<b>37</b>	<b>82</b>	<b>16</b>	<b>25</b>	<b>0</b>

## 4. DISCUSSION

### 4.1 Do the seasons influence the output of a mini rubber tree clonal garden?

Temperature regulates various biological activities, including photosynthesis (Taiz and Zeiger, 2017), nutrient availability and absorption (Malavolta, 2006), enzyme activation (Epstein and Bloom, 2004), and plant development (Brondani, 2012). At the same time, the clonal mini-garden's height, seasonality, and geographic layout influence the temperature (Alfenas et al., 2004; Assis and Mafia, 2007; Hartmann et al., 2011).

The optimal temperature range for rubber tree growth is 27 to 30 °C (Mason et al. 2001). At temperatures over 40 °C, the rate of respiration surpasses the rate of photosynthesis, resulting in the buildup of carbon dioxide due to enhanced photorespiration. Temperatures below 10 °C, on the other hand, can disrupt or diminish photosynthesis (Rao et al., 1993), affecting photoassimilate production and accumulation, and severely impacting stem growth (Medrado, 1992).

Several authors have noted the effect of winter, particularly temperature, on development in young rubber tree plants (Hua-Son, 1983; Lemos Filho, 1991; Medrado, 1992; Pezzopane et al., 1995). When Pereira and Leal (2012) investigated the early propagation of rubber trees in winter, they discovered that the season had a detrimental influence on the growth of clonal garden shoots utilized as grafts. These authors determined that trimming the clonal garden to develop branches for grafting should only be done during the warmer months of the year, never in the two months preceding the winter season.

After removing the apical bud, this study found that green rubber tree stems may be produced satisfactorily under mini clonal garden settings. This condition demonstrates that the rubber tree has the potential to be used in a mini clonal garden, and that, depending on the time of year, successive collections can result in a significant increase in yield and a 50% reduction in production time when compared to the traditional system. Summer and spring were shown to be the optimum periods to gather green rubber tree stems. In addition to the increased output in these seasons, these periods are widely employed to perform out budding grafting in Brazil

because of the higher success rate (Martins et al., 2017).

The productivity of the mini rubber tree clonal garden is determined by the electrical conductivity of the solution. Electrical conductivity in nutrient solutions affects both nutrient and water absorption, leading to physiological changes like as photosynthetic efficiency and dry matter production (Huett, 1994; Beltrão et al., 1997).

Fertilization must be applied on a continual basis to ensure seedling survival and must be adjusted based on the plant's metabolic rate. Silveira et al. (2014) suggest that the concentration of salts in the nutrient solution in mini clonal garden may be 20% greater in winter than in summer, depending on the clone employed. This is due to decreased evapotranspiration and nutrient absorption, which necessitates a more concentrated solution to suit the plants' requirements (Ciavatta et al., 2014).

In addition to environmental considerations, the medium for cultivation and container employed must be considered, since the rubber tree has a robust root system that, when restricted, results in delayed development and/or a high death rate.

### 4.2 Nutritional importance

Nutrition affected the formation of green stems in the rubber tree, emphasizing the need of fertilization under mini clonal garden settings. Higashi et al. (2002) found that well-nourished strains under mini clonal garden conditions impact minicutting productivity in *Eucalyptus* spp.

Regardless of the season, treatments with lesser quantities of fertilizer resulted in reduced nutrient levels in the leaves, impacting green stem formation in the mini clonal garden.

N, in general, is the nutrient demanded in the greatest quantities by plants (Taiz and Zeiger, 2017), being essential as a primary component of proteins, nucleic acids, enzymatic cofactors, and secondary metabolites (Hein et al., 2020), and is the nutrient most absorbed by the rubber tree (Medrado, 1992), in addition to playing a crucial role in the foliar expansion of mini clonal gardens (Silveira et al., 2014), in the productivity of mini clonal gardens (Rocha et al., 2015) and, therefore, in production of new vegetative buds, resulting in an increase

in chlorophyll content (Rezende et al., 2015). The most significant nutrients for rubber tree development are N, P, K, and magnesium (Thitithanakul et al., 2017).

Considering the optimal nutrient levels in rubber plants in the field (Mendes et al., 2012; Malavolta, Vitti, & Oliveira 1997; Raij et al., 1997), N is much higher than the average values recorded ( $31.7 \text{ g kg}^{-1}$ ). This happens because nutrient levels in mini clonal gardens might be greater than in the field (Silveira et al., 2014).

The overabundance of N that caused an imbalance in T5 may be explained by Bredemeier and Mundstock (2000), who state that when there is a large concentration of N near the roots, absorption is carried out by low affinity carriers that are not regulated. This causes an imbalance because the plant receives more nitrogen than it can metabolize.

K falls within the typical range reported in the literature ( $11.7 \text{ g kg}^{-1}$ ). The remaining nutrients did not exhibit quadratic behaviour; hence content ranges could not be determined.

In terms of micronutrient content, B was the greatest, followed by Fe, Mn, Zn, and Cu. In one experiment, Haag et al. (1986) found that even at low levels of B in the fertilizer solution, high quantities were identified in rubber tree leaves. According to Bergmann (1984), high B concentrations (100 to 1000  $\text{mg kg}^{-1}$ ) suggest toxicity, which may have resulted in death in this experiment, as the T5 seedlings perished and had levels over 140  $\text{mg kg}^{-1}$  across three seasons.

#### 4.3 Grafting success

Grafting reduced the output of the mini clonal garden, and fertigation had an impact on the survival of the grafted seedlings. Fertilization studies in mini clonal gardens of several species shown that nutrition has a favorable influence on vegetative growth (Brondani, 2012; Rocha et al., 2015; Oliveira et al., 2019; Pimentel et al., 2019).

The leaf stage influences the grafting procedure, with leaf stage D of the stems being preferred (Rocha et al., 2018). The period of leaf ontogeny is influenced by mineral nutrition and water availability. Moraes (1980) observed that in mature rubber tree plants, under favorable conditions, new leaf flows form within 45 days; but, with

low nutrient levels, even during the period of maximal water supply, the duration of the cycles is longer.

In this study, fertigated rubber trees with an electrical conductivity of  $2.0 \text{ mS cm}^{-1}$  in the solution exhibited higher stem productivity and grafting rates, highlighting the necessity of defining appropriate nutritional solutions in grafted seedling development. Solutions T1 and T2 were inadequate, however excess nutrients in T5 induced toxicity. However, the higher stickiness of the T1 graft might be attributed to the quality of the stems, which were less delicate than in other treatments, allowing the bud to be released from the stem.

Grafting procedures' poorer survival rates may be due to more than just nutrition. Grafting can be influenced by several parameters, including rootstock age, graft height, and tissue differentiation phases (Simão, 1971; Hartmann et al., 2011; Rocha et al., 2018; Nair, 2021). Furthermore, the leaf stage of the graft determines its placement (Rocha et al., 2018; Medrado, 1992).

During the plant growth cycle, cambial tissue activity is limited, resulting in poor grafting. During this time, the plant devotes its resources to the development of reproductive organs, resulting in a low supply of carbohydrates to help in graft healing (Oliveira et al., 2008).

#### 5. CONCLUSION

It is concluded that fertigation increased the productivity of the mini clonal garden, and the estimated electrical conductivity (EC) of  $1.64 \text{ mS cm}^{-1}$  resulted in greater production of green stems of rubber trees (*Hevea brasiliensis*), with a 50% reduction in harvesting time when compared to the traditional system. In the municipality of Piracicaba (SP), the most ideal seasons for generating rubber tree stems are summer and spring, whereas green stems cannot be produced in July and August, even under greenhouse conditions.

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## AUTHOR CONTRIBUTIONS

Karla Borelli designed the study. Discussions among all authors contributed to its subsequent conceptual and theoretical development. Data collection was performed by Karla Borelli. Karla Borelli and José Henrique Tertulino Rocha developed the data analyses and wrote the first draft of the manuscript with aid from Antonio Natal Gonçalves, to which, Magali Ribeiro da Silva, Erivaldo José Scaloppi Junior and Marco Antonio Tecchio contributed revisions.

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