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Effect of Different Varieties and Silicon Fertilizer on the Rooting Efficiency and Productivity of Mini-Cuttings and Powdery Mildew Disease of Eucalypt

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Authors' contributions

This work was carried out in collaboration among all authors. Author KFC did the work, did the statistical analysis and wrote the first version of the manuscript. Author HNP designed the study acting as advisor. Author LOS who corrected the bibliographic references, included some considerations in the work and translated it into English. All authors read and approved the final manuscript.

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ABSTRACT

Considering that silicon (Si) is a nutrient that stands out in the control of fungal diseases and in the increase in productivity of several cultures, this study intend to evaluate its influence on the productivity and severity of mildew in mini-stumps, as well as rooting of eucalyptus mini-cuttings. The experiment was carried out in a randomized block design with four replications in split plot scheme, the first factor related to Silicon doses (0, 0.5, 1.0, 1.5 and 2 mmol L^{-1}) and the second factor related to five clones of *Eucalyptus grandis* x *Eucalyptus urophylla* hybrids (I144, I224, 3334, GG100, GG680). The following variables were evaluated: Si content in mini-cuttings, powdery mildew severity and mini-stumps productivity, and rooting of mini-cuttings. The highest absorption

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of Si was obtained with a dose of 1.22 mmol L^{-1} (0.14%). There was no influence of the nutrient solution containing Si on the productivity of mini-stumps, powdery mildew severity or rooting of eucalyptus mini-cuttings. Clones GG100 and 3334 presented, respectively, the best and worst results for all evaluated variables.

Keywords: Mini-cuttings; silicate fertilization, Oidium eucalypti; clonal mini garden.

1. INTRODUCTION

In Brazil, clonal plantations of Eucalyptus began to stand out in the 1970s, following the development of the cuttings technique [1]. Improvement of clonal forestry provided more uniform and productive plantations, since superior genetic materials adapted to local conditions can be multiplied on a large scale with excellent cost-effectiveness.

Cutting was initially the most widely used technique for commercial cloning of Eucalyptus [1]. However, given its limitations, the mini-cuttings was then implemented, and currently, most of the large and medium-sized Brazilian forestry companies uses it on a commercial scale [2].

Due to their structure, clonal mini-gardens are subjected to the action of various phytopathogenic organisms. A very common disease in those places is caused by the fungus *Oidium eucalypti* Rostrup, which usually causes significant losses to forest seedling producers [3]. This pathogen mainly attacks young leaves and shoots, so the clonal mini-garden is a place that presents excellent conditions for its development. These fungi will hardly cause the death of their hosts, however, use their nutrients, reduce their photosynthesis, and maximize respiration and transpiration, which culminates in reduced plant development and productivity [4]. Due to the high spreading capacity of conidia, this fungus quickly infects healthy mini-stumps, causing infestation throughout the clonal mini-garden [5].

Many cultivated eucalyptus species are highly susceptible to powdery mildew and the most widely used control method today is the spraying of synthetic fungicides [6]. The use of fungicides, despite being efficient, causes several problems related to the selection of pathogen resistant strains besides impacting the environment and threatens human health [7]. The environmental and human-related hazardous of chemicals products have stimulated the reduction in chemical control and increased the use of other measures such as cultural control, biological control, genetic control, and alternative control [8].

In many agricultural crops such as rice, coffee, and sugar cane for example, silicate fertilization has been extensively studied for the control of various diseases [9,10,11,12,13,14]. In addition to its influence on the phytosanitary aspect, Silicon (Si) has also been identified as an important nutrient, responsible for increasing the productivity of those crops [15,16,17,18,19].

The beneficial effects of Si uptake and accumulation, are generally related to structural functions and plant defense, i.e. the element can affect plant production through various indirect actions, such as: improving plant architecture (upright leaves), reducing self-shading and lodging; increasing structural stiffness of tissues; decreasing toxicity of Fe, Mn, Al and Na; increasing resistance to pathogens; and increasing protection against herbivory, including phytophagous insects [20,21].

The use of silicon (Si) as a substitute for fungicide consumption has shown a very viable alternative, both from an environmental and economic point of view. Si management in plant nutrition could make a significant contribution to more sustainable and less polluting agriculture [22].

Despite all the beneficial effects already proven to be attributed to silicate fertilization for various crops, the effect of Si on forest species is poorly studied. Considering all these factors, the objective of the present study was to evaluate the efficiency of the application of silicon doses in the mildew severity in eucalyptus clonal mini-garden and to verify its influence on the productivity of mini-stumps and rooting of mini-cuttings.

2. MATERIALS AND METHODS

The experiment was carried out at the Research Nursery of the Department of Forest Engineering belonging to the Federal University of Viçosa - UFV, between February and October. The average temperature values in the city of Viçosa from 1961 to 1990 can be seen in Fig. 1.

The experiment was made in a complete randomized block design with four replications in split plot scheme, the first factor related to Si doses (0, 0.5, 1.0, 1.5 and 2 mmol L^{-1}), and the second factor related to the five clones of *Eucalyptus grandis* x *Eucalyptus urophylla* (I144, I224, 3334, GG100, GG680).

Before planting in the clonal mini-garden, the seedlings were pruned leaving only two pairs of leaves so that there was loss of apical dominance and lowering of the strain. Subsequently, 5 ml of mono ammonium phosphate at a concentration of 1.5% (15 g L^{-1}) was applied to each tube, and the seedlings were kept in these containers for another week.

Each plot was carefully isolated by fiber plates and plastic tarpaulin to avoid contamination by the nutrient solution applied to neighboring plots. In the first month after planting, a basic nutrient solution (without silicon) was used in all treatments in order to homogenize the physiological state of the plants and the formation of the crown. The irrigation system adopted was drip. During the entire duration of the experiment, pests and diseases were monitored, nutrient solutions exchanged and electrical conductivity of effluent (EC) measured for each plot, keeping it between 1.5 and 2.0 mS cm^{-1}.

At 30 days after planting, the silicon doses (0, 0.5, 1.0, 1.5 and 2.0 mmol L^{-1}) were then applied along with the basic nutrient solution and maintained until the completion of the experiment. The source of silicon used was monosilicic acid, obtained by passing potassium silicate through a column containing cation exchanger resin (Amberlite IRA 410) according to the methodology proposed by [24].

In the first two months, the mini-stumps were weekly pruned in order to make them cup-shaped. After this period, the number of mini-cuttings and then their rooting percentage began to be evaluated. The number of mini-cuttings was obtained by counting all shoots larger than 7 cm. After removal of these shoots, samples of 16 mini-cuttings per subplot were selected, which were later staked in polypropylene tubes (with a volume of 54.0 cm^3), placed in 96-unit grids and kept in a greenhouse for rooting. The selected cuttings were approximately 7 to 10 cm long, with two pairs of leaves, besides the apex.

Between collection and staking, shoots were packed in cold water in Styrofoam boxes to prevent loss of turgor. Leaf reduction was performed on the leaves that were too much large and prevented the mini-cuttings from remaining erect. Mini-cuttings and rooting percentage were evaluated every two weeks, however, pruning was performed weekly. In the weeks when there was no counting and stacking the collected material was discarded.

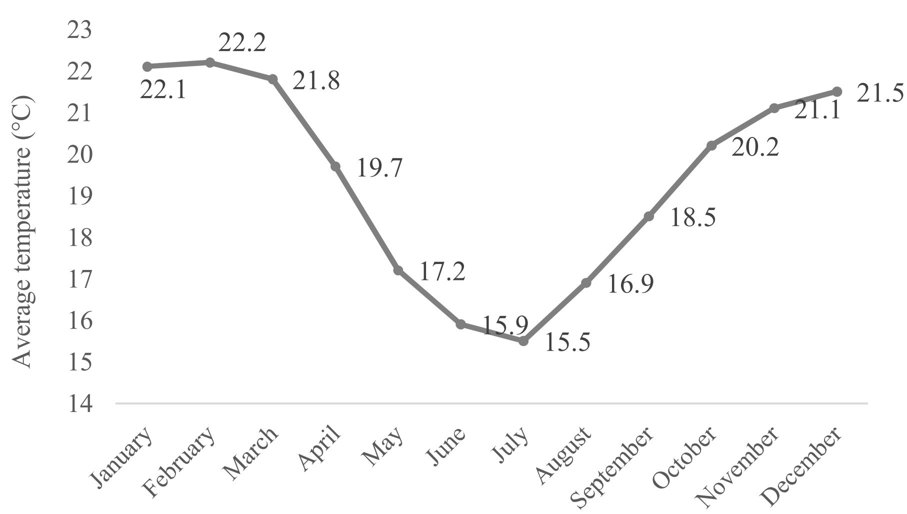


Fig. 1. Average maximum and minimum monthly air temperature in Viçosa - MG, considering the historical series from 1961 to 1990 [23]

The greenhouse had an automated mist irrigation system to control humidity (above 80%) and temperature (below 30ºC). After 25 days of staking, the rooting percentage was evaluated by counting the number of rooted mini-cuttings. This procedure was performed from May to October.

Throughout the evaluation period of productivity and rooting of the mini cuttings, the control of the powdery mildew in the clonal mini-garden was performed by applying neem oil and water. When these methods were not satisfactory, chemical control with fungicides was performed.

After the conclusion of this stage of the experiment (evaluation of mini-stumps productivity and rooting of mini-cuttings), mildew severity evaluations in the clonal mini-garden began. The inoculum was obtained from contaminated seedlings and kept in living tissue inside the clonal mini-garden. The inoculation method was modified from [24]. Plots containing the healthy seedlings under evaluation in the experiment were interspersed with plots containing eucalyptus seedlings contaminated with powdery mildew, thus, due to the proximity, the dispersion of the fungus spore was facilitated allowing a natural inoculation of the healthy seedlings.

To allow further infestation, no control of the powdery mildew was performed for a month. The assessment of the severity of this disease was performed using a grade scale as proposed by [25]. For this, a sample of five mini-stumps per subplot was selected, where all the young leaves emitted were evaluated assigning them grades according to the percentage of stains caused by the fungus on the leaves.

Si content was measured by evaluating the concentration of this nutrient in the mini-cuttings (leaves and stems). The whole process was performed according to the methodology proposed by [26].

Statistical analysis of the variables included analysis of variance (F test at 0.05 significance), polynomial regression analysis and Tukey test at 0.05 significance using the SISVAR program [27] after the data were analyzed and submitted to analysis of normality (Shapiro-Wilk at 0.05 significance) and homogeneity (Levene test at 0.05 significance) using the SPSS program [28].

3. RESULTS AND DISCUSSION

The Si leaf content in mini-cuttings in relation to the increase of Si concentration in the nutrient solution used for fertigation was similar for all genetic materials studied, that is, no interaction between these factors was observed. There was an increase in Si contents in mini-cuttings with increasing doses of this nutrient in the nutrient solution to a concentration of 1.22 mol L^{-1} (0.14 dag kg^{-1}), (Table 1, Fig. 2).

Although there was an increase in Si contents in the mini-cuttings with the increase of the applied dose with the nutrient solution, it is observed that these values are still low when compared to those obtained for some agricultural crops, mainly grasses [29,30,31,32].

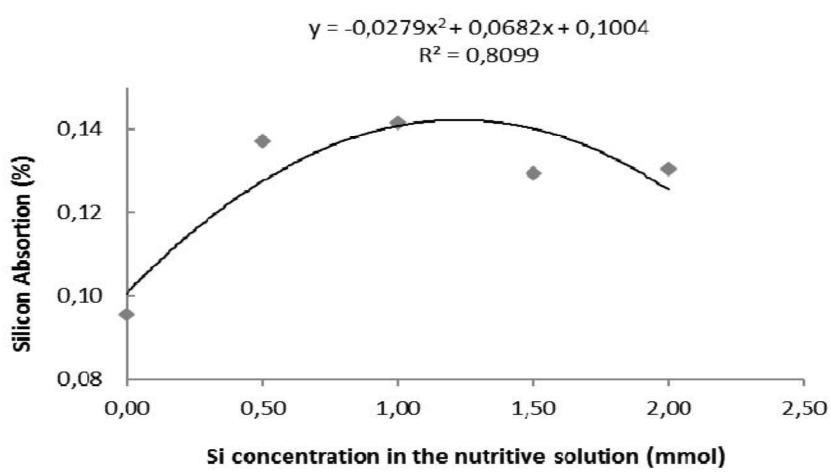


Fig. 2. Si concentration in eucalyptus mini-cuttings due to the increase in the doses of Si in the nutrient solution applied

Table 1. Analysis of variance of Si concentration in Eucalyptus clone minicuttings as a function of increasing Si doses

FV	GL	SQ	QM	FC	Pr>Fc
Plot	3	0,00818	0,00273	2,004	0,1672
Doses of Si	4	0,02642	0,0066	5	0,0147
Error 1	12	0,01634	0,00136	-	-
Clone	4	0,00165	0,00041	0,623	0,6479
Doses of Si * clone	16	0,01676	0,00105	1,586	0,1008
error	60	0,03963	0,00066	-	-
Total	99	0,10898	-	-	-
CV SI = 29,10 (%)			CV Clone = 20,27		
Total average = 0,13 dag kg -1		Number of observations = 100			

The mini-cuttings analyzed come from shoots that were pruned weekly. Thus, it is believed that the youthfulness of the sampled parts provided by periodic pruning did not allow the accumulation of larger amounts of silicon, since the mini-cuttings did not stay long enough in contact with the nutrient solution.

The absorption and accumulation capacity of Si varies depending on the species. Silicon-accumulator plants are those with leaf contents above 1 dag kg^{-1}, and non-accumulators plants with silicon content of less than 0.5 dag kg^{-1} [33]. Thus, considering the values obtained in this study, we can classify the genetic materials studied as plants non-accumulator of Si.

Similar results were obtained by Carvalho et al. [34]. According to the authors, eucalyptus is not a self-accumulator plant, although it is responsive to it. Duarte and Coelho [35] reported a relevant silicon leaf uptake in *Eucalyptus grandis* x *Eucalyptus urophylla* seedlings (0.23 dag kg^{-1}), allowing the authors to classify the studied plants as intermediate in the accumulation of this nutrient. Queiroz et al. [36] have concluded that *Eucalyptus camaldulensis* is capable of absorbing and translocating Si and that these processes are higher at 60 days after application of different sources of this nutrient. These same authors explain that foliar application is more efficient than soil application and, as in the present study, eucalyptus cannot be classified as a Si accumulator plant.

The average monthly productivity of mini-cuttings throughout the study period (from May to September) was 10.4 mini-cuttings per mini-stumps per month. In the period of lower temperatures (from May to July), the average productivity was 9.57 mini-cuttings per mini-stumps per month. In the warmer months (September and October), the average productivity was 15.2 mini-cuttings per mini-stumps per month.

Clones I144, GG100 and GG680 showed the highest monthly average productivity per mini-stumps. In the period of lower temperatures (May to July), these clones showed monthly productivity of 10.3; 10.2 and 9.9 mini-cuttings per mini-stumps per month, respectively. For the months of September and October (months when temperatures were highest) these same clones had monthly productivity of: 16.6; 17.9 and 17.1 mini-cuttings per mini-stumps per month. In both studied periods there was no interaction between the plot (Si) and subplot (clone) factors. Silicon did not influence the yield of mini-cuttings, which probably occurred due to the low absorption of this nutrient caused by the constant renewal of plant shoots, since prunings were performed weekly. Differences were observed only between clones, as shown in the following analysis of variance tables (Tables 2 and 3).

Clones 3334 and I224 had productivity of 8.6 and 8.8 mini-cuttings per month for the cold months, and 12.5 and 12.0 mini-cuttings per month for the warmer months (Fig. 3). The differences in productivity observed between these genetic materials are due to the genetic variability between them.

It was observed that the production of mini-cuttings in the present study was superior to most studies found in the literature. Brondani [37] in their work with *Eucalyptus benthamii* mini-cutting and micropropagation, found that during the spring and summer seasons, the evaluated clones had a minimum number of 7.5 shoots per mini-stumps and a maximum of 14.15 shoots per mini-stumps. In the fall and winter seasons, this

Table 2. Analysis of variance of eucalyptus cuttings yield as a function of Si doses and clone from May to June

FV	GL	SQ	QM	FC	Pr>Fc
Plot	3	5,0181	1,6727	0,539	0,6645
Doses of Si	4	18,0773	4,51931	1	0,2755
Error 1	12	37,2332	3,10276	-	-
Clone	4	55,5238	13,8809	7,904	0
Doses of Si * clone	16	20,3103	1,26939	0,723	0,7601
Error	60	105,375	1,74625	-	-
Total	99	241,538	-	-	-
CV SI = 18,42 (%)			CV Clone = 13,86 (%)		
Total average = 9,6 mini-cuttings -1 mini-stumps-1 month-1			Number of observations = 100		

Table 3. Analysis of variance of eucalyptus mini-cuttings yield as a function of Si doses and clone between September and October

FV	GL	SQ	QM	FC	Pr>Fc
Plot	3	861,317	287,106	17,67	0,0001
Doses of Si	4	19,915	4,97875	0	0,8682
Error 1	12	194,98	16,2483	-	-
Clone	4	609,365	152,341	11,83	0
Doses OF Si * clone	16	323,935	20,2459	1,572	0,1051
Error	60	772,625	12,8771	-	-
Total	99			-	-
CV SI = 26,47 (%)			CV Clone = 23,57		
Total average = 15,2 mini-stumps^{-1} mini-cuttings^{-1} month^{-1}			Number of observations = 100		

number was 5.45 shoots per mini-stumps (minimum) and 13.18 shoots per mini-stumps (maximum).

Cunha et al. [38,39] reported a monthly average productivity per mini-stumps in *Eucalyptus urophylla* x *E. grandis* hybrids, which ranged from 7.9 to 11.8 and 9.6 to 13.2, respectively. Wendling et al. [40] describe that the average production of mini-cuttings for sand bed clonal mini-garden system is 5.6 mini-cuttings per mini-stumps every 5-10 days. Souza [41] describes average productivity between 5 and 13 mini-cuttings per month, varying as a function of the spacing between mini-stumps and time of year.

For the other characteristics evaluated (rooting, number of mini-cuttings and powdery mildew severity), the response of the clones in relation to the increase of Si concentration in the nutrient solution was similar, that is, there was no interaction between the studied factors. In addition, there was no influence of increasing Si doses on any of these variables (Tables 4, 5 and 6).

The low Si content in eucalyptus mini-cuttings did not allow this nutrient to influence their rooting. For this same reason, it is believed that it was not possible to find a difference in the severity of powdery mildew in the mini-stumps irrigated with Si doses, since the pathogen mainly attacks young leaves and shoots.

The average rooting of mini-cuttings throughout the study period (from May to September) was 48%. An increase in rooting was observed as temperature increased. Between May and July (late autumn and early winter), the average rooting was 43%, while in September and October (spring), the average rooting was 75%, showing that this characteristic is very sensitive to these changes.

Clone GG100 presented the highest percentage of rooting in the cold period (66%). Because it presented a small variation in this percentage with the increase in temperature, in September and October its rooting was lower than the others (73%). The clones that showed higher sensitivity to temperature variations were 3334, followed by clone GG680, with a rooting percentage variation of 57% and 50%, respectively. In the warm period, clone I144 had the highest rooting percentage (87%) (Fig. 4).

The rooting percentages obtained in this study were found to be below most studies related to the subject [42,39,43]. This was due to the low temperatures normally recorded in the region during the winter period (Fig. 1).

The severity of powdery mildew is directly related to the genetic material used. Clones I224 and 3334 presented the highest percentages of leaf area infected by powdery mildew (55.15% and 49.5%, respectively), while the smaller clones GG680 and GG100 (5.2% and 11.4%) (Fig. 5).

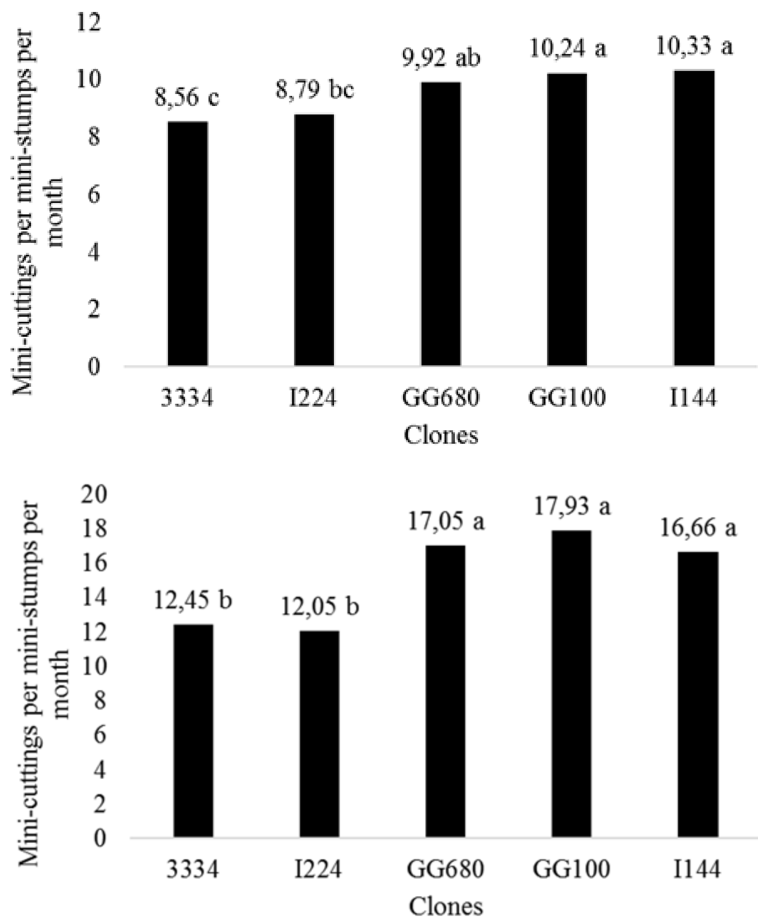


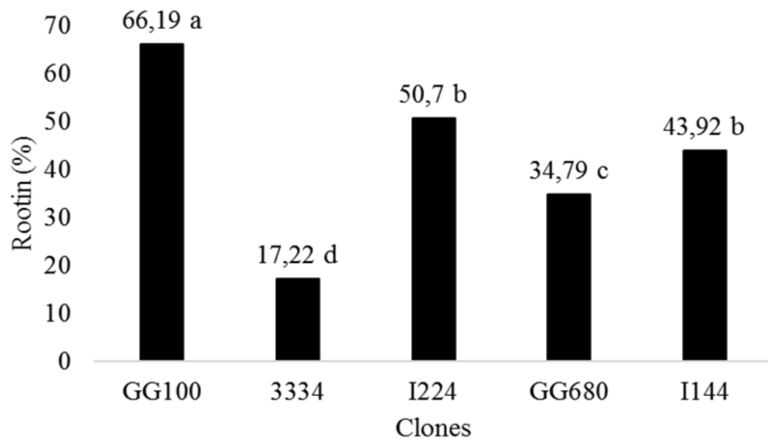
Fig. 3. A: Number of mini-cuttings per month for each clone from May to July (DMS = 1.18 mini-cuttings per month; CV = 13.86%) and B: Number of mini-cuttings per mini-stumps per month for each clone in the months of September and October (DMS = 3.19; CV = 23.57%)

Table 4. Analysis of variance of rooting of eucalyptus mini-cuttings as a function of Si doses and clone from May to June

FV	GL	SQ	QM	FC	Pr>Fc
Plot	3	4409,42	1469,81	6,149	0,0089
Doses OF Si	4	189,652	47,413	0	0,9344
Error 1	12	2868,16	239,013	-	-
Clone	4	26574	6643,625	84,707	0
Doses OF Si * Clone	16	892,21	55,7631	0,711	0,07719
Error	60	4705,82	78,4304	-	-
Total	99	39639,7		-	-
CV SI = 36,32 (%)			CV Clone = 20,81 (%)		
Total average = 42,6%		Number of observations = 100			

Table 5. Analysis of variance of rooting of eucalyptus mini-cuttings as a function of Si doses and clone from September and October

FV	GL	SQ	QM	FC	Pr>Fc
Plot	3	641,796875	13,9323	0,699	0,5705
Doses OF Si	4	346,09375	86,5234	0	0,8836
Error 1	12	3672,65625	6,05469	-	-
Clone	4	22346,09375	586,523	34,628	0
Doses OF Si * clone	16	2177,34375	136,084	0,844	0,6331
Error	60	9679,6875	161,328	-	-
Total	99	38863,67188		-	-
CV SI = 23,27 (%)			CV Clone = 16,89 (%)		
Total average = 75,2%		Number of observations = 100			



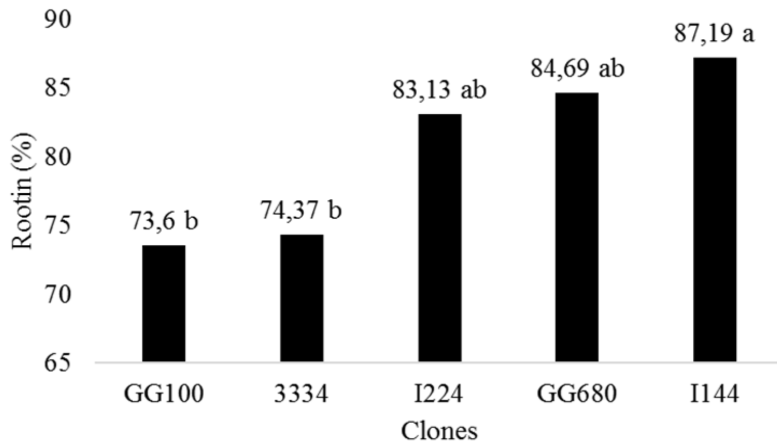


Fig. 4. A: Rooting percentage of eucalyptus clones mini-cuttings submitted to five doses of silicon 25 days after staking from May to July (CV = 20.81; DMS = 7.87) B: Percentage of rooting of mini-cuttings of eucalyptus clones submitted to five doses of silicon 25 days after staking in September and October (CV = 16.89; DMS = 11.30%)

The Si action in disease prevention is related to the accumulation of this nutrient in the cell wall of the transpiration organs, thus leading to the formation of a double layer of silica and, consequently, increasing the rigidity of the plant epidermis, promoting mechanical protection for plants [44]. Furthermore, Si can induce the formation of phytoalexins, which act in defense of the plant similar to the action of human antibodies [45]. For some crops, especially those considered non-accumulative, such as eucalyptus, the benefits of Si are not yet well defined, and depend on more applied studies.

The effect of Si on powdery mildew in forest species is still poorly studied, however, when Rosa [46] researching silicon on powdery mildew resistance in a clonal eucalyptus garden, concluded that the incidence of the disease during warmer temperatures is not influenced by Si applications. Only when the temperature increased was it possible to observe the influence of Si doses on the rate of disappearance of powdery mildew, which was faster when the seedlings were fertilized with higher doses of the nutrient.

For other crops, quite different results are found in the literature, such as those obtained by Pereira [47] who observed a 44% reduction in rust severity when 35 g L^{-1} of potassium silicate was sprayed at pH 5.5 in soy. The author reports that potassium silicate at this pH proved to be as efficient as the commercial resistance induction standard Acibenzolar-S-Metil in reducing the severity of Asian soybean rust.

Menzies et al. [48] reported that the application of potassium silicate in nutrient solution as well as in foliar sprays significantly increased the latent period of *Podosphaera xanthii* in pumpkin leaves, besides reducing the number of colonies of this fungus. Menzies et al. [49] also found that potassium silicate foliar sprays were effective in reducing the severity of powdery mildew on cucumber.

From the results presented in previous studies [50,51,52,53] it is believed that the absorbed Si doses were too low to have any effect on the incidence of powdery mildew, since absorption of this nutrient in the plants that responded to Si application were those that presented higher leaf contents than those observed in the present study.

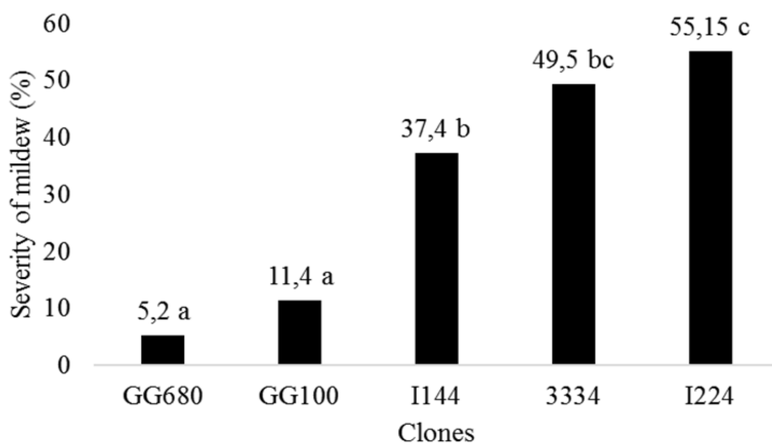


Fig. 5. Severity of mildew in eucalyptus clones (CV = 56.77; DMS = 16.03%)

Table 6. Variance analysis of powdery mildew severity in eucalyptus mini-cuttings as a function of Si doses and clone

FV	GL	SQ	QM	FC	Pr>Fc
Plot	3	4175,63	1391,88	4,564	0,0235
Doses OF Si	4	1488,56	372,14	1	0,3529
Error 1	12	3659,92	304,993	-	-
Clone	4	40271,36	10067,8	31,029	0
Doses OF Si * clone	16	3140,54	196,284	0,605	0,8675
Error	60	19467,7	324,462	-	-
Total	99			-	-
CV SI = 55,04 (%)			CV Clone = 56,77 (%)		
Total average = 31,73%		Number of observations = 100			

4. CONCLUSIONS

Eucalyptus is not a Si accumulator plant since the concentration in the mini-cuttings was less than 0.5 dag kg^{-1}. The productivity of the mini-stumps, as well as the severity of the powdery mildew and the rooting of the mini-cuttings are not influenced by the application of this nutrient with the nutrient solution, however, it was verified that these characteristics are directly influenced by the genetic material used. There was a clear increase of the productivity of mini-stumps and the rooting of mini-cuttings with the increase of the temperature.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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