

# Morphophysiological changes in seedlings of two wood species after application of salicylic acid<sup>1</sup>

Maria Eunice Lima Rocha<sup>2</sup>\*<sup>10</sup>, Fernanda Ludmyla Barbosa de Souza<sup>2</sup>, Maria Soraia Fortado vera Cruz<sup>2</sup>, Pablo Wenderson Ribeiro Coutinho<sup>2</sup>, Marlene de Matos Malavasi<sup>2</sup>, Ubirajara Contro Malavasi<sup>2</sup>

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#### ABSTRACT

The forestry production sector uses several strategies to minimize post-planting seedling losses. Some practices can modulate characteristics of interest for plant growth and defense, including changes in the light exposure, reduced watering, fertilization, and chemical or mechanical stimulus. This work quantified morphophysiological changes on *Schinus terebinthifolius* and *Cedrela fissilis* seedlings resulting from the application of salicylic acid for eight weeks. The experimental design was completely randomized, composed of four treatments (0, 100, 200, and 300 mg L<sup>-1</sup> of salicylic acid). The quantified variables included height, diameter, and dry matter mass of the root and aerial parts. Additionally, leaf area, root-cell electrolyte loss, lignin content in roots, and stem plus phenolic compounds were quantified. On *aroeira* seedlings, the height, aerial, and radicular dry matter masses and leaf area were reduced while the diameter and the lignin content increased directly as a function of salicylic acid doses. In *cedro* seedlings the dose of 100 mg L<sup>-1</sup>, however, resulted in a better balance of plant biomass and would, therefore, be the most indicated dose for the species. The application of salicylic acid for 8 weeks on seedlings of both species resulted in the most relevant morphophysiological modifications correlated with quality characteristics of seedlings of woody species.

Keywords: forest species; lignin; morphometric parameters; regulators; seedlings.

#### INTRODUCTION

Schinus terebinthifolius R., known as Brazilian "peppertree", is a native woody species belonging to the *Anacardiaceae* family. In some regions of Brazil, the species is known as "aroeira vermelha", "pimenteira", "aroeira da praia", and "aroeira mansa". The species can be found in the Atlantic Forest, the *Cerrado*, and the *Caatinga* biomes, mainly in the area between the states of Rio Grande do Sul and Pernambuco (Carvalho *et al.*, 2013).

*Schinus terebinthifolius* is classified as heliophyte, perennial, and a pioneer woody species. As a result of its phenotypic plasticity, rapid dispersion and growth, the species has great adaptability to the most diverse habitats, including wet and dry, deep and shallow, clayey and sandy soils, as well as being found from sea level up to 2,000 m of altitude (Gilbert & Favoreto, 2011; Carvalho *et al.*, 2013).

*Cedrela fissilis* V. belongs to the *Meliaceae* family. It is a non-endemic species, native to Brazil and popularly known as Brazilian "cedarwood" or "cedro-rosa". The species is classified as initial secondary, but it can be found on degraded soils in primary forests. When present in capoeira pastures, it can behave as a pioneer (Biernaski *et al.*, 2012). The species shows wide distribution including the Amazon, the *Cerrado*, and the Atlantic Forest biomes. In addition, the species is widely used in landscaping and in the composition of heterogeneous reforestations in degraded areas (Caires *et al.*, 2011).

The forest production sector uses several tactics to maximize production and minimize seedling post-planting mortality by altering seedlings morpho-physiological attributes (D'Avila *et al.*, 2011), which are related to edaphic and climatic conditions, namely "the target seedling concept" (Landis *et al.*, 2010). Recent studies have

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<sup>&</sup>lt;sup>2</sup> Universidade Estadual do Oeste do Paraná, Programa de Pós-Graduação em Agronomia, Marechal Cândido Rondon, Paraná, Brazil. eunice\_agronomia@yahoo.com.br; myllananda@hotmail.com; soraiaf12@hotmail.com; pablowenderson@hotmail.com; marlenemalavasi@yahoo.com.br; biramalavasi@yahoo.com.br

<sup>\*</sup>Corresponding author: eunice\_agronomia@yahoo.com.br

highlighted the potential of plant regulators to induce plant defense mechanisms against biotic or abiotic stresses, altering metabolic pathways linked to plant development and growth (Oro *et al.*, 2012; Mazzuchelli *et al.*, 2014).

Salicylic acid (SA) is an endogenous growth regulator that participates in physiological processes and acts on the plant resistance, promoting biological alterations and regulation of enzymes, in addition to being directly involved in plant protection (Rivas-San & Plasencia, 2011).

SA is synthesized from the amino acid phenylalanine. SA is considered a signal amplifier, which at low concentrations ( $10^{-3}$ - $10^{-6}$  M) can improve the antioxidant capacity in plants and attenuate the effect of adverse conditions. At high concentrations, however, SA can be harmful by accelerating cell death or by increasing susceptibility to pests, diseases, and abiotic stresses (Hara *et al.*, 2012; Miura & Tada, 2014; Wittek *et al.*, 2015). When exposed to stress, seedlings may present several physiological responses, such as increased peroxidase activity, cell lignification, oxidation of phenolic compounds, ethylene biosynthesis, and maintenance of membrane integrity (Taiz *et al.*, 2017).

The phenolic compounds are part of a highly varied group, some of which are soluble in organic solvents, while others are soluble in water, and other insoluble, including flavonoids, phenolic acids, simple phenols, coumarins, tannins, and lignins considered multifunctional due to structural variability of these compounds. In addition, those compounds can give natural durability to wood (Taiz *et al.*, 2017).

Lignin production is a process related to the evolution of vascular tissues, formed from phenylalanine, present in the cytoplasm and accumulated in the cell wall by the action of peroxidase, laccase or phenol oxidase enzymes (Marjamaa *et al.*, 2009). Studies have shown that the use of chemical stimulus may be related to the production of lignin and thus favor seedling development and field survival (Dranski *et al.*, 2013; Mazzuchelli *et al.*, 2014; Cadorin *et al.*, 2015).

Despite the great economic potential and superior quality of their wood, native woody species are little considered in commercial plantations when compared to exotic ones. Therefore, it is important that studies on the former species are carried out so that their use occurs in a balanced way with recommended species. Additionally, it is interesting to pay attention to factors related to the conservation and protection of native woody species that are at risk of extinction (Bobato *et al.*, 2008).

In addition, seedlings subjected to chemical stimulus can alter morphometric, physiological, biochemical, and cellular characteristics, in addition to inducing adaptive responses in plants subjected to the most diverse stresses. The objective of the experiment was to quantify morphophysiological changes in *Schinus terebinthifolius* and *Cedrela fissilis* seedlings resulting from the application of salicylic acid for eight weeks at the plant nursery.

### **MATERIAL AND METHODS**

The experiment was conducted in a region located at 24° 33' 24" S and 54° 03' 24" W with an altitude of 420 m, at the *Universidade Estadual do Oeste do Paraná*, Marechal Cândido Rondon, Paraná. According to Iapar and Köppen climate classification, local climate is considered Cfa, subtropical, with an average annual temperature between 22 °C and 23 °C, well distributed rainfall during the year and hot summers (Alvares *et al.*, 2013; Nitsche *et al.*, 2019).

The values of relative humidity and air temperature during the conduction of the experiment were obtained daily with the aid of a datalogger (KlimaLogg Smart Model) (Figure 1).

The experiment used 400 4-months-old *Schinus terebinthifolius* and 400 4-months-old *Cedrela fissilis* seedlings obtained from *Itaipu Binacional* nursery and from the *Instituto Ambiental do Paraná* (IAP). Seedlings were propagated in 120 cm<sup>3</sup> plastic tubes. The commercial substrate used was the Humusfertil vermicompost based on pine bark, sand as a source of substrate, and vermiculite, with the respective guarantees, electrical conductivity of 1.5 mS cm<sup>-1</sup>; density of 480 kg m<sup>-3</sup>; hydrogen potential (pH) of 6.5, and; maximum humidity and retention capacity (CRA) in weight/weight equal to 60%.

Seedlings of both species were transported from the original nurseries and placed in a shade house for 30 days between the months of August and September 2016 (acclimatization period). During that period, each seedling received 3 mL of a nutrient solution (Hoagland & Arnon, 1950) weekly. Before submission to treatments, we recorded mean values of 14.60 and 8.09 cm for height, 2.99 and 2.51 mm for stem diameter, and 13.34 and 6.62 leaves for *Schinus terebinthifolius* and *Cedrela fissilis* seedlings, respectively.

The treatments were applied once a week from September 26 to November 14, 2016 (8 weeks), at the same time, on 5-month-old seedlings. The treatment solution consisted of salicylic acid, in the concentrations indicated for each treatment, deionized water, and adjuvant (Agral-Syngenta®), 30 mL in 100 L of water, according to the manufacturer's recommendation. The adjuvant was used in order to increase the efficiency of the product, increasing the spread of the drops and facilitating the foliar absorption of the product. The acid was applied by using a manual sprayer between 6:00am and 6:30am due to the milder weather conditions at this time. The applied amount was 100 L ha<sup>-1</sup> on both species, following the recommendations for the pesticide application technology.

Throughout the experiment, cultural treatments were carried out according to the demand of the species, among which feature cleaning the container, application of nutrient solution, and irrigation via micro-sprinklers at five daily 10-minute intervals: at 06:45am, 09: 45am, 12:45pm, 03:45pm, and 05:45pm. After the acclimatization process, the seedlings remained in a protected environment for the application of treatments and evaluations.

Measurements on the seedlings included increments in height (HI), diameter (ID), above ground dry biomass (AGDB), and below ground dry biomass (BGDB). The height measurement was performed from the base of the seedlings, lower region, close to the plastic container, up to the last insertion of the apical growth, while the diameter was determined at the base of the stem, lower region, with a digital caliper, determined over 400 seedlings per species.

At the end of the treatments period, twenty seedlings per treatment were randomly selected for determination of leaf area using an LI-3000A portable meter (Li-Cor, United States). Afterwards, seedlings had above- and below ground tissues separated for determination of dry biomass at 60 °C for 72 hours. The same samples selected for dry biomass determination were used for the physiological evaluations described next. Additionally, root electrolyte loss was determined according to Wilner (1955). We also quantified phenolic compounds proposed by Georgé *et al.* (2005) and lignin content according to Van Soest (1994). The physiological evaluations were carried out at the end of the application period of salicylic acid.

The experiments followed a completely randomized design formed by four treatments (doses) of salicylic acid (0, 100, 200, and 300 mg  $L^{-1}$ ), with five repetitions over 20 plants each, totaling 400 seedlings per species.

The results were submitted to the Bartlett and Shapiro-Wilk tests in order to test for homogeneity and normality of the data, followed by the analysis of variance. Treatment averages were compared by the F-test and the Dunnett's test.

### **RESULTS AND DISCUSSION**

The highest dose of SA reduced height increments of *Schinus terebinthifolius* seedlings (Figure 2A). SA may have stimulated specific proteins, enzymes, and genes that act to protect plants, creating a stress condition. Therefore, excessive or incorrectly exogenous application of SA may cause hormonal imbalance in terrestrial plants, affecting the route of other metabolites (Campos, 2009).

With *Cedrela fissilis* seedlings, results indicated a reduction of height increment in seedlings treated with salicylic acid. The averages of the increments were 1.93, 1.72, 1.71, and 1.68 cm for treatments with 0, 100, 200, and 300 mg L<sup>-1</sup>, respectively (Figure 3A). With *Schinus terebinthifolius* and *Cedrela fissilis* seedlings, the reduction in height was 27.47% and 12.95%, respectively, between control (0 mg L<sup>-1</sup>) and those receiving 300 mg L<sup>-1</sup> of SA. Contrasting results were reported by Mazzuchelli *et al.* (2014), where the application of SA resulted in an increase in height of *Eucalyptus urophylla x Eucalyptus grandis* (H13 clone) seedlings with doses of 0, 100, 200, and 300 mg L<sup>-1</sup>.

The increased doses of SA applied to *Cedrela fissilis* seedlings resulted in an increase on the stem diameter increment (Figure 3B). Such trend is likely to be advantageous because seedlings with larger diameters usually present higher survival rates when exposed to field planting stress, mainly because they present greater capacity for new root formation according to Del Campo *et al.* (2010). On the other hand, *Schinus terebinthifolius* seedlings did not present changes on the stem diameter

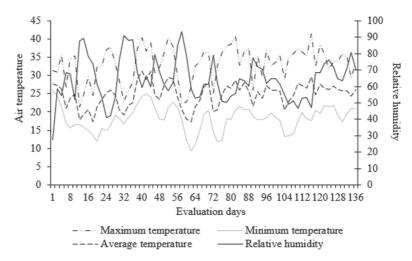
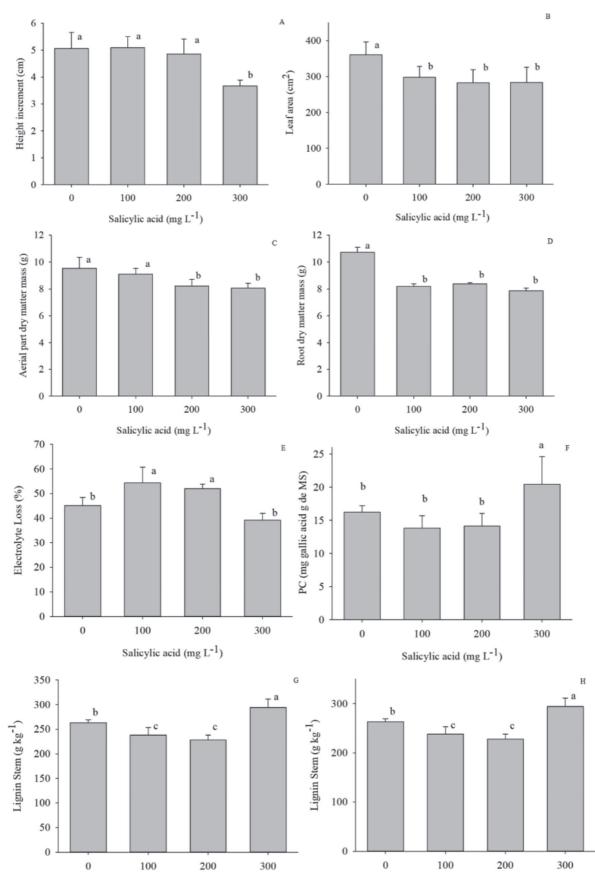


Figure 1: Values of temperature and average of the relative humidity of the air during the experiment carried out with *Schinus terebinthifolius* and *Cedrela fissilis* in a protected environment.

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Different letters above bars mean statistical difference from each other by the Dunnett test at the 5% probability level

**Figure 2**: Height increment (A), leaf area (B), Aerial part dry matter mass (C), root dry matter mass (D) electrolyte loss (E), phenolic compound (F), lignin stem (G) and lignin root (H) in *Schinus terebinthifolius* seedlings after application of salicylic acid.

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increment as a function of SA treatments with values varying from 1.71 mm to 1.86 mm.

The larger the leaf area, the greater the production of photo-assimilates, despite the greater water loss due to transpiration. However, *Schinus terebinthifolius* seedlings showed an inverse relationship between SA tested doses and leaf area. We interpreted that trend as an induction of stressful condition which resulted in senescence or appearance of new leaves that are not photosynthetically active (Mazzuchelli *et al.*, 2014). Despite this, statistically, there was no significant difference between the doses of SA, differing only from the control treatment; their averages were 360.98, 297.87, 283.01, and 283.69 cm<sup>2</sup> (Figure 2B). The analysis of leaf area from *Cedrela fissilis* seedlings showed no statistical difference (P > 0.05) as a function of SA doses, presenting a mean value of 285.16 cm<sup>2</sup>.

Aboveground dry biomass showed a reduction of approximately 15.41% from treatment 1 (100 mg L<sup>-1</sup>) to treatment 4 (300 mg L<sup>-1</sup>) in Schinus terebinthifolius seedlings (Figure 2C). These results can be related to the decrease on shoot height, leaf area, and number of leaves, since SA may have promoted a reduction of aboveground tissues in order to balance plant growth. Substances applied to plants or produced in a quantity greater than ideal can induce leaf fall and decrease leaf area, decreasing above- and belowground dry biomass, as plant hormones present synthesis and inhibition mechanisms in terms of concentrations (feedback mechanism) according to Taiz et al. (2017). Opposite results were obtained by Saracho et al. (2021) and for the species Schinus terebinthifolius, where the highest average of aerial biomass was obtained at a dose of 200 mg L<sup>-1</sup> (2.71g/plant) with seedlings irrigated every 12 days.

The analysis of aerial dry biomass from *Cedrela fissilis* seedlings showed no statistically significant difference (P > 0.05) as a function of SA doses and control treatment and the averages were equal to 3.39, 3.44, 3.51, and 3.20g.

In Schinus terebinthifolius seedlings, there was no difference across the doses of salicylic acid. These differed only from the control treatment for Root dry matter mass. These values decreased as the doses of the acid increased (Figure 2D). Similar results were obtained by Oro et al. (2012) for the root dry matter mass parameter on Cariniana estrellensis R. seedlings, where the control showed higher values in relation to the other treatments with the application of ethephon, which can be justified by the reduction of the aerial part. Hence, the plant, in order to balance the shoot/root system growth also showed a decrease on the root. In addition, reducing dry root biomass is a strategy for reducing energetic costs in situations of stress. In the case of Schinus terebinthifolius, at the maximum dose (300 mg L<sup>-1</sup>), there was an increase on the concentration of phenolic compounds and lignin, on the stem and on the root, components that are linked to the defensive responses of plants.

*Cedrela fissilis* seedlings, on the other hand, that received the two highest SA doses (200 and 300 mg L<sup>-1</sup>) increased in values of 3.70 and 3.73 g of dry root biomass, respectively (Figure 3C). According to Jacobs & Landis (2009), seedlings in the nursery phase undergo a period of acclimatization, in which they redirect part of the energy that would be used in the development of the aboveground tissues for root growth. Thus, the biomass of the roots tends to increase due to photoassimilate accumulation.

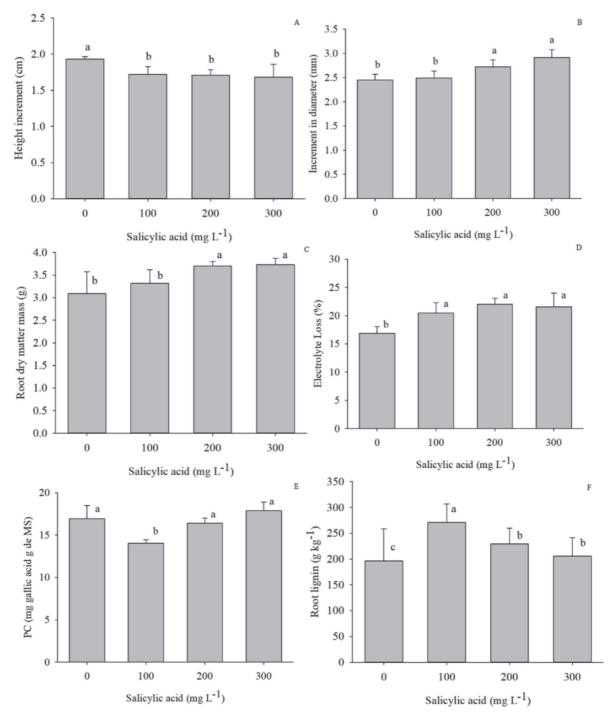
The seedling root tissues electrolyte loss experiment evaluates the integrity of cell membrane (Landis et al., 2010). In Schinus terebinthifolius seedlings (Figure 2E), application of 100 and 200 mg L<sup>-1</sup> resulted in higher ion extravasation, since both (100 and 200 mg L<sup>-1</sup>) did not show a significant difference, which is directly related to the low field survival capacity (Dranski et al., 2013). In previous studies, authors such as Guo et al. (2010) have argued that, under certain circumstances, the transit of solutes in the cells could favor osmotic balance inducing tolerance to several environmental stresses. Thus, entrance and exit of those substances may be beneficial, as long as membrane selectivity is not completely corrupted. That is, losing the ability to control what enters and what leaves the cell, for example, the release of cytoplasmic content and disorganization of the organelles.

*Cedrela fissilis* seedlings subjected to the application of salicylic acid differed only from the control seedlings (16.90%) (Figure 3D). Thus, it is inferred that the application of SA generated a stressful condition resulting in the release of solutes from the cells. In turn, this may result in the plasmolysis and destabilization of the electrochemical potential depending on the intensity at which these tissues reach. Evidently, ions are translocated from one cell to another, and this transport is mediated by cell membranes or through carrier proteins. Under atypical conditions, membrane selectivity is compromised (Taiz *et al.*, 2017), and therefore, high values of ion extravasation may indicate higher stress as a function of the treatments or a strategy to inform the plant of these adverse conditions.

Phenolic compounds are secondary metabolites present in plants and formed from the bond between an aromatic ring and one or more hydroxyl groups (-OH) (Silva *et al.*, 2010; Taiz *et al.*, 2017). For the quantification of the phenolic compound content, application of 100 and 200 mg L<sup>-1</sup> of SA in *Schinus terebinthifolius* seedlings did not differ from the control seedlings (P > 0.05) with values of 16.23, 13.84, 14.15, and 20.46 mg of gallic acid per gram of dry biomass (Figure 2F). The maximum dose resulted in greater synthesis of phenolic compounds, and this can be justified by the characteristic of each species and hormonal requirements. Since the hormones are required in small amounts by plants and concentrations close to 0.5 to 1.0 mM can activate, inhibit, or modify important metabolic routes, high doses can stress plants and activate defense mechanisms (Yuan & Lin, 2008). gallic acid per gram of dry matter. The lowest value (14.03 mg of gallic acid per gram of dry matter) was calculated from seedlings subjected to  $100 \text{ mg L}^{-1}$  of SA, reinforcing the fact that *Cedrela fissilis* were more sensitive than *Schinus terebinthifolius* seedlings and responded earlier to the application of salicylic acid (Figure 3E).

In *Cedrela fissilis* seedlings 0, 200, and 300 mg  $L^{-1}$  of SA resulted in no difference in values of milligrams of

In both *Schinus terebinthifolius* and *Cedrela fissilis* seedlings, the highest mean values for phenolic



Different letters above bars mean statistical difference from each other by the Dunnett test at the 5% probability level

**Figure 3**: Height increment (A), diameter increment (B), Rood dry matter mass (C), electrolyte loss (D), phenolic compound (E) and Root lignin (F) in *Cedrela fissilis* seedlings after application of salicylic acid.

compounds were obtained with the application of 300 mg  $L^{-1}$  of SA. Nevertheless, the composition of phenolic compounds varies according to the species, cultivar, phenological stage, and edaphic and climatic conditions (Gomes *et al.*, 2013). The amounts of phenolic compounds may increase under conditions where biological processes are impaired and may vary according to temperature, water availability, luminosity, nutrient availability, pest and pathogen infestation, and mechanical and chemical damage (Ramakrishna & Ravishankar, 2011; Ncube *et al.*, 2012).

Very high temperatures, above 35 °C, for example, influence the stomatal opening and closing. This stress condition can trigger a series of responses, including activation or inactivation of enzymes and production of phenolic compounds linked to the defense of plants. At high temperatures, presence of light or other stress related to the oxidation of plant pigments, the excess of phenolic compounds can attenuate these effects, acting on the photoprotection and development of plants (Ferreira et al., 2016). The same authors detailed that the higher the temperature, the greater the risk of degradation or inactivation. High temperatures and luminosity can interrupt the electron transport chain, contributing to the formation of phenolic compounds. Cedrela fissilis and Schinus terebinthifolius seedlings were exposed to thermal oscillations reaching 37.4 °C during the experimental period, and it was inferred that other factors contributed to the results (Figure 1).

Originating in the metabolic pathway of phenolic compounds, lignin is present in most tissues. Nursery practices to increase seedling lignin content may reduce water and solutes cavitation under drought stress (Rogers & Campbell, 2004). Stem lignin content from Schinus terebinthifolius seedlings increased with the application of 300 mg L<sup>-1</sup> of SA (Figure 2G). While the concentrations of lignin in the roots of Schinus terebinthifolius were higher for most treatments in relation to the levels observed on the stem and from the dose of 200 mg L<sup>-1</sup>, these levels were increased, however not differing from the control treatment. As obtained previously, the dose of 100 mg L<sup>-1</sup> showed the lowest average for this parameter (Figure 2H). Although lignin is not considered a quality attribute in seedlings of woody species, the molecule performs a number of activities in plant metabolism, mainly under water deficit, improving the tolerance of vegetables to this condition (Malavasi et al., 2016).

In *Cedrela fissilis* seedlings, stem lignin content was not changed (P > 0.05) in response to the application of SA (mean value of 177.59 g kg<sup>-1</sup>). For the roots of *Cedrela fissilis* seedlings, the lowest lignin content (196.41 g kg<sup>-1</sup>) was quantified in control seedlings, while the highest (271.46 g kg<sup>-1</sup>) was for seedlings that received 100 mg L<sup>-1</sup> of SA (Figure 3F). The highest SA treatment may have generated a great change on the metabolism of the plant, signaling a stress condition and increasing lignin content as a defense strategy. In research work developed by Awate & Gaikwad (2014), a similar result was observed, where, with 12 months old *Simarouba glauca* DC., the application of 50 mg L<sup>-1</sup> of SA increased secondary metabolites such as coumarins, lignin, sterols, xanthoproteins, cardiac glycosides, and saponins.

Therefore, it can be inferred that the species of the seedlings, the edaphic and climatic conditions, as well as the stage of development will be determinant for the use of plant regulators. Thus, it is imperative that further studies be conducted in order to quantify the most suitable doses, the period of exposure, and the time of exogeneous application of plant regulators.

## CONCLUSION

Application of salicylic acid for 8 weeks on *Schinus terebinthifolius* and on *Cedrela fissilis* seedlings resulted in morpho-physiological changes, which were indicative of high-quality seedling characteristics.

On seedlings of *Schinus terebinthifolius*, the application of 300 mg L<sup>-1</sup> of SA resulted in a greater balance between the tissues above and below the ground, since it reduced the height and increased the diameter of the seedling, even though the latter parameter did not show any significant difference between treatments. The content of lignin and phenolic compounds increased as the doses of salicylic acid increased. However, the exception to the results obtained in these seedlings was the loss of electrolytes that reduced when 300 mg L<sup>-1</sup> were applied, demonstrating that the maximum dose for the evaluated plants did not act as stress and therefore no signaling in the plants happened.

In the case of this research, the dose of 300 mg L<sup>-1</sup> is the most recommended for mastic trees because this species is more rustic and less susceptible to external variations. Furthermore, the treatments have generated morphophysiological changes that favored the development and growth of seedlings.

On *Cedrela fissilis* seedlings, the relation between height and diameter was similar to the previous species. The expected behavior in cases where the plants are stimulated or placed in adverse conditions is an increase in secondary growth and the detriment of primary growth. At the dose of 300 mg L<sup>-1</sup> there was a greater leakage of electrolytes, and the production of phenolic compounds was increased, resulting from a plant defense mechanism. The lignin amount was reduced after the dose of 100 mg L<sup>-1</sup>. For cedar, the doses of 100 and 200 mg L<sup>-1</sup> showed similar results, and these could be recommended, as they stimulated plant defense through the production of phenolic compounds, lignin, and loss of electrolytes, which is advantageous, since these make the plant more reactive later if subjected to these conditions again. These treatments also presented a good relationship when the vegetal biomass was evaluated.

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