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# Beyond the Wood Log: Relationships Among Bark Anatomy, Stem Diameter, and Tolerance to Eucalypt Physiological Disorder (EPD) in Cultivated Clones of *Eucalyptus grandis* Hill Ex Maiden and *E. urophylla* T. Blake

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## Abstract

*Eucalyptus* plantation forests play an important role in the global trade balance, and have been challenged with the Eucalypt Physiological Disorder (EPD) exhibiting symptoms on barks. Despite of that, there is little information on the anatomical features of phloem and periderm associated with this disorder. Although tolerant and susceptible commercial clones exhibited similar anatomical structures, they differed in the proportions of conducting and total phloem tissue and the amount of phloem containing Calcium oxalate (CaOx) crystals. The frequency and diameter of sieve tube elements (STEs) also varied among the tested clones. The increased area of phloem with non-collapsed STE and CaOx crystals were linked to the EPD tolerant phenotype. Bark, secondary phloem, and periderm thickness were correlated with EPD scores. Structural characteristics of phloem cells is correlated with increasing stem diameter. Bark and phloem thickness exhibited significant and positive associations with EPD-tolerant clones and stem diameter, while negative correlations with EPD scores. These connections corroborate the positive impact of increasing the proportion of total phloem thickness on stem diameter growth and EPD tolerance. The present results were based on restricted, yet commercially important, *Eucalyptus* species (*E. grandis*, *E. urophylla* and *E. grandis* × *E. urophylla* hybrids) highlighting bark and phloem traits linked to plant growth and EPD tolerance.

**Keywords:** bark anatomy; functional anatomy; plant applied anatomy; eucalypt physiological disorder; forestry; plant stress response

## 1. Introduction

*Eucalyptus* comprises about 700 wood species [1] that make up planted forests for various purposes [2–4]. The exploration of new frontiers for the forest settlement [5] comes along with challenges. Climate change shows an increase in the temperatures and water deficit in several regions of the world [5–8]. These changes have a potential impact on eucalypt plantations [9] as water deficit is one of the triggers of eucalypt disorders [6,10–15]. This is particularly important as there is caution, and even restrictions, to eucalypt plantations due to the association with drying up water resources and other soil and environmental issues [16]. On the other hand, there are compilation of researches that ascertain that eucalypt cultivation is sustainable and with economic and environmental benefits [17]. Nine species comprise more than 90% of the planted eucalypt forests [18,19] *E. grandis*, *E. urophylla* among them, which have been reported as the most frequently used *Eucalyptus* species used in water deficit resistance experiments [20].

The Eucalypt Physiological Disorder (EPD) has a complex etiology and exhibits symptoms such as depressed lesion and bark cracks, leaf abscission, sprouting, dieback, and plant death [8,21,22]. Except for bark secondary metabolite profiling and cell characterization [23–26], there is a lack of statistical analysis of anatomical traits applied to abiotic stress [20] and EPD tolerance. EPD has negative effects on tree growth [6,15,22,27], and wood production and quality [27,28]. There are initiatives that seek to understand the disorder and venues for selection of resistant and tolerant clones [28–34]. Although EPD symptoms are observed in the bark, there is no research associating bark anatomy with EPD.

The suitability of eucalypt plantation areas is expected to remain unaltered up to 2050, but changes in the precipitation, air temperature, evapotranspiration, and water availability are foreseen, which imply restriction or compromise *Eucalyptus* cultivation in Brazil by the end of the 21st century [9]. Stress triggers contribute to EPD [10,15,35,36] and their respective structural [29,31], metabolic [7,31,37], and nutritional [32,36] responses. Less adapted individuals are expected to have decreased growth and survival [20,38,39]. When these stresses are amplified over time, there is a reduction in tree growth and an increase in tree mortality [38,39], which can be traced back to anatomical and physiological characteristics.

Adaptative measures are proposed, such as genotype selection, but there is no information on the role of phloem in *Eucalyptus* tolerance to increased air temperature, water use, and nutrient efficiency. Anatomical and histological phenotyping has potential applications for physiological and genetic approaches [40,41]. Almeida et al. [28] evaluated wood properties, reporting that heartwood volume and syringyl/guaiacyl ratio that differed between EPD tolerant and susceptible clones EPD. Despite Almeida et al. [28] observed similar bark density, EPD is also expected to influence phloem properties and cambium activity as there are cambium responses to environmental stresses [5,9,42].

On the other hand, there are successful reports on the use of anatomical data aligned with plant responses to abiotic stresses [27,31,43,44], and contributing to selection of plants tolerant to abiotic stresses [37]. Among these, the statistical analysis of anatomical traits from xylem, petiole and leaf blade samples were effective in finding biomarkers associated with tolerance to water deficit tolerance in eucalypts [27,37,45]. Phloem is acknowledged for its role and importance to plant development [42,46–49], environmental [42,50,51] and stress responses [48,51–53], and other ecological or anatomical developmental features [42,54]. Biochemical and laboratory analysis, such as electrical conductivity, pH, amino acids and phenolics content, have also been associated with EPD tolerance [34].

Anatomical and allometric traits may therefore contribute to clone selection [40,41] in breeding programs aimed at improving EPD tolerance. In the present work, allometric treatment and quantitative analysis of bark and phloem anatomical traits of *Eucalyptus*

commercial clones with contrasting EPD phenotypes were conducted to obtain traits that contribute to the understanding and selection of EPD-tolerant genotypes.

## 2. Materials and Methods

### 2.1. Experiment

This research is part of a collaborative induced project “Anatomical description of leaves and stems of semi-commercial clones of Suzano SA” aiming at the characterization of leaf, bark, and wood anatomy of commercial and semi-commercial clones. The semi-commercial clones are being tested by the company breeding staff before release for full commercial plantation. The experiment was conducted in a commercial plantation area selected due to its record of EPD occurrence (Edival A. V. Zauza, personal information). This area is located in Itabatã district, Mucuri municipality (DMS coordinates-18°05′11.00″ S-39°33′2.99″ W; DD coordinates-18.08639-39.55083), in the Southern of Bahia State, Brazil. The soil classification is Abruptic Yellow Argisol (Typic, Dystrophic, Tb), moderate A horizon, sandy/loamy/clayey texture. The experiment was conducted from 2015 to 2020. The site has a climate described as Aw (tropical with dry winter) and exhibited, between 2017 and 2020, an average air temperature of 24 °C, precipitation of 915 mm year<sup>-1</sup>, and a water deficit (estimate) of 380 mm year<sup>-1</sup>. The rainfall regime in the experimental area was presented in Almeida et al. [28]. The clones were planted with a spacing of 3 × 3 m, following cultural practices adopted by Suzano SA. According a confidentiality agreement, further information of eucalypt plantation management should be addressed to Suzano staff.

In a preliminary approach, thirty commercial genotypes, derived from Suzano’s breeding program, were screened from which 9 different representative clones, from *E. grandis*, *E. urophylla* and *E. grandis* × *E. urophylla* hybrids, were selected based on their different EPD scores in commercial stands (historical record), ranging from tolerant, intermediately tolerant to susceptible (Table 1). This classification was based on the occurrence of the disorder in commercial plantations and was provided by Suzano SA. The experimental design consisted of four blocks for each clone. Three of the 4 original blocks were sampled, while the fourth block was preserved according to the company’s directives. To reduce uncontrolled bias, the plants evaluated were grown in this commercial field under standardized management conditions, at the same site, plant age, and at the same or equivalent position used for stem sampling.

**Table 1.** *Eucalyptus* clone/sample relationship with the respective phenotypes according historical data on EPD occurrence in commercial fields and species/pedigree. (Adapted from Nascimento et al. [34]).

| Clone—Samples       | Phenotype       | Pedigree                                |
|---------------------|-----------------|-----------------------------------------|
| E1-1, E1-2, E1-3    | tolerant        | <i>E. urophylla</i>                     |
| E2-4, E2-5, E2-6    | tolerant        | <i>E. urophylla</i>                     |
| E3-7, E3-8, E3-9    | tolerant        | <i>E. grandis</i> × <i>E. urophylla</i> |
| E4-10, E4-11, E4-12 | semi-tolerant   | <i>E. grandis</i>                       |
| E5-13, E5-14, E5-15 | semi-tolerant   | <i>E. grandis</i> × <i>E. urophylla</i> |
| E6-16, E6-17, E6-18 | semi-tolerant   | <i>E. grandis</i> × <i>E. urophylla</i> |
| E7-19, E7-20, E7-21 | mod-susceptible | <i>E. grandis</i>                       |
| E8-22, E8-23, E8-24 | susceptible     | <i>E. urophylla</i>                     |
| E9-25, E9-26, E9-27 | susceptible     | <i>E. grandis</i>                       |

Six plants of each genotype (clone) were conducted in each plot. One living representative tree of commercial *E. grandis*, *E. urophylla* and *E. grandis* × *E. urophylla* hybrids clones in each plot was evaluated according EPD symptoms, felled and sampled for anatomical analysis. EPD symptoms and respective scores are found in Table 2. Dead trees were discarded for sampling. The EPD score and symptom description followed Almeida et al. [28].

**Table 2.** EPD scores and symptoms used for the evaluations of trees in the commercial areas and in the Eucalypt Physiological Disorder experiment, and corresponding evaluation of the tree samples according to the EPD scores. (Adapted from Almeida et al. [28]).

| EPD Score | Symptoms                                                                                                                                                                    | Samples                                                                                                 |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| Level 0   | asymptomatic plants                                                                                                                                                         | E1-1, E1-2, E1-3, E2-4, E2-5, E2-6, E3-7, E4-10, E4-11, E5-13, E5-14, E5-15, E6-16, E6-17, E6-18, E7-21 |
| Level 1   | depressed surface lesion, cracking and slight detachment of the bark (“scaling”), randomly distributed on the trunk or branches                                             | E3-8, E3-9, E4-12, E7-19, E7-20, E8-23, E8-24                                                           |
| Level 2   | drying of the basal third leaves of the crown, cracking of the bark and swelling at specific points on the stem or randomly distributed along the main stem or branches     | E8-22                                                                                                   |
| Level 3   | dieback, bifurcation of the main trunk, sprouting, formation of corky bark, release of bark (exophylactic periderm) and edema (callosity or rough appearance) on the leaves | E9-25, E9-26, E9-27                                                                                     |
| Level 4   | drying canopy and plant death                                                                                                                                               | - *                                                                                                     |

\* Dead plants were not collected/sampled.

2.2. Sample Processing

There was a total of 27 plots and 3 plots sampled for each clone. The EPD score range and symptom description [28] are shown in Table 2. Selected trees were evaluated according the EPD score (Table 2) and bark tissues were sampled.

The trees were felled (Figure 1A,B) and bark samples (Figure 1B) from three heights on the stem, DBH (Diameter at the Breast Height); 50%, and 75% of the commercial height, were collected, stored, and processed for anatomical and statistical analysis. The diameter of the stem at each position was recorded. These discs were cut into wedges and packed in 300 mL bottles with 70% ethanol (Distribuidora Jandaia, Jandaia do Sul, Brazil), with 10 times the sample volume. The samples were submitted to vacuum, and the solution was replaced for a new 70% ethanol solution, which was maintained until processing. Square to rectangular samples (4–8 mm side) containing bark, cambium and a small amount of xylem were obtained, dehydrated in an increasing graded ethyl series, and embedded in methacrylate (Historesin—Leica, Nussloch, Germany) according to manufacturer’s recommendations, with adaptations. The embedding period was extended to at least 8 weeks, and the historesin renewed 3 times. During this process, the samples were submitted to vacuum and kept at 8 °C until use.

Samples were cross-sectioned (10 µm) in a rotary microtome (RM2265—Leica). Two to four slides with four to six sections for each tree and stem height position sample were stained with toluidine blue (Fluka—Buchs, Switzerland) [55], and mounted with synthetic resin (Permount—Fisher, Fair Lawn, NJ, USA). A representative cross section was photographed and used for the statistical analysis of anatomical traits. A detailed descriptive anatomical analysis of *Eucalyptus* bark tissues was conducted and will be reported elsewhere. The measurements of anatomical traits were carried out on the cross-sections of at least 81 samples and 162 images (9 genotypes × 3 repetitions × 3 positions × 2 sets of

images from normal/polarized light) containing the outer bark up to the most recently formed xylem. Images were obtained using a photomicroscope (AX70 TRF, Olympus Optical, Tokyo, Japan) with the U-PHOTO system connected to a digital camera (AxionCam Carl Zeiss, Gena, Germany).

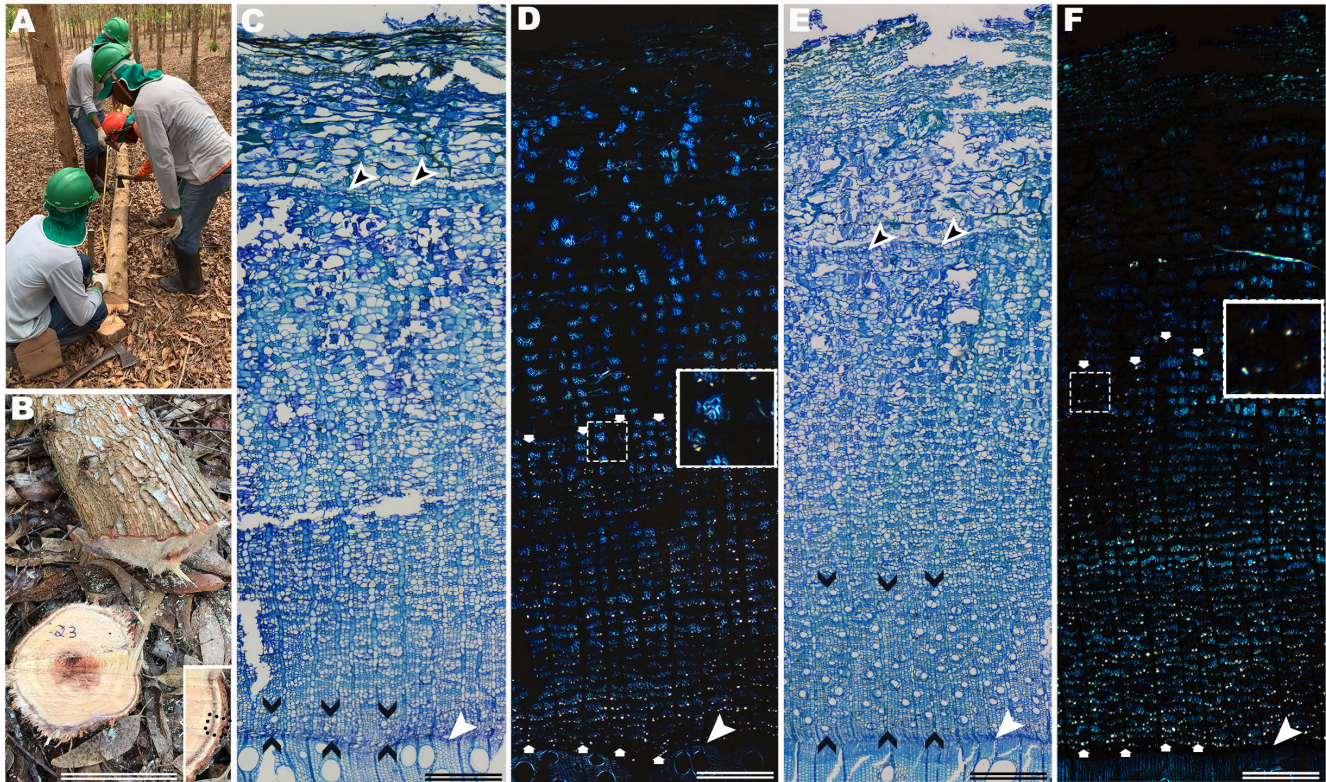


**Figure 1.** Suzano's *Eucalyptus* experimental field, data collection and sampling of stem discs. (A) Measurements for sampling stem; (B) detail of felled tree and region of bark sampled (*E. urophylla*, sample E8-23); (C) cross section of the bark of a susceptible clone showing the cambium and the conducting phloem region under normal light (*E. grandis*, sample E7-21); (D) cross section of the bark of a susceptible clone highlighting the cambium and the phloem region with CaOx crystals under polarized light. Hatched area was amplified to visualization of the CaOx crystals (*E. grandis*, sample E7-21); (E) cross section of the bark a tolerant clone, cambium and the conducting phloem region under normal light (*E. urophylla*, sample E2-6); (F) cross-section of the bark of a susceptible clone highlighting the cambium and the phloem region with CaOx crystals under polarized light. Hatched area was amplified to visualization of the CaOx crystals (*E. urophylla*, sample E2-6). Bars: B = 10 cm; C, D, E, and F = 400  $\mu$ m. White arrowheads evidence the cambium; black arrowheads evidence the limits of conducting phloem, and white small arrows the limits of the phloem with CaOx crystals; black/white arrowheads evidence the newly formed periderm; white arrowheads evidence the cambium; and black arrowheads evidence the limits of conducting phloem.

The cell structure, the presence of CaOx crystals, and the position of cambium and phellogen (Figures 1C–F and 2) were adopted as references for the identification of the histological regions in the *Eucalyptus* bark. The organization of the phloem tissue (Figure 2A,B), the preserved STE form, and the presence of CaOx crystals under polarized light for each previously photographed section (Figures 1D,F and 2E) were adopted for the identification the nonconducting, conducting, and total phloem thickness, and regions of the periderm or rhytidome.

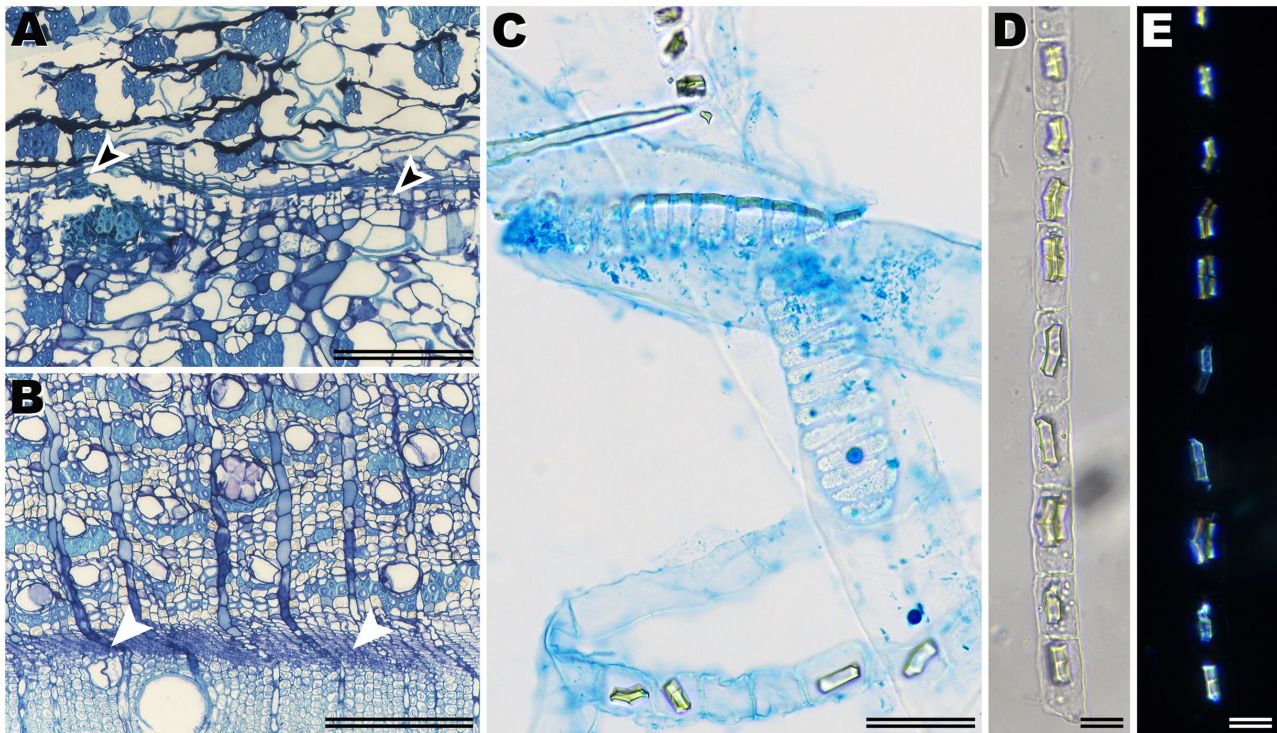


**Figure 2.** Bark and secondary phloem anatomy. (A) outer bark and newly formed periderm (*E. grandis* × *E. urophylla*, sample E6-16); (B) inner bark and phloem tissue near the cambium, note the preserved integrity of the sieve tube elements (*E. grandis* × *E. urophylla*, sample E6-16); (C) detail of sieve tube elements and the sieve plate (*E. grandis*, sample E7-19); (D) axial parenchyma with CaOx crystals under normal light (*E. grandis*, sample E7-19), and (E) the same axial parenchyma with CaOx crystals under polarized light (*E. grandis*, sample E7-19). Bars: A and B = 200 µm; C = 50 µm, and D and E = 20 µm. White arrowheads evidence the cambium; black/white arrowheads evidence the limits of conducting phloem/phellogen.

### 2.3. Analysis of Bark Variation and Data Collection

Image Pro-Plus® software (version 4.5, Media Cybernetics, Silver Spring, MD, USA) was used to measure the thickness of the different histological regions identified in the bark: the periderm (or rhytidome), phloem with CaOx crystals, and with collapsed and non-collapsed STE. These last two referring to the nonconducting and conducting phloem. The periderm or rhytidome region was defined by the position of the last phellogen formed in the outer bark; the nonconducting phloem defined by the region presented collapsed STE; the conducting phloem defined by the region outside the cambium and that presented non-collapsed STE observed under the light microscopy; and the phloem with crystals the region outside the cambium that presented CaOx crystals observed under polarized light. Thickness estimates were obtained based on 3 measures for each image combination of variable and stem height. The thickness of the phloem tissue (nonconducting and conducting) was measured based on the limits of the cambium and phellogen as references.

The diameter of the sieve tube elements was established by the average of two perpendicular measurements of the diameter of each STE. Fifty STE were sampled in each section. The identification and selection of the STE was based on cell size, and presence of sieve plate. Characteristics such as proximity to the cambium, and presence of turgid companion cells [56] and presence of plastids were also used as reference. The number of non-collapsed STE per area was obtained by counting the elements observed in cross sections of the bark. The area of non-collapsed STE was delimited and, subsequently, the frequency of STE and number of STE in a stem cross section were estimated.

The number of STE, area and thickness of the phloem regions per stem cross sections were calculated based on the stem diameter (bark and xylem) and the differences in the respective areas at each commercial height sampled. Conducting phloem was defined based on the non-collapsed STE, while the total phloem length was defined based on presence of living cells with CaOx inclusions, and phloem cells that redifferentiated into the last formed phellogen layer. The most recently formed phellogen was adopted as a reference for the outer border of the phloem.

The stem diameter (SD), in cm, and the following anatomical variables were evaluated in *Eucalyptus* bark and secondary phloem: bark total thickness (Btt), periderm or rhytidome thickness (P/Rh), total phloem tissue thickness (TPh), conducting phloem thickness (CPh), thickness of phloem with crystals (PIC), thickness of nonconducting phloem (NCoP), average thickness of conducting and phloem with crystals (CPPC/2); average diameter of sieve tube elements (SED); in  $\mu\text{m}$ , estimated fraction of conducting phloem in stem cross section (CPA), and estimated area of total phloem tissue in stem cross section (TPCA); in  $\text{cm}^2$ , number of parenchyma rays per 100  $\mu\text{m}$  (NR\_C); number of rays 100  $\mu\text{m}^{-1}$ , ratio of phloem with crystals thickness per total phloem thickness (PIC/PI); ratio of conducting phloem per total phloem thickness (CPh/PI); dimensionless, estimated number of sieve tube element (in the conducting phloem) per stem cross section (SESCS);  $\text{SE} \times 10^5$ , frequency of sieve tube element per  $\text{mm}^2$  (FSmm);  $\text{STE mm}^{-2}$ .

#### 2.4. Statistical Analysis

Histological data, stem diameter and EPD scores were compiled and a descriptive analysis of the data was performed to identify outliers and examine the distribution of the variables, allowing the detection of possible inconsistencies. In the following, a multicollinearity analysis was performed, the objective of which was to verify the variables with high correlation, identifying redundancies that could compromise the accuracy of the statistical models. The selection of attributes was adjusted, eliminating or transforming those that showed excessive interdependencies and thus ensuring the robustness and validity of the subsequent modeling.

The data sheet was subjected to tests for variance homogeneity, normal distribution, and subsequently to analysis of variance (ANOVA), based on the following mixed model:

$$y = Xb + Zg + Us + Vp + Wc + e$$

where ( $y$ ) represents the vector of observed data for the trait; ( $b$ ) is the vector of fixed repetition effects; ( $g$ ) denotes the random vector of genetic effects; ( $s$ ) captures the random effect of the species or pedigree; ( $p$ ) corresponds to the random vector of the effects of the assumed phenotype; ( $c$ ) is the random vector of the cutting height effect; and ( $e$ ) represents the vector of residuals. The matrices ( $X$ ), ( $Z$ ), ( $U$ ), ( $V$ ) and ( $W$ ) are responsible for mapping, respectively, each observation to the fixed, genetic, species (or pedigree), assumed phenotype and cutting height effects, allowing a detailed analysis of the different sources of variation present in the model. A diagonal structure for the residual variance matrix was assumed so that each height could have a specific residual variance. BLUPs for each genotype within each commercial height were obtained. Variances for each random effect were obtained by restricted maximum likelihood (REML).

The averages of the reported EPD phenotypes (scores associated with the tolerance and susceptibility) were used as reference and compared using the  $t$  test ( $p \leq 0.05$ ) in the R software (Version 3.6.1) [57].

## 2.5. Correlation Analysis

A correlation analysis was performed using two complementary methods: for continuous traits, the Pearson coefficient was applied, while the Spearman coefficient was used for variables represented by integers. The correlation coefficients of all variables present in the data were evaluated; for this, the “corr\_plot” function of the “metan” package in the R software was used. To verify the significance of each correlation, the *t* test was applied [57,58].

An auxiliary technique to visualize the associations between traits that led to the establishment of the common factors was the analysis and establishment of the correlation network [59] constructed from correlation matrix, which made it possible to visualize, explicitly, the pattern of relationship between the determinant variables in the explanation of the common factors used as selection criteria. The positive correlations between the variables were represented by green lines and the negative ones by red lines. The fine lines represent lower correlations, with no highlighting distinguishing them.

## 2.6. Kohonen Self-Organizing Maps (SOM)

Kohonen Self-Organizing Map (SOM) grouping and distribution traits cluster analyses were performed based on the same dataset. The SOM, unsupervised learning neural network method that detects similarities between input patterns through a competition process [60,61], grouping and distribution traits cluster analyses were performed. The value of each individual evaluated for the nine traits for each alleged phenotype was used as input. The number of neurons equal to 25 neurons, the standardized average Euclidean distance was used and for the iterative process, the number of 500,000 iterations was stipulated.

# 3. Results

## 3.1. Descriptive Statistics Preliminary Analysis

The average bark thickness of the commercial *Eucalyptus* clones ranged from 3.06 to 4.63 mm from 75% of commercial height to DBH in 5-year-old trees. Bark thickness is slightly thinner in susceptible (4.17 mm) and thicker in EPD-tolerant clones (5.72 mm) (Supplementary Table S1).

STE could be easily identified in the conducting phloem region (Figure 1C,E). The nonconducting to conducting phloem border was identified using the neighboring STEs; position near the cambium; wall thickness, cell size and round or round faceted shaped and, ultimately; presence of the sieve plate (Figure 2B,C). The sieve plate was always inserted into the periclinal cell wall and slight tilted about 30° (Figure 2C) to the right or left. The presence of CaOx crystals was easily identified under polarized light (Figures 1D,F and 2E) as well as cell collapse in the outer phloem cells (Figure 2A). The presence of the sieve elements was adopted as reference to identify the area of conducting phloem.

The total phloem length was measured as the distance between the cambium and the phellogen (Figures 1C,E and 2A,B). The cambium and the last phellogen formed were easily noticed. The non-collapsed STE region exhibited CaOx crystals, which were observed in the inner regions of nonconducting phloem, although they were faded or absent in the outer regions of nonconducting phloem (Figure 1D,F).

In the multicollinearity analysis, it was identified that some variables presented VIF (Variance Inflation Factor) (Supplementary Table S1) greater than 10, thus excluded from the subsequent analysis. These were the thickness of nonconducting phloem, total bark thickness, total phloem area in cross section, average thickness of conducting and phloem with crystals, conducting phloem/total phloem ratio, and phloem with crystal/total phloem ratio.

Descriptive statistical analysis, average and standard deviation, was performed considering the different stem heights (DBH, 50%, and 75%) for all sampled trees, according to their expected EPD phenotype (historical data), and tolerant samples with a score of “0” and susceptible clone samples with scores from “1” to “3” (Supplementary Table S1).

When tolerant clones with score “0” were compared to susceptible clones with scores “1” to “3”, all traits were higher in numerical value for tolerant clones at DBH, except for number of rays. The frequency of STE behaved oppositely to all traits, as it increased in susceptible samples at all heights. Total and conducting phloem thickness, average diameter of STE, CPh/Pl ratio, CPPC/2, estimated areas of conducting and total phloem, and the estimated number of STE per stem cross section increased from susceptible to the tolerant clone samples among the stem heights evaluated (Supplementary Table S1).

These results outline the importance of the phloem as it is increased in tolerant and top-to-bottom stems. Except for the number of rays, the STE differentiation and structure are involved with tree growth as well as EPD tolerance.

There seems to be a trade-off between STE frequency and other phloem traits, as there is an increase in STE number from base to the top of the tree and from tolerant to susceptible clones, while there is an increase in conducting phloem area at all tree heights from susceptible to tolerant clones, and from top to bottom of the trees. Similarly, although there is no trend for the STE diameter in relation to tree heights, there is a slight increase of SED across tree heights, from susceptible to tolerant clones.

Regardless of sampling, EPD phenotype or plants exhibiting or not EPD symptoms, the presence of one or more periderm (rhytidome) implied in the P/Rh variation. Despite this, P/Rh exhibited increasing thickness from the top to the base of the trees, and at DBH of susceptible to tolerant clones. All the EPD phenotypes (tolerant, semi-tolerant, moderately susceptible and susceptible) and sample score (SP) evaluations were assigned by Suzano team according to the behavior of the clones in the field, and were maintained in the descriptive analysis. The moderately susceptible was removed from the following statistical analysis. Statistical mean tests followed to access significant differences and confirm the trends among the evaluated traits.

### 3.2. Analysis of Variance and Comparison Between Means

The interaction effect between the attributed phenotype and sampling height was significant, so was the comparison of averages between the phenotypes for each height (Supplementary Table S2). Despite the specie effect being significant, this was not the primary goal of the experiment, and it would not be possible to conduct the analysis of the interaction between species and EPD tolerance, as our initial experimental design did not foresee this effect. There were not enough observations to estimate all combinations in a model considering the species effect and its interactions, since it would mean to estimate more parameters than the information available in the sample.

The variation of the anatomical traits of the barks were evaluated along eucalypt trees (DBH, 50% and 75% commercial height), associated with EPD scores, and with stem diameter as a reference to tree growth (Figures 3–6). Stem diameter, number of STE in the conducting phloem, and thickness of the conducting phloem (Figure 3) and stem diameter, thickness of the phloem with crystals, thickness of the phloem tissue, and frequency of STE  $\text{mm}^{-2}$  (Figure 5), showed at least one significant difference when EPD and tree height were considered, respectively. The average diameter of the STE, total phloem, phloem with crystal and periderm thickness, frequency of STE and number of rays (Figure 4) were similar among EPD phenotypes and tree height, respectively.

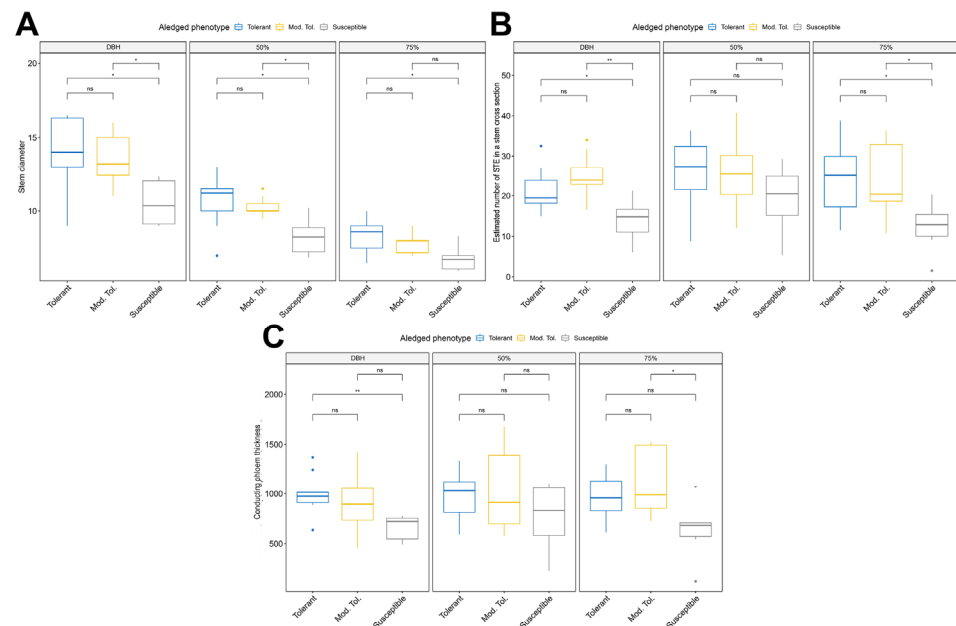


**Figure 3.** Boxplots showing the effect of EPD phenotype on (A) stem diameter (SD), cm; (B) Estimated number of STE in a stem cross section; and (C) conducting phloem thickness,  $\mu\text{m}$ . \* Indicates statistically significant differences according to  $t$  test at  $p \leq 0.05$ ; ns, non-significant differences; and \*\* Indicates statistically significant differences according to  $t$  test at  $p \leq 0.01$ .

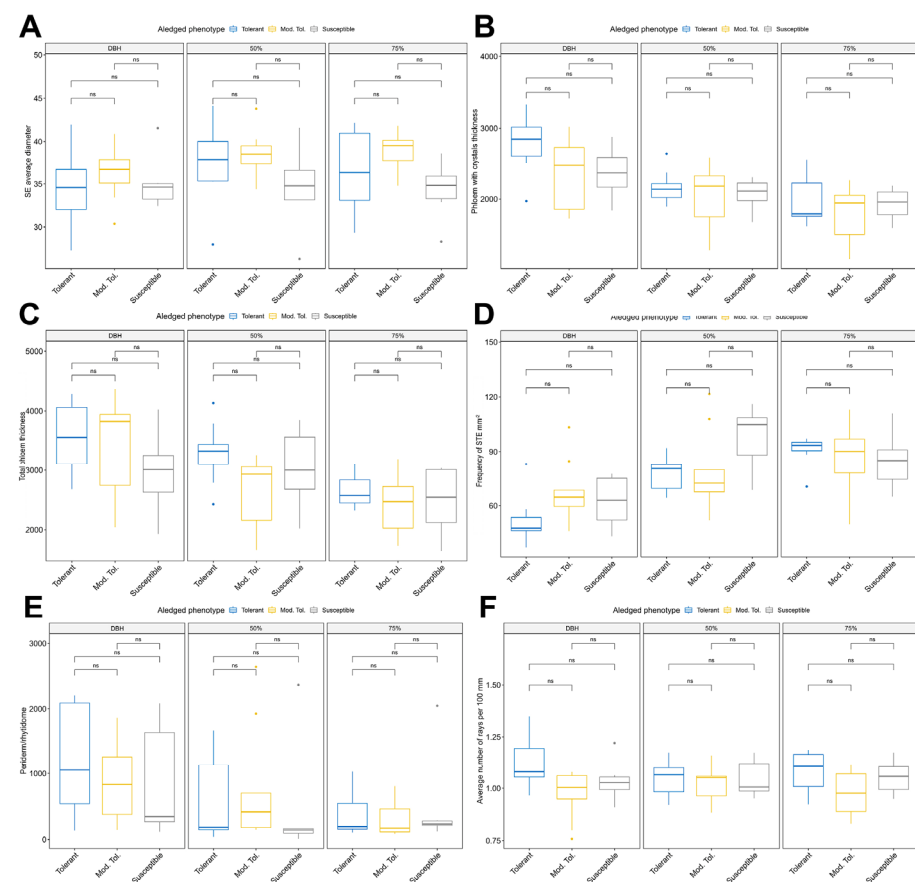


**Figure 4.** Boxplots showing the effect of EPD phenotype on (A) Sieve tube element average diameter (SED),  $\mu\text{m}$ ; (B) phloem with crystals thickness (PIC),  $\mu\text{m}$ ; (C) total phloem thickness (TPh),  $\mu\text{m}$ ; (D) frequency of sieve tube elements  $\text{mm}^{-2}$ ; number of STE  $\text{mm}^{-2}$ ; (E) periderm/rhytidome thickness,  $\mu\text{m}$ ; and (F), average number of rays per 100 mm (NR\_C). ns, non-significant differences.

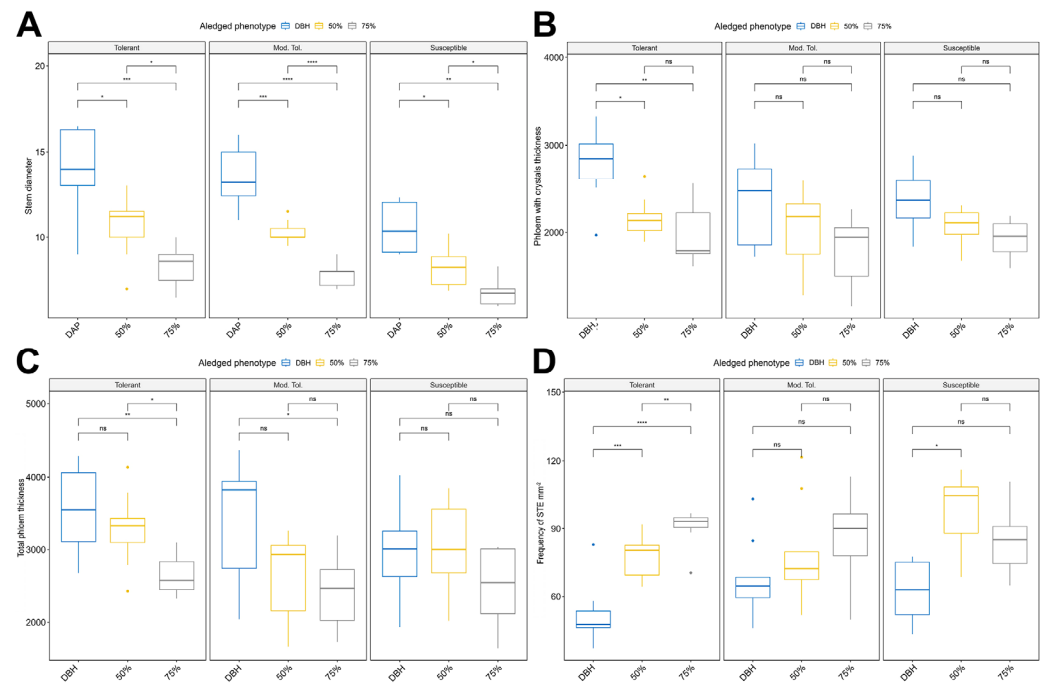


**Figure 5.** Boxplots showing the effect of tree height on (A) stem diameter (SD), cm; (B) phloem with crystals thickness (PIC),  $\mu\text{m}$ ; (C) total phloem thickness (TPh),  $\mu\text{m}$ ; and (D) frequency of sieve tube elements  $\text{mm}^{-2}$ . \* Indicates statistically significant differences according to  $t$  test at  $p \leq 0.05$ ; \*\* Indicates statistically significant differences according to  $t$  test at  $p \leq 0.01$ ; ns, non-significant differences, and \*\*\* Indicates statistically significant differences according to  $t$  test at  $p \leq 0.001$ ; \*\*\*\* Indicates statistically significant differences according to  $t$  test at  $p \leq 0.0001$ ; ns, non-significant differences.

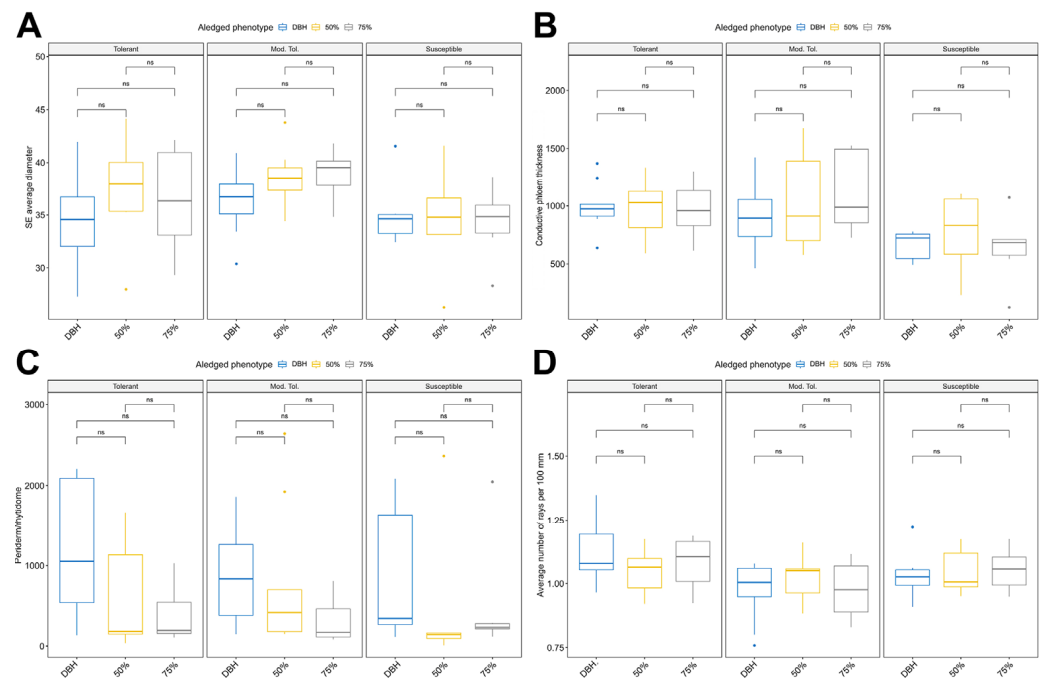


**Figure 6.** Boxplots showing the effect of tree height on (A) sieve tube element average diameter (SED),  $\mu\text{m}$ ; (B) conducting phloem thickness (CPH),  $\mu\text{m}$ ; (C) periderm/rhytidome thickness,  $\mu\text{m}$ ; and (D) average number of rays per 100 mm. ns, non-significant differences.

Historical information on the EPD occurrence (scores) of the 9 commercial eucalypt clones in commercial fields (Table 1) was analyzed concomitantly with tree sampling according to EPD symptoms in the sampled trees in the plots (Table 2). There are outliers that are attributed to the small number of samples, 27 trees and 3 heights evaluated, or that may be connected with the oscillation of the EPD occurrence or intensity in the sampled clones. The traits of bark and secondary phloem accommodated a variation linked to tree height and the EPD phenotype (Figures 3 and 4). Stem growth based on stem diameter and phloem traits (SESCS and CPH) are significantly associated with EPD-tolerant genotypes, and tree height may be linked with the thickness of phloem with crystals and total phloem, and frequency of STE, in addition to the expected increase in stem diameter.

The DBH position was more informative, since stem diameter and phloem traits showed statistical differences in the samples of this stem height. There are variables that exhibited significant differences and decreased from DBH to 75% of the commercial height, such as stem diameter (Figure 5A), thickness of phloem with crystals (Figure 5B), and total phloem thickness (Figure 5C). On the other hand, the frequency of STE increased from DBH to 75% of the commercial height (Figure 5D). Other variables (Figure 6) did not show such trends.

### 3.3. Correlation Analysis

Pearson correlation was adopted for continuous traits, and Spearman correlation for discrete traits. There are weak to strong correlations of variables which did not follow normal distribution and homogeneity of variance. There is a strong, significant and positive relationship of stem diameter and total bark thickness, phloem area presenting CaOx crystals and conducting phloem. The correlation analysis allowed to align the phenotypes with bark and phloem anatomical features and EPD scores, as the conducting phloem area was correlated with the average of historical EPD scores ( $-0.41$ ) and the score of sampled plants ( $-0.56$ ).

The correlation analysis was conducted based on traits with significant differences among EPD phenotypes or plant height (Figure 7), and which presented homogeneity of variance and normal distribution. There are significant (0.1 to 5.0%), and weak to strong correlations among the variables, from which positive and negative correlations ranged from  $-0.62$  to  $0.81$ . The expected phenotypes (PHD) were maintained as a reference for the correlation analysis, as well as the EPD score attributed to the sampled trees in the experiment (SP).

There is a strong ( $0.81$ ) and significant ( $0.1\%$ ) association between PHD and SP, and other associations with bark and phloem anatomical traits. Most of these correlations expressed anatomical and histological features of bark linked with stem diameter growth and EPD phenotype. The thickness of the periderm, conducting phloem, and phloem with crystal thickness are correlated with stem diameter, as are variables associated with secondary growth. Further, the frequency of STE and the estimated number of STE in a stem cross section, phloem structural features connected to secondary growth, are also correlated with stem diameter, and all being traits that are derived from the bark. There is a cohort increase in stem diameter, periderm, total; phloem with CaOx crystals, average diameter of STE, and the estimated number of STE per stem cross section. There is also a reduced frequency of STE per area with increasing bark thickness (Figure 7) and negative correlations of SD with historical EPD records (PHD) and sample EPD scores (SP),  $-0.36$  and  $-0.44$  respectively.

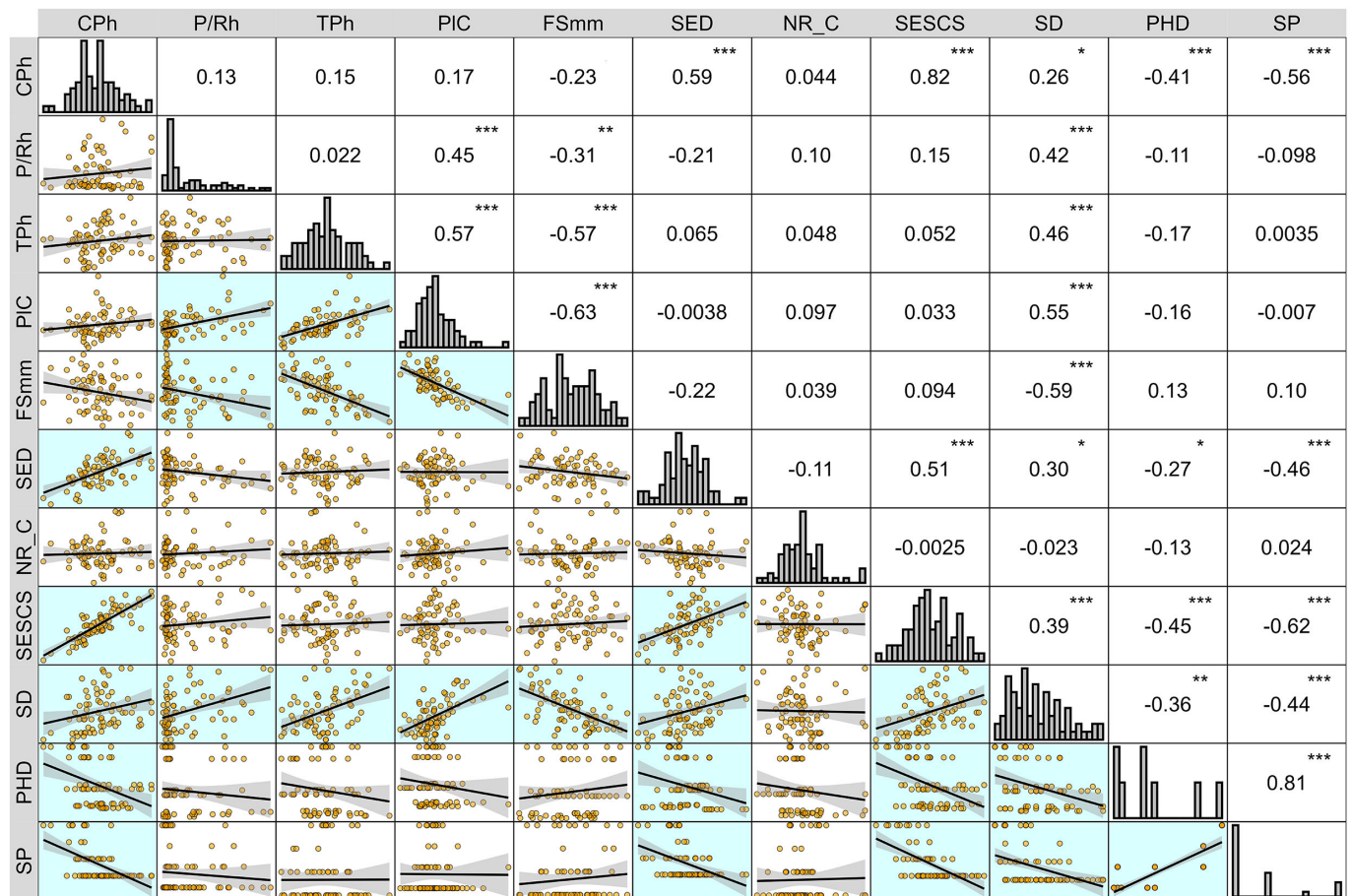


**Figure 7.** Correlation analysis table of anatomical traits of the bark of commercial *Eucalyptus* clones with different EPD phenotypes (scores). Upper-right rectangles display the correlation and significance values, the lower-left rectangles display the graphical dispersion of the variables used in the correlation. CPh: Conducting phloem, thickness ( $\mu\text{m}$ ); P/Rh: Periderm or rhytidome, thickness ( $\mu\text{m}$ ); TPh: Total phloem, thickness ( $\mu\text{m}$ ); PIC: Phloem presenting CaOx crystals, thickness ( $\mu\text{m}$ ); FSmm: frequency of sieve tube elements per square mm (STE  $\text{mm}^{-2}$ ); SED: average diameter of sieve tube elements, diameter ( $\mu\text{m}$ ); NR\_C: average number of rays found per 100  $\mu\text{m}$  cambium; SESCOs: estimated number of STE per stem cross section, area (STE  $\text{mm}^{-2}$ ); SD: measured stem diameter, diameter (cm); PHD: average score of the evaluated genotypes, empirical data from commercial fields; SP: EPD score attributed to the sampled plants. Smaller numbers and no tags, not significant, \* significant at 5%; \*\* significant at 1% and, \*\*\* significant at 0.1% probability by *t* test. The larger the correlation the bigger the numbers are presented.

The positive association of total phloem and phloem with crystal thickness in the stems supports the idea of a balanced process, since it is positively correlated with stem diameter. Similar reasoning is applied to the thickness of phloem presenting CaOx crystals that augments with the increase of stem diameter. On the opposite hand, it reduces with the increase of the number of STE per area. There are positive correlations of the average diameter with the number of STE per stem cross section which also has consequences to STE differentiation. This supports the existence of a trade-off between phloem traits linked to STE differentiation. On the same hand, the average diameter ( $-0.46$ ) and number the of the STE per stem cross section ( $-0.62$ ) are negatively correlated with SP, supporting that the STEs differentiation process is inversely associated with the disorder. A concomitant increase of STE average diameter with the estimated number of STE in a stem cross section is described, since there are positive and significant correlations among them (0.51). Phloem is also associated with the stem diameter growth (0.46).

Positive correlations of conducting phloem thickness with the number of STE per stem cross section (0.82) and average diameter of the STEs (0.59) are observed (Figure 7) and related to phloem tissue and sap transportation. The negative correlation of thickness of the conducting phloem with the sample (SP) ( $-0.56$ ) and historical score evaluation (PHD) ( $-0.41$ ) expresses the importance of the preserved structure of secondary phloem tissue for EPD tolerance. Despite the inherent variability of periderm and rhytidome thickness, it was positively correlated (0.45) with the phloem with crystals (Figure 7). This suggests a connection of the amount of CaOx crystals and the formation of new phellogen layers. A negative correlation with the frequency of STE and its average diameter ( $-0.22$ ) describes phloem structure playing a role in healthier trees or EPD tolerant clones. This is reinforced because phloem tissue and phloem with crystal thickness exhibited a significant positive correlation (0.56). The negative associations between the frequency of STE and total phloem thickness; FSmm and phloem with crystal thickness, and FSmm and STE diameter are clues to trade-offs between sieve tube elements features in live phloem tissue. The importance of phloem is emphasized as the number of STE per stem cross section increases with mean STE diameter.

PHD is derived from the information of average EPD intensity under field conditions provided by the forestry company, while SP is the EPD score of the sampled trees for each clone evaluated. The 81% positive correlation between these variables indicate the congruence of historical data from commercial eucalypt plantation fields and the phenotype evaluated in the collected samples. Variables such as the conductive phloem thickness, diameter and number of STE per area are negatively associated with the EPD scores. This means that a larger number of differentiated STE cells comes along with the reduction of EPD incidence or vice versa.

The importance of bark and secondary phloem tissues in plant growth is revealed by the positive correlations of stem diameter (SD) with periderm (P/Rh), total phloem thickness (TPh), phloem with CaOx crystals thickness (PIC), frequency of STE (FSmm), diameter of STE (SED), and the estimated number of STE in a stem cross section (SESCS) (Figure 7). There is a reduction in the frequency of STE per area with increasing stem diameter, supporting a trade-off relationship. These traits have an impact on the assessment of plant growth and eucalypt production, as are strongly correlated with stem diameter.

The correlation network of bark/secondary phloem traits, stem diameter and EPD score (Figure 8) is complex, although it can be represented with phloem traits, physiological disorder and associations with the increased stem diameter (Figure 8). This corollary reflects an intrinsic relationship of phloem tissue with *Eucalyptus* growth that may benefit the selection process and the understanding of EPD occurrence and intensity.

The strongest correlations (Figure 8) highlight interesting features. On the other hand, the correlation between CPh and SESCO (0.82) implies an increased amount of STE associated with a larger conducting phloem.

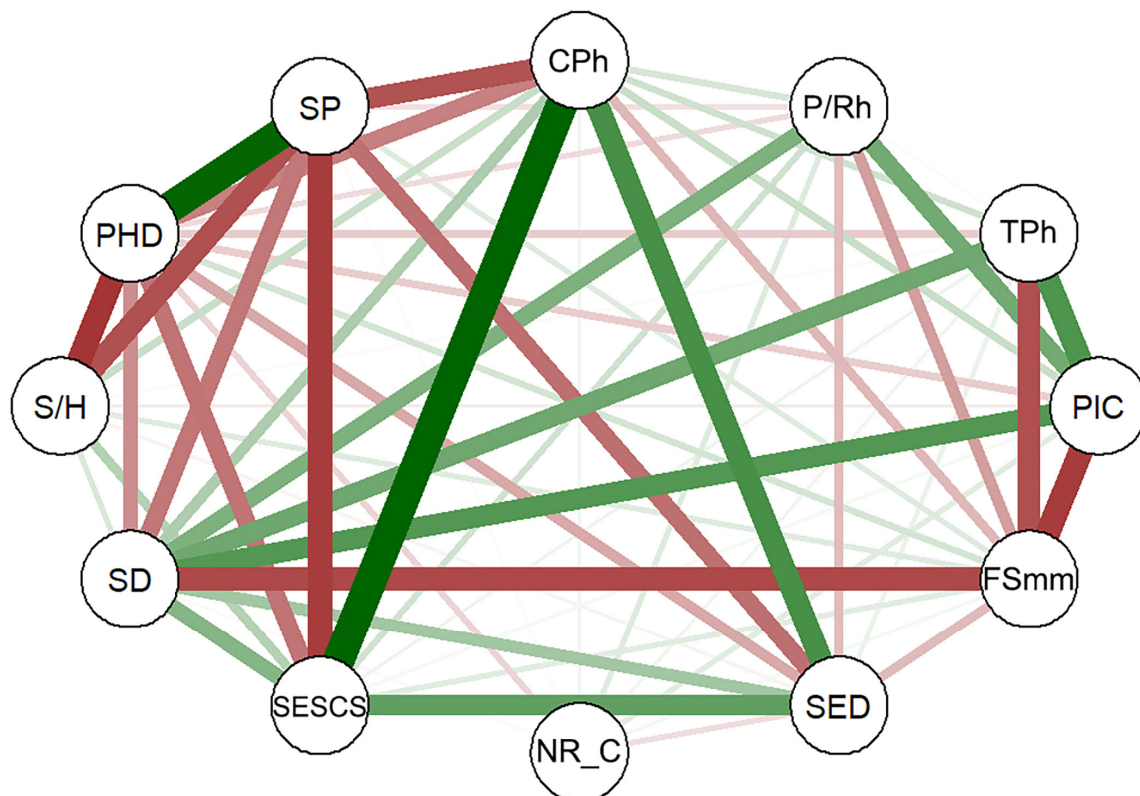


**Figure 8.** Correlation network of the main anatomical traits of the bark of commercial *Eucalyptus* clones with divergent EPD phenotypes (scores). CPh: Conducting phloem, thickness; P/Rh: Periderm or rhytidome, thickness ( $\mu\text{m}$ ); TPh: Phloem, thickness ( $\mu\text{m}$ ); PIC: Phloem presenting CaOx crystals, thickness ( $\mu\text{m}$ ); FSmm: frequency of sieve tube elements per square mm (STE  $\text{mm}^{-2}$ ); SED: average diameter of sieve tube elements, diameter ( $\mu\text{m}$ ); NR\_C: average number of rays found per 100  $\mu\text{m}$  cambium; SESCO: estimated number of STE per stem cross section, area (STE  $\text{mm}^{-2}$ ); SD: measured stem diameter, diameter (cm); PHD: average score of the evaluated genotypes, empirical data from commercial fields; S/H origin or pedigree of the clone, pure specie or a hybrid; SP: EPD score attributed to the sampled plants. The higher the correlation, the wider the lines connecting the traits. Red lines represent negative correlations and green lines represent positive correlations.

### 3.4. Pattern Recognition (SOM)

Consistent clusters, or neurons, were formed based on *Eucalyptus* bark traits and clones were classified according to their expected historical EPD phenotype and scores assigned to the sampled trees. Self-Organizing Map (SOM) clustering analysis exhibited clone samples of the same EPD phenotype being grouped into independent neurons (Figure 9A). This clustering highlights the similarity of tolerant, medium tolerant, and susceptible clones, respectively, gathered into clusters of isolated neurons (Figure 9B). This result supports the use of phloem anatomical traits to identify contrasting EPD eucalypt phenotypes (Figure 9). The clusters allowed the identification and visualization of clones sharing similar EPD responses in a two-dimensional map. This is a user-friendly graphical display of the data. The blank neurons separating the groups and the clones being correctly divided according to their expected phenotype (Figure 9), reproduce the confidence of the clone tolerance/susceptibility inference based on the anatomical variables.

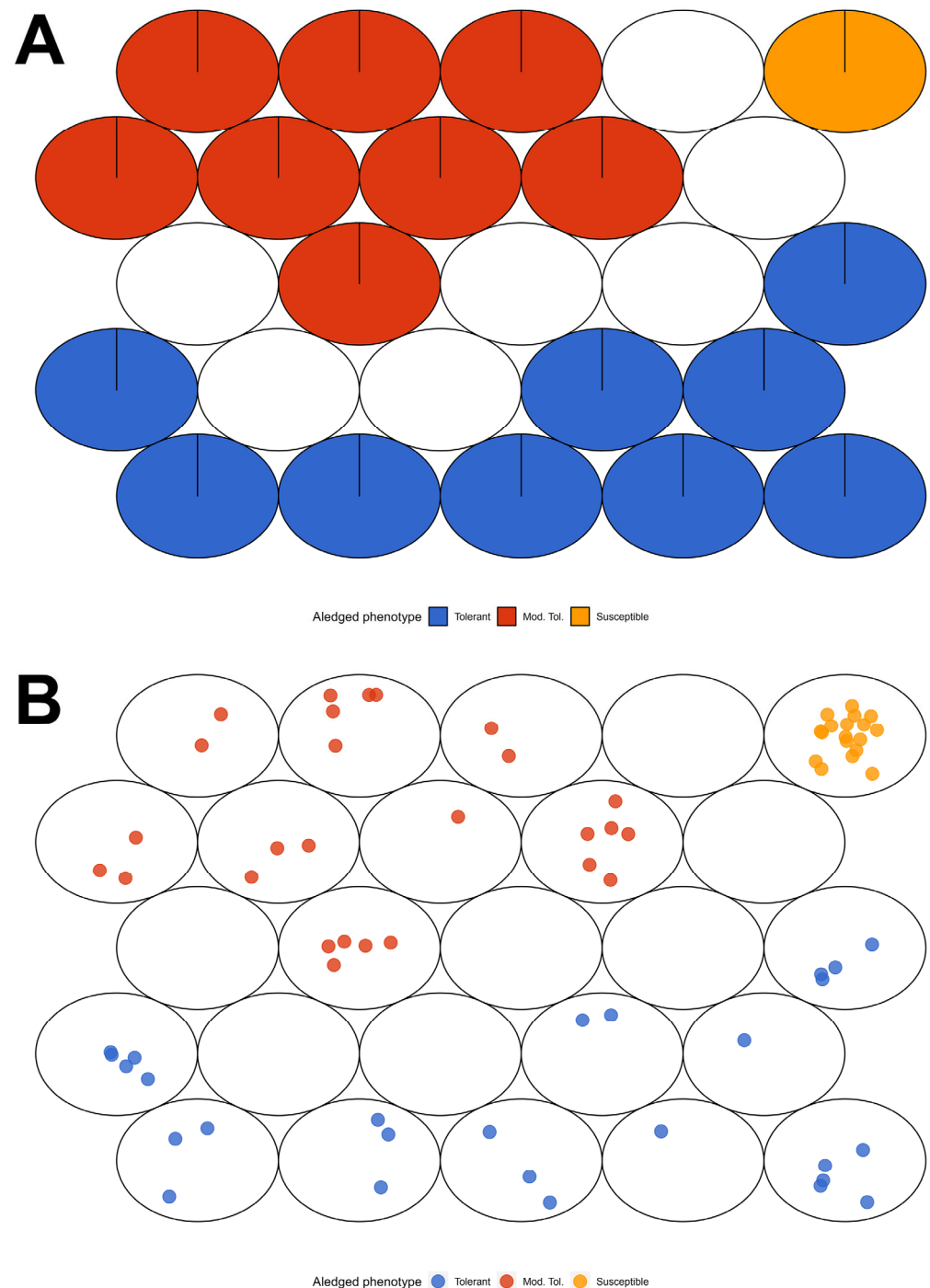


**Figure 9.** Five-by-five Kohonen self-organizing map. (A) Clustering of neurons according to expected phenotypes, and (B) SOM revealing the phenotypic similarity of the *Eucalyptus* plants based on anatomical traits of bark and phloem. Colors are associated with expected phenotypes: blue, neurons composed of tolerant samples; red, neurons composed of semi-tolerant samples; and orange, susceptible samples.

The variables that contributed most to the formation of each neuron were based on phloem and STE characteristics, such as conducting phloem thickness, phloem tissue thickness, thickness of phloem with CaOx crystals, frequency of sieve tube elements per  $\text{mm}^{-2}$ , average diameter of sieve tube elements, average number of rays per 100  $\mu\text{m}$ , estimated number of STE per stem cross section. The periderm or rhytidome thickness and stem diameter contributed significantly to the distribution of the samples in the neurons (Figure 10), despite the inherent variability of this trait (Supplementary Table S1). NR\_C

contributed to the neuron definition, despite no statistically significant difference among the EPD phenotypes (Figure 4F) and no significant correlation between the tested traits (Figure 7).

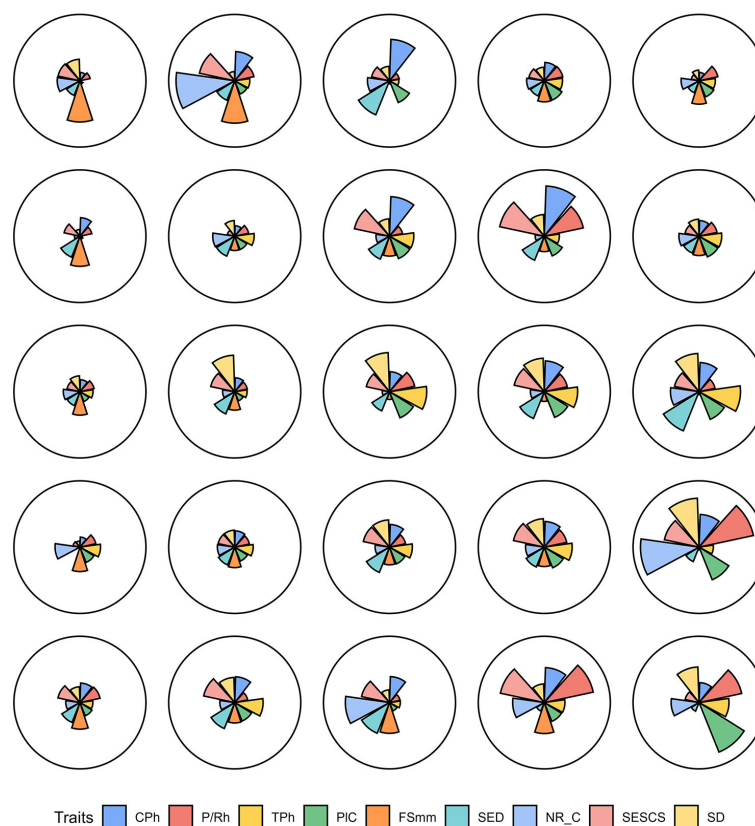


**Figure 10.** Kohonen distribution trait cluster and relative contribution of each trait to the neuron formation. CPh: Conducting phloem, thickness ( $\mu\text{m}$ ); P/Rh: Periderm or rhytidome, thickness ( $\mu\text{m}$ ); TPh: Phloem tissue, thickness ( $\mu\text{m}$ ); PIC: Phloem presenting Ca oxalate crystals, thickness ( $\mu\text{m}$ ); FSmm: frequency of sieve tube elements per square mm ( $\text{STE mm}^{-2}$ ); SED: average diameter of sieve tube elements, diameter ( $\mu\text{m}$ ); NR\_C: average number of rays found per 100  $\mu\text{m}$  cambium; SESCO: estimated number of STE per stem cross section, area ( $\text{STE mm}^{-2}$ ); SD: measured stem diameter, diameter (cm).

Samples of susceptible clones could be assembled into a single neuron, but the individual contribution of each variable was small (Figure 10). SD, TPh, PIC, CPh, SESCO, FSmm and P/Rh were central features to group the semi-susceptible clones. Interestingly, NR\_C contributed to a neuron harboring samples of the tolerant and semi-susceptible clones. All traits impacted the assembly of neurons of the tolerant samples, although PIC, P/Rh, SD, SED, SESCO, CPh exhibited a greater contribution in this assortment (Figure 10).

#### 4. Discussion

The bark thickness observed for 10-year-old *E. urophyla* [54] corresponded to 110% to 140% of the bark thickness observed for the 5-year-old *E. grandis*, *E. urophyla* and their hybrids in our report. There was a wide variation of bark thickness from *E. macrocarpa* (6–23 mm), *E. leucoxylon* (3–25 mm), and *E. tricarpa* (8–36 mm) in native forests [62]. Commercial clones exhibited bark thickness in the BHD (2.2–6.7 mm) close to the lower limit of the *Eucalyptus* plants in a natural environment. This thinner bark in fast-growing clones may represent a structural vulnerability. Physiologically, a thinner bark offers less thermal insulation, mechanical protection and conductive phloem, making the transport tissue more susceptible to damage from abiotic stresses which are known EPD triggers. The lack

of information on the age of the sampled trees prevented more in-depth comparisons. The average proportion of the conducting phloem was 25% at DBH for the 9 clones evaluated, slightly less from the 36.4% observed for *E. urophylla* [54]. Age is known as having influence on bark features and growth [63], as well as environment [64–66] and genotype [21,65,67], can be sources of variation.

Regardless of tree age and number of samples, conducting phloem thickness is a promising trait to be used for the selection of EPD-tolerant clones. Despite the restricted number of commercial *Eucalyptus* clones evaluated in the present report, the link of bark characteristics to EPD tolerance is highlighted. Our central hypothesis is that a greater CPh represents a structural strategy that confers physiological resilience. A thicker conducting phloem may provide a greater carbon transport capacity, ensuring that the supply of photosynthates to sinks (such as roots and shoot apices) is maintained even under stress conditions that could compromise part of the conductive tissue. Other species and a greater number of accesses should be tested to validate the hypothesis of a structural strategy supporting EPD tolerance and other abiotic stress-related tolerance trait. Picoli et al. [20], observed that most reports approaching water deficit resistance in *Eucalyptus* were restricted to 1 to 3 genotypes besides highlighting the scarcity of applied and functional approaches on phloem and bark anatomy.

The general organization of *Eucalyptus* bark was similar among the clones tested, an outer bark consisting of the periderm or rhytidome, and an inner bark, involving nonconducting phloem without CaOx crystals, a phloem region with collapsed STE and crystals, and an inner phloem region with non-collapsed STE and CaOx crystals. Notably, the conductive phloem was increased in the tolerant clones tested. Similar observation applies to phloem with CaOx crystals, which suggests this compound active metabolic and possible defense role, rather than being merely a passive byproduct in the tolerant clones.

Stem diameter and anatomical traits such as conducting phloem and number of STE per stem cross section area may be associated with plant environmental responses related to EPD. In this context, we propose that tolerant clones maintain a phloem architecture (e.g., greater CPh, more STEs) that is structurally more robust or has a greater capacity for seasonal adjustment. This would make them physiologically less susceptible to disruptions in carbon transport during stress periods, an ecological advantage in clonal plantations. Gričar and Prislan [66] observed that seasonal changes in the proportion and structure of the conducting tissue had a significant impact in the phloem sap transport and storage function of *Picea abies*, *Fagus sylvatica*, and *Quercus petraea*. Hence, the value of these traits as an indicator of the morphological and physiological strategies of tree performance in different environments depends on uniform sampling protocol and methodology.

Attention is drawn to phloem variables such as thickness of bark, total, conducting phloem and phloem with crystals thickness, and relationships between these traits. The central discovery of this study is the structural difference in the transport system between tolerant and susceptible clones. The correlation analysis sets ground for a tolerance mechanism based on a superior capacity for photosynthate transport. A more robust phloem architecture (greater CPh and SESCO) can mitigate stress effects (e.g., increased sap viscosity), preventing the ‘carbon starvation’ of sink tissues, what is hypothesized as one of the causes of the physiological disorder. Beyond the correlation analysis, there were significant differences among the tolerant and susceptible EPD clones for stem diameter, number of STE in a stem cross section, and conducting phloem thickness across the different tree heights evaluated that support this hypothesis.

On the other hand, there were differences across the tree height for the stem diameter for the different EPD clone phenotypes, for the thickness of phloem with crystals for tolerant clones, total phloem tissue for tolerant and intermediate tolerant clones; and for the

frequency of STE for the tolerant and susceptible clones. Ramalho et al. [65] reported that a decrease in bark thickness in base-top direction that was associated with tree spacing. This evidence that management will also influence the amount of bark, although there is not information on the amount of phloem tissue. Despite the same spacing was used for the 9 clones evaluated, in a commercial plantation spacing may contribute to an environment that account for differences in EPD occurrence.

The adaptative importance of the bark is highlighted in addition to a linear association of bark thickness with stem diameter [68] and a reduction of the biomass of the bark from base to top direction [69]. This relation varied with the plant functional group [68], with the plant height [69], and between EPD tolerant and susceptible clones [28]. There was an increase in inner bark area with the increase of temperature and decrease in soil fertility whereas a decrease in precipitation would result in an increase of the secondary phloem area from different species in environments ranging from 213 to 2969 mm in annual precipitation, and 6.5° to 26 °C mean annual temperature [69]. Our data, show a strong link between phloem anatomy and EPD tolerance, aligned with these studies, suggesting that tolerant clones have phloem anatomical features analogous to those populations adapted to ecologically more stressful environments.

The contribution of species to tolerant responses [12] cannot be ruled out, depending on plant or stand age [36,62], and the nature of the stress triggers. There also appear to be differences as the evaluation is conducted under controlled stress [29,31,70] or natural conditions [62,71,72], what favored EPD resistance strategies to be found in selected genotypes [29,32,34]. It is worth noting that in previous reports, the species evaluated in natural environment were not among the nine most important *Eucalyptus* species in commercial plantations [18,19].

As complied by Picoli et al. [20], natural ecosystems approaches help, but the complexity of the competition for resources, may be different from an anthropic (planted forests) environment. Under standardized conditions, the differences observed in bark percentage [28] and variability in secondary phloem, inner bark features and functionality [68,69] could have its application extended to anatomical features significantly associated with the EPD tolerance, as different clones exhibited significant differences for the stem diameter, number of STE and conducting phloem. Stem diameter, thickness of phloem with crystals, total phloem, and frequency of STE followed the same trend, accentuating a different behavior of phloem anatomical features among tolerant, moderate tolerant and susceptible *Eucalyptus* clones.

Matusick et al. [73] evaluated stem functional traits among co-occurring species, *E. marginata* and *Citriodora calophylla*, and observed little variation in bark area, and no significant effect and interaction of drought vulnerability and bark area between them. In an approach to dieback occurrence at a population level of *E. piperita*, the environmental and genotype—environment interaction on the outcome of physiological disorder were emphasized [72]. In contrast, *Eucalyptus* specie-related such as bark thickness, moisture and density, were recognized as important traits as defense against fire [62]. Meaningful associations and evaluation of stress resistance responses in *Eucalyptus* could certainly use a histological approach, as observed for EPD resistance.

In a planted forest, the genotype effect may be blur as different species presented representatives ranging from EPD-tolerant/semi-tolerant to susceptible, especially considering that selected commercial clones were evaluated. To evaluate the association of the genetic background with *Eucalyptus* species to stress triggers, a quantitative genetic analysis should be conducted. But first, the number of associated loci and the favorable allele frequency in *Eucalyptus* species should be evaluated. This should also be justified considering that there is an environment effect, since not all plants from the same genotype express the

disorder, and those that do express it exhibit a different level (scores) of the disorder. This is a challenge to evaluate EPD responses. For this reason, despite our sample came from selected commercial clones, different genotypes from the same species (or hybrids between them) exhibited divergent EPD responses, historically in the production field and in the experimental plots.

Genotype [21,67] can be associated with EPD tolerance, and environment [66] can be a source of variation, influencing bark features [68,69]. Yield and tolerance traits can also benefit from both these features [9,21]. For example, the evaluation and selection of tolerant, productive clones that are adapted to specific environmental conditions clones are the ground for eucalypt clone zoning for plantations [9,74,75].

This scenario fits EPD and traces back to the phloem tissue, as it has been connected with water deficit stress in other plant species [50,52,53,76] and water deficit being associated with EPD [6,11–13,15]. Despite the similar organization of secondary phloem in EPD-tolerant and susceptible genotypes, there is a difference in the conductive phloem and phloem with CaOx crystals among these plants. The histometric approach confirmed this contrast. We propose that these characteristics, reflecting differences in cell differentiation and metabolism, are mechanistically linked to EPD tolerance. Caetano-Madeira et al. [32] highlighted plant structural, metabolic and physiological adjustments of EPD divergent clones subjected to water deficit, whilst Nascimento et al. [34] reported significant correlations of electrical conductivity, pH, total amino acids and quercetin content in bark samples. Regardless of the cause, effect or EPD triggers, the phloem tissue is linked to plant growth as inner bark thickness variations revealed signals strongly correlated with photosynthetic production and, consequently, phloem transport [77].

Considering water deficit is one of the triggers associated with EPD, it is worth put in evidence the strong and highly significant correlations among conducting phloem thickness (CPh) and the number of STE per stem cross section (SESCS), CPh and the average diameter of STE (SED), and SESCO and SED. Salmon et al. [49] and Sevanto [52] reviewed the association and impact of drought and phloem transport. The tight balance of carbon and water flux are evidenced, while there are reports that support the decrease in phloem transport and unloading as well as it being not impaired under drought conditions. Despite the poor understanding of phloem transport and interplay of its structure and function under water deficit conditions, Gričar et al. [51] reported phloem cell size being influenced by site and Dannoura et al. [50] reported smaller STE under prolonged drought.

Considering that transport in phloem follows as described by Hagen-Poiseuille [49,52], the phloem hydraulic conductivity will be modeled by STE diameter [mechanism/physics]. According this reasoning, *Eucalyptus* plants with increased CPh (tolerant clones), will have higher SESCO and STE diameter, hence conductivity capacity increased to the fourth power of STE radius. This represents a direct mechanism to reduce carbon starvation under stress conditions that triggered EPD.

Allometric variation allowed us to confirm an increase in stem diameter with conducting phloem and total phloem thickness, in addition to a negative correlation of CPh and SD with EPD scores. These associations describe anatomical traits of phloem and bark as linked to stem diameter growth and disorder tolerance. Phloem may be intrinsically related to EPD tolerance as carbon deprivation may be responsible for the reduced root apical meristem activity [78]. Furthermore, the metabolism in clones susceptible to EPD is altered, since, despite higher photosynthetic rate, there was less dry mass accumulation and less efficient use of carbon [32]. Sevanto et al. [79] reported that hydraulic failure could be associated with the lack of carbohydrate supply for osmoregulation and, consequently, the failure to maintain hydraulic integrity in *Pinus edulis*. Plants could die from both hydraulic failure and carbon starvation. This hypothesis is raised based on the review of the

available information as support for the integration of carbon resources under water deficit conditions [49]. The authors observed that the symptoms and survival time in response to drought stress can vary with individual trees even in similar environmental conditions.

There is a significant difference of conducting phloem thickness and number of STE comparing EPD tolerant, moderate tolerant and susceptible clones, as well as correlation of conducting phloem STE diameter, number of STE in a stem cross section with EPD scores. As phloem tissue is responsible for photosynthate transport and nutrient redistribution in plants, it will be accountable for or associated with nutritional [30,32,35,36] and physiological [32] triggers of EPD. Therefore, an increased number of STE or conducting phloem in the tolerant *Eucalyptus* clones can be one of the structural strategies that grant them an increased capacity and resilience of phloem sap transport.

Phloem tissue, its structure and function, is among the pathways for plant survival and production. Bark and phloem traits can be correlated with stem diameter growth and EPD scores. Photosynthesis, transpiration and stomata conductance, vascular, xylem and phloem areas in the petiole cross section, were important traits for EPD tolerance as they exhibited high accuracy and medium heritability [37]. In this context, bark anatomical traits are reasoned as a key trait for EPD tolerance, as they represent the physical infrastructure for carbon transport that sustains all other physiological responses.

The STE diameter averages of *E. grandis*, *E. urophylla* and hybrids are larger compared to the average of 27.1  $\mu\text{m}$  in *E. urophylla* [54], and in agreement with the range of 34 to 42  $\mu\text{m}$  observed in *E. globulus* [80]. In this report, it was not possible to establish a trend within the *E. globulus* tree height and STE diameter, and plant height was not considered a significant source of variation for STE length and tangential diameter. Although there was no difference in STE diameter comparing the EPD phenotypes, there were significant correlations with other traits such as stem diameter (SD), linking it to tree growth, and the EPD scores. Wang et al. [81], on the same hand, observed a trend of increased sieve element diameter and a decrease of sieve element density with the distance to the tip of the stem/shoots in samples of 188 angiosperm wood species. The variability of the traits according the EPD phenotypes is less clear, despite the outliers being depicted. This is attributed to the EPD occurrence and intensity differences in eucalypt plants. It is observed that among the different clones tested there were differences among the EPD scores. So, lowering the status of any of nutritional, physiological, environment, and stress features, may result in a favorable condition to the occurrence of different levels of the disorder, evaluated as EPD scores. As a complex trait, EPD shows an incomplete penetrance and variable expressivity, which can also be related to the variation in STD diameter. In short, penetrance is the percentage of individuals of a specific genotype and express the phenotype associated with that underlying genotype, and expressivity refers to the degree to which a specific genotype is expressed as a phenotype within an individual [82].

Tolerant clones displayed phloem with increased CaOx in the histological cross sections bring dynamic roles of Calcium and oxalic acid to the spotlight. Despite there was no significant difference among the EPD clones, there as a positive correlation of the PIC and stem diameter. The significant and positive correlation between phloem with crystals thickness (PIC) with stem diameter (SD) put in evidence the importance of phloem tissue to stem secondary growth. It is reasonable to assume that the parenchyma cells with CaOx crystals are alive as these inclusions are consumed in the outer phloem and as they are able to contribute to the formation of new phellogen layers. Essentially, being alive, these cells may also actively contribute as part of a structural strategy to stress responses, growth and differentiation processes. In fact, the negative correlation of the frequency of STEs per  $\text{mm}^{-2}$  (FSmm) and PIC with SD pave the way for an additional role of CaOx in the cell differentiation process that seems to be overlooked. EPD is triggered by stress conditions (e.g.,

water deficit) and anatomical traits associated with growth (CPh, P/Rh, and SD) and cell differentiation (SED and SESCS) processes have a negative correlation with the increase of EPD. These observations lead to hypothesize that CaOx may be considered more than histological markers potentially acting as a metabolic regulator or defense mechanism. Hudgins et al. [83] reported the frequency of CaOx crystals worked as a constitutive defense that, combined with phloem fibers, provided a barrier to bark-boring insects in stem of Pinaceae and nonPinaceae plants. It is worth to note that eucalyptus also exhibited considerable sets of phloem fibers and part of the secondary phloem crowded with CaOx crystals.

Tooulakou et al. [84] reported diurnal volume changes of mesophyll CaOx crystals in *Amaranthus hybridus*. This was attributed to a biochemical mechanism allowing carbon storage in the form of CaOx crystals, mainly during the night when stomata were closed and photosynthesis shut down. The authors argue that during the day, the degradation of crystals would provide supplementary carbon resources for photosynthetic assimilation, particularly under carbon starvation conditions. Despite there was not a follow up of the CaOx crystals in the *Eucalyptus* bark, there is indeed more phloem tissue with CaOx crystals in the EPD-tolerant clones, as well as increased stem diameter, compared to the susceptible clones. Hence, this is strong evidence that CaOx crystals may have important contributions to plant stress resilience and growth. The reported an “alarm photosynthesis” [84] that is triggered under limited CO<sub>2</sub>. Increased CaOx may cope with stress tolerance, as EPD tolerant *Eucalyptus* had more Ca in their leaves [32] and has been identified as a possible biomarker for water deficit [33]. Injuries to phloem tissues can induce protein plugging of the STE as well as callose deposition. These are caused by electro potential waves and consequent Ca<sup>2+</sup> release into the STE [85]. The increase in CaOx in *Eucalyptus* phloem can contribute to signaling or other strategies associated with EPD tolerance, since, similarly, the presence of CaOx in phloem cells has been associated with insect resistance in several Pinaceae [83].

Further, it is intriguing that the frequency of STE mm<sup>-2</sup> reduced with the stem diameter, conducting phloem, periderm, total phloem, phloem with crystal thickness, and the average diameter of the STEs. There is a significant, yet negative, correlation of FSmm with SED and SD, while Liesche et al. [46] reported a weak correlation of STE radius and stem length, in addition to larger sieve pores and longer STEs of woody angiosperms with compound sieve pores. We interpret the negative correlation between FSmm and SED/SD as an ontogenetic “size vs. number” trade-off in phloem organization. We assume that the negative correlation between FSmm and SED/SD may be based on a trade-off of size and number of functional conductive elements, were a smaller number of STEs would have the transport more easily regulated under unfavorable conditions. It is highlighted that FSmm behaved oppositely to all traits, and increased in susceptible samples at all tree heights while there is an increase in conducting phloem from susceptible to tolerant from top to bottom of the trees. This configuration (fewer, wider elements) is functionally consistent with the maintenance of flow under stress. Nevertheless, this is a hypothesis yet to be tested. The evaluation of number and diameter of sieve pores and STE length in *Eucalyptus* also consist traits to be evaluated in future research approaching plant growth or response to stress conditions. Wang et al. [81] raised evidence that phloem anatomical traits in wood angiosperms, such as STE diameter, was correlated with water availability. Further, the opposite trend, increasing sieve element diameter and decreasing its density according the distance to the stem tip, evidence a trade-off involving STEs differentiation that could apply to stress or EPD tolerance and with impact on the overall conductive capacity of plants. Savage et al. [86] also observed that hydraulic resistance in phloem was inversely proportional to sieve element radius and plant height. Hence, water deficit triggers and EPD-tolerance phenotype are linked to STE and phloem characteristics.

The observed negative association between the frequency FSmm and both the mean SED and SD should be interpreted as a “size vs. number” ontogenetic trade-off in phloem organization. In our data, increasing sieve element diameter and stem thickening are associated with a reduction in sieve element density per unit area, indicating that increasing conduit caliber can compensate for the reduction in number, maintaining or increasing transport capacity per area. This pattern is consistent with general principles of the increase of internal flow increases with conduit radius, so increasing diameter is an efficient way to increase transport capacity without the need for a linear increase in conduit number. Conversely, producing many small conduits increases frequency per area and may result in higher construction costs or greater susceptibility to clogging or collapse of the STE. This interpretation fits why clones with a pattern associated with tolerance have larger SED and SD, but smaller FSmm—i.e., fewer and wider sieve elements—a configuration functionally consistent with the maintenance of flow under stress conditions. These findings are consistent with descriptions of phloem scale and function that emphasize trade-offs between conduit geometry, hydraulic resistance, and vulnerability [46]. Thus, a negative correlation between FSmm and SED/SD may be understood as a reflection of vascular developmental constraints, in which the plant balances transport efficiency and structural costs in response to environmental pressures.

The intensity of the disorder can vary between different trees [27], but also between trees of the same clone, as there are clones that are more frequently affected by EPD than others. In our report, the association of EPD occurrence by field and clone, and the evaluation (scoring) of the sampled trees, benefited the search for the relationship between variables and possible cause-and-effect impacts. To reduce bias, the evaluated plants were conducted in a commercial field with standardized management conditions, in the same location, plant age and in the same position used for stem sampling. It is observed that the concepts of penetrance and expressivity [82] apply to the occurrence and intensity of eucalypt disorders in commercial and natural fields [21,71]. The variability in the occurrence of the disorder [86,87] also emphasizes several environmental features [6,13,15,35,36] that contribute to the incidence and intensity of disorders. Despite there are significant correlations and association of histological variables with EPD tolerance and stem diameter, there were evaluated a limited number of samples, genotypes and species. The significant associations highlight that phloem and bark traits are relevant but additional studies should be conducted before generalizations to other species and environmental conditions.

Even though only nine genotypes were screened in the present experiment, they are part of selected genotypes of a breeding program, aiming at and gathering traits that fit commercial plantations and stands. In this breeding program, *E. grandis* and *E. urophylla* were favored, hence the results here apply for these species and respective hybrids. Generalizations and application of the reported traits to EPD should follow additional experiments to prove the robustness of these applied to other *Eucalyptus* species.

The negative correlation of SD with the historical EPD records (PHD) and the EPD scores (SP) of sampled trees is a result of the expected impaired growth of plants with increasing EPD scores. The PHD and SP correlation reports historical data based on average clone behavior in commercial stands and is adequately addressed by the occurrence and intensity of EPD the sampled trees. The high and significant correlation (81%) between SP and PHD validates our approach, indicating that the observed anatomical differences are not short-term artifacts but rather stable structural traits that predict the clone's long-term ecological performance in the field. The assigned SP and PHD scores are aligned and denote that the clone response to a set of triggers that will favor the physiological disorder. The high and significant correlation between SP and PHD is a reference for future evaluations and genotype screening, as EPD are unlikely to be available for the clones being tested.

Both the genotype, the environment and, consequently, the management, may interact with the intensity and occurrence of EPD as there is an increase from 0.682 to 0.825, but a decrease from 0.735 to 0.15 in the correlations of bark thickness and bark content with spacing between trees, respectively [65].

In the correlation network, different variables were cross-linked evaluating developmental and growth outcomes. The dependence between phloem anatomical traits may benefit stem diameter growth, as may also involve trade-off between the phloem with crystal thickness and frequency of STE or the identification of traits significantly linked to EPD tolerance. Water deficit influences the sieve element differentiation as size and number of sieve tube elements were affected by the amount of precipitation [88] and the diameter of STE were reduced under prolonged water deficit conditions. The negative correlations of eucalypt STE with the frequency of STE and the EPD score of the evaluated trees support that similar phloem responses and trade-offs involving STE differentiation are expected for EPD as water deficit is one of its triggers [11,12,14,15].

Sevanto [52] speculated that, under water deficit, there is an increase in phloem sap viscosity, considering independent conduits. In the EPD context, a greater conducting phloem thickness (as in the tolerant clones) implies more conduits, which would allow higher total transport rates, mechanistically compensating for the increased sap viscosity. Phloem failure has been recognized as one of the mechanisms causing tree mortality under drought, where smaller sieve tubes and high-viscosity sap may slowdown phloem transport, reducing hydraulic conductivity and force between sink and source organs of *Fagus sylvatica* trees [50]. The cessation of phloem transport would not only affect the allocation ability of the trees to access carbohydrate resources. Salmon et al. [49] reported significant alterations in the phloem structure of trees that were submitted to water deficit stress. There was a reduction in the bark and functional phloem thickness in addition to pronounced phloem cell collapse and deformation that affected photosynthate transportation. Since EPD can be triggered by a stress condition, plants with larger phloem area and non-collapsed STE would contribute to higher transport rates in tolerant plants, conferring physiological resilience. Similarly, there is a reduction in the carbon availability that may have a negative impact on secondary growth [49]. Although phloem anatomy is poorly characterized [20,49,76], the high and significant correlation of total phloem tissue thickness and stem diameter may address clone selection in breeding programs. This is supported as bark thickness is associated with phloem vulnerability where thicker bark would act as a physical protection for phloem tissue [49].

Vázquez-Segovia et al. [69] reported that the higher the diameter, the greater is the thickness and the contribution of bark to the total biomass of the plant, and that bark could be a good predictor of the stem diameter depending on the tree size and development. Stress could be an additional feature to influence the carbon allocation in the stem as carbon allocation in phloem in relation to wood varied little across species, although other tissues and outer bark were strongly affected by environmental features. These features should be considered in the evaluation and management of commercial areas, nevertheless, under experimental conditions the environmental conditions are expected to be standardized.

Kohonen's self-organizing map (K SOM) allowed the visualization of three clone clusters based on bark anatomy and their expected EPD phenotypes. K SOM is a multivariate analysis whose algorithm allows the organization of complex data into groups according to their similarities [89]. Anda et al. [90] reported it was developed to predict the water stress index for soybean plants based on minimal amount of input data, providing reliable results. This clustering technique has been successfully used to represent different varieties from other plant species submitted to water deficit stress where genotypes were classified into defined locations on the map, as well as stressed plants separated from control plants

based on a set of high-throughput selected traits [91] or across multiple seasons [90]. The SOM two-dimensional map was successful in separating groups of clones with similar EPD phenotype. This is an alternative to Principal Component Analysis [31,32] and the Mulamba and Mock ranking [33,37] for the output of different EPD clones.

This is the first report of the using phloem and bark anatomy to compare contrasting EPD clones. Neuron clustering was benefited by easily identified and tissue-related traits such as conducting phloem thickness, live phloem thickness, and thickness of the phloem with CaOx crystals. Other traits that may be more related to the cell differentiation process, such as the number of sieve tube elements per  $\text{mm}^{-2}$ , average diameter of sieve tube elements, and the estimate number of STE per stem cross section, were also important for neuron clustering. Significant contribution of NR\_C to the definition of neurons, despite the absence of significant statistical differences between EPD phenotypes or correlations between traits, illustrates the ability of SOM to detect and provide complex, nonlinear interactions that may not be evident through traditional statistical analyses. This complexity may be associated with the radial transport of nutrients from phloem to xylem tissues. Nascimento et al. [34] observed variation in starch in wood samples of different *Eucalyptus* clones. Although it was not possible to differentiate the clones, there was significant and negative correlations ( $-0.45$  to  $-0.48$ ) of the starch content in the wood samples and EPD scores. In addition, xylem histological sections revealed starch in the tolerant, and absent or less evident in the susceptible genotypes. These traits may work as biomarkers for clone selection as they directly reflect the plant's structural capacity for carbon transport and stress response. Decreased value on wood yield and quality are reported due increased bark content [92,93], although no phloem mensuration is described. It appears that a historical negative selection for 'bark' (to maximize wood) [94–96] may have inadvertently led to the selection of clones with a reduced proportion of conducting phloem. However, recognizing only the importance of wood, the main commercial product, the significance of phloem tissue as part of plant growth processes, and responses to stresses or triggers leading to the EPD have been neglected.

Although NR\_C (number of rays per 100  $\mu\text{m}$ ) did not show significant differences in univariate ANOVA, nor evident bivariate correlations with phenotypic scores, nor key phloem variables, its relevant contribution to the definition of clusters in the self-organizing map constitutes a methodologically informative result. Unsupervised multivariate models, such as SOM, preserve topology and capture patterns of multidimensional covariation and nonlinear interactions between variables that univariate tests and pairwise correlations may not detect. Thus, a variable with a marginally small effect can consistently integrate an anatomical syndrome when considered in combination with other traits. In the case of NR\_C, it is plausible that this trait covaries with radial and structural aspects, distinguishing tolerant and susceptible phenotypes in the multivariate space. Therefore, the contribution of NR\_C to SOM is interpreted as evidence that the abundance of parenchyma rays participates in a coordinated anatomical configuration—related to radial structure, storage, and/or transport pathways—that only becomes apparent when multiple dimensions of tissue architecture are simultaneously examined. This result reinforces the importance of integrating multivariate methods in the analysis of functional traits, as they reveal emergent patterns of covariation (anatomical syndromes) that remain invisible in univariate approaches [97].

Phloem structure differs among *Eucalyptus* clones associated with EPD, even in asymptomatic individuals, as phloem traits allowed the grouping of different EPD phenotypes based on historical data of commercial field plantations. Similar outcomes should be expected for abiotic stresses such as tolerance to water deficit as water deficit is one of the main physiological triggers for EPD [6,11,15].

The etiology of EPD is complex [21], therefore other agents [11,35,36] will also influence the occurrence and intensity of its symptoms. EPD will depend on genotype (available tolerance strategies), the environment (combination of environmental stressors), and their interactions. However, the features and behaviors contributing to tolerance strategies remain to be clarified. To select these strategies, one must have access to the stress trigger prevalent in a specific environment. The basic reasoning here is that, as in hypoxia, mineral imbalance or water deficit, the stress starts at the cellular level, which will define the plant's performance. As an example, when faced with multiple stresses such as hypoxia, genotypes of the same plant species can adopt different adaptive strategies, characterized as escape (LOES) or quiescence (LOQS), as discussed by Voesenek et al. [98] and demonstrated by Harguindeguy et al. [70] in *Eucalyptus* clones. The Low-O<sub>2</sub> Escape syndrome (LOES) and Low-O<sub>2</sub> Quiescence syndrome (LOQS) are strategic behaviors that respectively imply, in short, energetic investment trying to physically escape stress with structural modifications, conserve energy and mobilize antioxidant and protective responses.

Under a heat stress scenario, protein denaturation [99] and HSP activation [100], and altered stomatal opening [101] are expected, although the perception of stress differs among organs. For instance, ABA transport and the ability to induce stomatal closure were still better in leaf than in root during root water deficit stress [102]. It is worth to note that the degradation of CaOx crystals during the day and under carbon starvation conditions seemed to be regulated by ABA [103]. Similar results are expected for heat stress, as leaves are directly exposed to solar radiation compared to roots. In the succession, at some point in the development of the stand (natural or planted), there will be a stress due competition for resources, being it water, light, nutrients, or others. This can result in etiolation; apical dominance; suppression of lateral branches; and redistribution of assimilates, the latter involving the phloem transport capacity. Similar analysis may be applicable to EPD. Localized and reduced growth, cellular and metabolic actions appear to be more effective, while actions that imply in greater risk and energy expenditure may be effective or lead to collapse, depending on the context, stress triggers and period of exposure.

The present report covers a gap in the literature associating quantitative bark and phloem traits to *Eucalyptus* development-growth and to the resistance to a physiological disorder launched by a stress component such as water deficit. It is possible to draw an ordered sequence from growth and developmental processes, through tissue, cellular traits, and metabolism aspects akin to that, ultimately, are triggered by environmental stresses or stimuli. Under stress triggers, better adapted plants capable of expressing phenotypic plasticity resources [20] will exhibit increased growth compared to, for instance, EPD-susceptible plants [7,29,32]. We highlight that vegetative growth is one of the main aspects of the *Eucalyptus* culture. There are trade-offs observed in the *Eucalyptus* xylem that influence the plant hydraulic architecture such as decrease in vessel elements (VE) concomitant to increase in the number of VE per mm<sup>-2</sup> [31]. Yet, Andrade-Bueno et al. [31] also reported that VE frequency and diameter classes were more evenly distributed for the tolerant and medium tolerant compared to the susceptible genotypes. Hence, a different hydraulic architecture, and seemingly, providing safer sap transport under the stress condition. Wang et al. [81] and Almeida et al. [88] reported that STE diameter was associated with water availability and it is known that water deficit influences cell differentiation [103]. For this reason, the number, frequency and differentiation aspects of STEs may be linked to water deficit triggering the EPD and its developmental and growth outcomes. In fact, inner bark area augmented with the increase of the temperature and decrease of soil fertility [69]. It is reported that there are correlations between water deficit and plant competitiveness with the physiological disorder [12]. Further, there are cellular and metabolism attributes that differ between EPD-susceptible and tolerant genotypes such as pH, electrical conductivity,

total amino acids, among others [34], that may have a role in signaling and initiate a stress response. For instance, the significant correlations of starch in xylem and EPD scores consist of a clear association of the carbohydrate metabolism with EPD response. Carbohydrate transport is associated with the disorder as we observe that STE and phloem traits differ between EPD-tolerant and susceptible *Eucalyptus* clones. This will have an impact on other plant cell and organism structural features such as mineral nutrition and transport. Caetano-Madeira et al. [32] reported differences in Calcium concentration, among other mineral nutrients, when comparing tolerant and susceptible *Eucalyptus* clones under water stress conditions. This contrast is aligned with the CaOx occurrence, as tolerant *Eucalyptus* clones harbor increased phloem with crystals. Hence, xylem and phloem hydraulic properties will be influenced by environmental stimuli [31,69] respectively in their capacity for mineral transport and redistribution. They will also modulate mineral nutrition that is also linked to physiological disorder occurrence [30,32,35,36]. The above-mentioned elements are part of a loop that, supported by our results, help to explain how adapted genotypes thrive under stress conditions that favors EPD.

In summary, there are multiple strategies to cope with stress and a functional cohort system to recognize and respond to different stresses. The access and distribution of photosynthates, as well as signaling, that occurs through phloem is part of this core. The difference in nature of the plant traits (morphological, nutritional, physiological, and metabolic) being associated with the disorder's response [29,32] contributes to the idea of multiple strategies associated with EPD tolerance. As there is a lack of reports associating anatomical features with EPD phenotype, score, occurrence and intensity, standardized management and genotypes available were provided to reduce bias for site, plant age, management conditions and sampling position. Similar approaches should be conducted with other accessions before assuming these traits are effective for selecting tolerance in other *Eucalyptus* species. Only anatomical features of bark and phloem from commercial eucalypt genotypes were highlighted here, however, they exhibited significant differences and correlations with stem diameter growth and EPD tolerance. Thus, there are different anatomical biomarkers that are strongly and significantly associated with EPD tolerance. This report is part of a broader research where wood property traits [28] and analysis of chemical and bromatological [34] have already been conducted and successfully associated with EPD tolerance. Leaf and petiole anatomy; leaf, bark and wood minerals; and analyses of growth variables are in progress from samples and data from the same plants and experiment. The combined analysis of this complete set of traits will allow a holistic view of EPD tolerance.

## 5. Conclusions

There is a similar organization of bark and secondary phloem of contrasting EPD clones, despite a trend of the increase bark and phloem regions from the top of the tree to the base. There are significant anatomical differences in the number of sieve tube element and conducting phloem thickness that indicate the maintenance of structurally preserved cells and tissue in the EPD tolerant clones. Additionally, there are significant correlations of *Eucalyptus* stem diameter and EPD tolerance with anatomical traits such as conducting phloem, phloem with Calcium oxalate crystals and periderm thickness, number of sieve tube elements per square millimeter, average diameter of sieve tube elements. The significant differences and correlation of these characteristics indicate the importance of phloem tissue to EPD tolerance and stem growth.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijpb16040124/s1>, Table S1: Statistical descriptive analysis, average

and standard deviation, of bark histometric traits of commercial eucalyptus clones with divergent EPD tolerance phenotypes (scores) and Variance Inflation Factor (VIF) for the preliminary (VIF1) and final data (VF2) analysis, as a measure for detection of multicollinearity among independent variables in a regression analysis, and Table S2: Analysis of Variance table. Btt: bark total thickness ( $\mu\text{m}$ ); CPh: region of conducting phloem, thickness ( $\mu\text{m}$ ); NCoP: region of nonconducting phloem, thickness ( $\mu\text{m}$ ); P/Rh: region of periderm or rhytidome, thickness ( $\mu\text{m}$ ); TPh: region total phloem, thickness ( $\mu\text{m}$ ); CPh/Pl: ratio of NCP per total phloem thickness; PIC: region or domain of phloem presenting CaOx crystals, thickness ( $\mu\text{m}$ ); CPPC/2: average of conducting phloem and phloem with crystals, thickness ( $\mu\text{m}$ ); PIC/Pl: ratio of phloem with CaOx crystals and phloem total thickness; FSmm: frequency of sieve tube elements per square mm ( $\text{STE mm}^{-2}$ ); SED: average diameter of sieve tube elements, diameter ( $\mu\text{m}$ ); NR\_C: average number of rays found per 100  $\mu\text{m}$  cambium; CPA: estimated area of conducting phloem per stem cross section, area ( $\text{mm}^2$ ); TPA: estimated area of live phloem per stem cross section, area ( $\text{mm}^2$ ); SESCO: estimated number of STE per stem cross section, area ( $\text{STE mm}^{-2}$ ); SD: measured stem diameter, diameter (cm); AP: attributed phenotype (score) of EPD in commercial fields according historical data; PHD: average score of the evaluated genotypes, empirical data from commercial fields; SP: EPD score attributed to the sampled plants.

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**Data Availability Statement:** The datasets for this manuscript are available upon request to Suzano S/A staff. Some of the information may not be disclosed according to a confidentiality contract. Requests to access the datasets should be directed to Lucio Mauro (lucio@suzano.com.br).

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**Conflicts of Interest:** Author E.Â.V.Z. was a former employed of Suzano S/A, now an independent researcher. The authors declare that this study received funding from Suzano S.A. and that these resources were managed by *Sociedade de Investigações Florestais* (SIF)/UFV. The funder had no role in the analysis and decision to publish. The funder had the following involvement with the study: provided part of the plant material, gathered information on the behavior of the genotypes in field conditions and (E.A.V.Z. and L.M.d.S.G.) contributed to the review of the manuscript. This experiment was part of a collaborative research and induced demanded project that was also funded by the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) and the National Council for Scientific and Technological Development (CNPq), project “Applied Plant biology: a bridge to an integrative approach to the water deficit and eucalypt physiological disorder tolerance”. J.d.S.L. and F.A.J. received an undergraduate fellowship during part of the conduction of this project funded by Suzano S.A. and M.N.F.A. received a post-Doctoral fellowship during part of the conduction of this project funded by Suzano S.A. This study was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001* as it was part of a Visiting Scholar

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## Abbreviations

The following abbreviations are used in this manuscript:

|        |                                                                    |
|--------|--------------------------------------------------------------------|
| EPD    | Eucalypt Physiological Disorder                                    |
| CaOx   | Calcium oxalate crystals                                           |
| STE    | sieve tube element                                                 |
| DBH    | Diameter at the Breast Hight                                       |
| Btt    | bark total thickness                                               |
| P/Rh   | periderm or rhytidome thickness                                    |
| TPh    | total phloem tissue thickness                                      |
| CPh    | conducting phloem thickness                                        |
| PIC    | thickness of phloem with crystals                                  |
| NCoP   | thickness of nonconducting phloem                                  |
| CPPC/2 | average thickness of conducting and phloem with crystals           |
| SED    | average diameter of sieve tube elements                            |
| CPA    | estimated fraction of conducting phloem in stem cross section      |
| TPCA   | estimated area of total phloem tissue in stem cross section        |
| NR_C   | number of parenchyma rays per 100 $\mu\text{m}$                    |
| PIC/PI | ratio of phloem with crystals thickness per total phloem thickness |
| CPh/PI | ratio of conducting phloem per total phloem thickness              |
| SESCS  | estimated number of sieve tube element per stem cross section      |
| FSmm   | frequency of sieve tube element per $\text{mm}^2$                  |
| SOM    | Kohonen Self-Organizing Map                                        |
| VIF    | Variance Inflation Factor                                          |

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