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Postfire Alterations of the Resin Secretory System in *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae) [†]

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Abstract: Fire is a natural disturbance in the Brazilian Cerrado that modulates the vegetation structure. *Protium heptaphyllum*, a woody species of the family Burseraceae, is common in this biome. The resin produced in secretory canals immersed in the phloem of the stem and leaves of this species plays important ecological and industrial roles. The aim of this study was to investigate the influence of fire on the development of resin canals in the leaves and stem of *P. heptaphyllum* and on the chemical profile of substances produced in the leaves. Young plants were subjected to controlled fire experiments. Leaf and stem portions were analyzed using light microscopy; the chemical compounds in the leaves were identified through gas chromatography–mass spectrometry. The percentage area occupied by secretory canals in the leaf midrib was higher in fire-treated plants than in control plants. Similarly, the density of secretory canals and their lumen area were higher in young stems (primary growth) of fire-treated plants. By contrast, although the canal density in the secondary phloem was lower in older stem portions (secondary growth) in fire-treated plants, their lumens were larger, resulting in similar data regarding the total lumen area of the secretory canals in fire-treated and control plants. The main chemical compounds identified in the leaves were vitamin E, sitosterol, α -amyrin, squalene, and β -amyrin. Three compounds showed significant increases in fire-treated plants, with vitamin E being the only one reduced by fire. Our findings reveal the plasticity of the secretory system and of the biochemical properties of the leaves of *P. heptaphyllum* in response to fire. These results are important when considering the current increase in fires caused by climate change and human activity in different ecosystems around the world.

Keywords: fire; leaves; phytochemistry; secretory canals; stem



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1. Introduction

The Brazilian Cerrado is the largest and most significant savanna formation in the Americas [1] and is considered one of the world's hotspots for conservation [2]. It is a mosaic of vegetation physiognomies, varying from savanna woodland to grassland [1]. Rainfall seasonality, nutrient-poor acidic soils with a high concentration of aluminum, and constant fire events characterize the Cerrado area [3,4]. Among those, fire is a determinant force

driving plant evolution in the biome [4,5], i.e., many plants in the Cerrado are pyrophytic or fire-adapted [1]. The effects of fire on vegetation are well known, and morphological adaptations have evolved for fire resistance, including thick cork bark, protective cataphylls on buds, underground systems with protective buds, and a high ability for vegetative propagation [1,6–8]. However, the influence of fire on several morphological and functional aspects of plants naturally occurring in the Cerrado, especially concerning their secretory systems, remains unknown.

Burseraceae is an angiosperm family commonly recorded in floristic surveys and phytosociological studies in diverse areas of the Brazilian Cerrado [9–12]. Their representatives produce aromatic resins consisting of terpenes of great ecological and economic importance [13]. *Protium* (tribe Protieae) is the most heterogeneous genus in the family and the main Burseraceae genus in South America, encompassing the largest number of species [14]. *Protium heptaphyllum* (Aubl.) Marchand is a tree species common in the Cerrado [9–12,15,16], where it fits among species with a high importance value [17].

The resin produced throughout the vegetative axis of *P. heptaphyllum* trees has a high percentage of volatile chemical compounds, composed of a mixture of triterpenes mainly from the α - and β -amyrin series [18]. It is used for commercial, industrial, and pharmaceutical purposes [19,20] and is a source of raw material for hygiene products, varnishes, incense, and candles [19,21]. Several pharmacological studies have demonstrated its anti-inflammatory, gastroprotective, antinociceptive, antihyperglycemic, photochemoprotective, antimicrobial, antioxidant, and antimutagenic properties [22–25]. Furthermore, the chemicals produced by *P. heptaphyllum* display important ecological roles, providing an effective defense against herbivores and pathogens [26]. Such substances are produced in secretory canals associated with the primary and secondary phloem of the aerial [27] and underground [28] organs of *P. heptaphyllum*. Secretory canals originate through schizogenesis, develop through a schizolysigenous process, and form an anastomosed secretory network in the stem [27]. The secretory process begins early in the undifferentiated portions of the stems and leaves, with canals active in secretion occurring at the shoot apex and close to the vascular cambium [27].

Studies have shown that biotic and/or abiotic environmental factors can influence the formation and distribution of secretory structures in resin-producing species, in addition to the quantity and chemical composition of the secretions produced [13,29,30]. A recent study highlighted the developmental plasticity of the secretory system in *P. heptaphyllum*, showing differential features in plants under contrasting environmental conditions [31]. Therefore, considering (a) the importance of fire in determining the structure of Cerrado vegetation, (b) the recent increases in the frequency and intensity of fires in the Cerrado area in Brazil owing to the expansion of agriculture, and c) the developmental plasticity of the secretory system in *P. heptaphyllum*, the aim of this study was to investigate the influence of fire on the development of resin canals in the leaves and stem of this species and on the chemical profile of substances produced in the leaves. We hypothesize that fire can induce the formation of more abundant secretory canals with a larger lumen area in the leaves and stems (primary and secondary growth) of *P. heptaphyllum*, in addition to inducing changes in the chemical profile of the substances produced in the leaves.

2. Materials and Methods

2.1. Plant Material

Individuals of *P. heptaphyllum* were obtained from the germination of seeds. They were kept in plastic bags with commercial substrate for cultivation (vegetable soil) in a greenhouse in the Department of Biology and Animal Science, Faculty of Engineering of Ilha Solteira (FEIS), São Paulo State University (UNESP), Brazil. When the individuals

were nine months old, they were subjected to the fire experiment. Voucher specimens were deposited in the Herbarium of the University of São Paulo (SPF).

2.2. Experimental Draw

The experiment was carried out at the Fazenda de Ensino, Pesquisa e Extensão, São Paulo State University (UNESP), Ilha Solteira city, Brazil, on 26 August 2022. Individuals of *P. heptaphyllum* that were 9 months old with an aerial axis measuring approximately 16.50 ± 0.48 cm in height were placed in the field 1 m apart. The experimental design comprised three fire plots (three blocks, 10×10 m each) with eight individuals each, in a randomized block design, totaling 24 individuals. Four-meter-wide firebreaks were prepared around each plot to prevent the fire from spreading. Before starting the experiment, the amount of combustible material in each plot was measured by collecting aerial biomass in 5 subplots of 0.30×0.30 m. When necessary, more combustible material (dried grasses collected in neighboring areas) was added to each plot, until reaching 600 g/m^2 [32].

The seedling bags were put below the ground and the stem base was kept on the soil line, exposing the aerial part of the plants. The controlled fire experiment was carried out in the late afternoon, in the direction of the wind ('head fire') [33]. According to data from the meteorological station located next to the experiment (height: 337.0, latitude: $20.0^\circ 25.0' 24.4''$, longitude: $51.0^\circ 21.0' 13.1''$), the relative humidity was approximately 26%, with an average air temperature close to 34°C , 0 mm of precipitation, and winds of around 4 km/h. The plots were burned separately to maintain independence between the replicates. In each plot, a data logger was used with two sensors attached to measure the fire temperature during the experiment.

The duration of the fire in each plot was, on average, 8 min and 11 s. The maximum fire temperature was 136°C , and the flames remained at a maximum height of 0.5 m for most of the time. After being burned, the plants were removed from the field and taken back to the greenhouse (average temperature of 29.5°C and 8 mm of irrigation per day). Plants in the control group (12 individuals) were also divided into 3 blocks; these plants were not burned and were kept in a greenhouse under the same cultivation conditions.

2.3. Light Microscopy and Morphometrical Analysis

Six months after the treatment (plants 15 months old), when the plants subjected to fire already showed robust regrowth, samples of aerial vegetative organs were collected for studies under light microscopy (Figure 1A–C).

Samples of the young stem portion (located 1 cm below the shoot apex) and the basal stem portion (secondary growth; 3 cm above soil level) were collected from the main stem of plants in the control group and from the stem regrowth of plants subjected to fire. In addition, samples of leaf blades were collected from the first pair of fully expanded leaves located below the apex in control plants; in fire-treated plants, samples were collected from the first pair of completely expanded leaves formed after the application of fire. Samples of the median region of the leaf blade of the central leaflet were excised for anatomical procedures.

The samples were fixed in a mixture of formaldehyde, glacial acetic acid, and 50% ethanol (FAA 50) [34] for 24 h, dehydrated in an ethyl series, and embedded in hydroxyethyl-methacrylate (Historesin, Leica, Nussloch, Germany). After that, they were cross-sectioned (6 μm thick) using a RM2245 rotary microtome (Leica, Nussloch, Germany). Slides were stained with 0.05% toluidine blue in phosphate buffer and citric acid pH 4.5 [35] and mounted using Entellan synthetic resin (Merck, Germany). Then, they were analyzed using a Primo Star photomicroscope (Zeiss, Oberkochen, Germany) and the relevant results were recorded with an AxioCam ERc 5s attached camera (Zeiss, Oberkochen, Germany).



Figure 1. Visual documentation of postfire resprouting in *Protium heptaphyllum* (Aubl.) Marchand. (A). Resprouting two weeks after the fire experiment. Note the burned basal portion of the original stem and the emergence of new shoots from its base. (B). Individual one month prior to sampling, showing continued growth of shoots from the base. (C). Appearance of an individual of *P. heptaphyllum* the day before sampling, six months after the fire experiment. The original stem remains dormant and visibly damaged, while the plant exhibits vigorous vegetative growth of basal shoots.

In the midrib of the leaves, the total number of canals present in the cross-section was visually counted. The total areas of the midrib of the leaves and the lumen area of the secretory canals present in the leaves and stems were measured in cross-sections using the Digimizer program (version 5.9.3).

The relationship between the total lumen area of the canals and the total area of the midrib was calculated and the resultant data were multiplied by 100 to find the percentage ratio of area occupied by the secretory canals in the midrib. The density of secretory canals in samples of young stem and stem in secondary growth was calculated in an area of 0.2 mm².

2.4. Chemical Analyses

The analysis of the chemical constituents present in the leaves of plants from both the control and the fire treatments were carried out at the Pluridisciplinary Center for Chemical, Biological and Agricultural Research (CPQBA) at the State University of Campinas (UNICAMP).

At the end of monitoring in a greenhouse, all leaves were collected, dried in an oven at 60 °C, and then pulverized in a knife mill. Due to the small size of the seedlings, it was necessary to pool leaves from multiple individuals from the same plot (specified in item 2.2) to obtain sufficient material—following the plot design. Thus, three composite samples of 0.5 g were separated for the control group and three composite samples of 1 g were separated for the fire-treated group, which had a greater quantity of leaves. Next, 5 mL of ethyl acetate was added to each plastic tube, and the compounds were extracted for 2 min in the Ultra-Turrax IKA T10 Basic apparatus. Then, the samples were centrifuged for 7 min at 3000 rpm, subsequently filtered with a syringe with a specific Unichro filter (Cobetter, Hangzhou, China) for organic solvent (with hydrophilic PTFE/L membrane and 0.45 µm pore size), packaged in 5 mL glass vials, and kept under refrigeration.

The analyses were conducted on a HP-6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a HP-5975 mass spectrometer) and an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium gas was used as the carrier gas (1 mL/min), and the injection mode operated under the following conditions: injector at 250 °C, column at 60 °C, heating rate at 5 °C/min up to 280 °C (26 min), and detector at 300 °C.

Data acquisition and processing were performed using GC/MSD ChemStation software, version D.02.00.275. The identification of chemical constituents was carried out

through comparative analysis of the mass spectra of the substances with the National Institute of Standards and Technology (NIST 11) library.

2.5. Statistical Analysis

Considering the data related to the morphometry of the secretory canals, the analyses were performed using RStudio software (version 4.2.2). Initially, the data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene’s test, both at a 5% significance level. Since at least one of the assumptions was not met, group comparisons were carried out using the non-parametric Mann–Whitney test. The statistical model used to describe the morphometry of secretory canals was:

$$Y_{ijk} = \mu + T_i + b_j + \varepsilon_{ijk}$$

where Y_{ijk} is the value of the morphometry of secretory canals of plant k , under treatment i , in plot j ; μ is the overall mean; T_i is the fixed effect of the treatment ($i = 1$ and 2); b_j is the random effect of plot j ($j = 1, 2, 3, 4, 5$ and 6); and ε_{ijk} is the experimental error associated with each observation.

Data on the chemical compounds identified in leaf extracts were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene’s test, both at a 5% significance level. Then, they were subjected to an analysis of variance (ANOVA), at 5% significance, using RStudio software (version 4.2.2). The statistical model used to describe the chemical constituents was:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ij} is the value of the chemical constituent of the plant j , under treatment i ; μ is the overall mean; T_i is the fixed effect of the treatment ($i = 1$ and 2); and ε_{ij} is the experimental error associated with each observation.

3. Results

3.1. Anatomy and Morphometry of Secretory Canals

Resin secretory canals were present in the primary phloem of the leaf midrib (Figure 2A–C) and young stem (Figure 2D–E) and in the secondary phloem of older stem portions (Figure 2F) of *P. heptaphyllum* plants under both the control and fire treatments. The secretory canals comprised a uniseriate secretory epithelium and a wide lumen (Figure 2C,D).

In the leaf midrib, the number of secretory canals in plants subjected to fire was smaller than in control plants (Table 1). However, the total area of the midrib was also smaller in fire-treated plants than that in control plants (Table 1). The lumen area of the secretory canals in the midrib did not differ between plants in either treatment (Table 1). Nevertheless, when analyzing the total lumen area of all secretory canals present in the midrib, the leaves exposed to fire presented lower values. Thus, the percentage of the area occupied by the secretory canals in the midrib was higher in fire-exposed plants than in control plants (Table 1).

In the young stem portions, the density of secretory canals in the primary phloem was higher in individuals from the fire-treated group in comparison to control plants (Table 2). By contrast, the density of secretory canals in the secondary phloem of older stem portions was higher in plants from the control group than in fire-treated plants (Table 2).

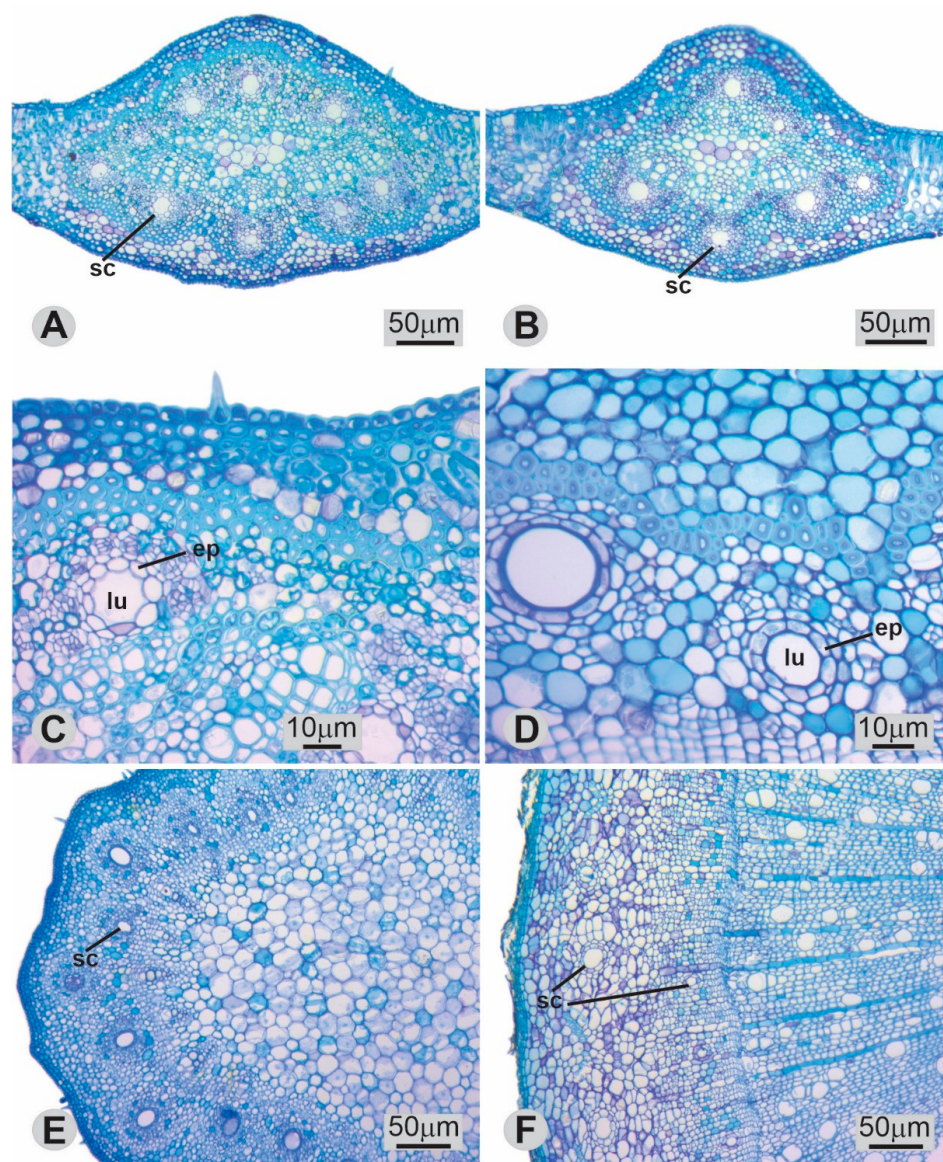


Figure 2. Cross-sections of the leaf (A–C) and stem (D–F) of *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae). (A–C). General view of the leaf midrib showing secretory canals in the phloem in control (A,C) and fire-treated plants (B). (D–E). Secretory canals in the primary phloem of young stem in fire-treated plants. (F). Secretory canals in the basal portion of the stem in secondary growth in fire-treated plants. ep: epithelium; lu: lumen; sc: secretory canal.

Table 1. Influence of fire treatment on the morphometric characteristics of the secretory system in the leaf midrib of *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae). Measurements represent the median and quartiles [Q1–Q3].

Morphometric Characteristics	Treatment	
	Control	Fire
Number of secretory canals	8 (7–9) ^A	7 (6–8) ^B
Lumen area of secretory canals (μm^2)	104 (85.2–121) ^A	103 (82.8–131) ^A
Total lumen area of the secretory canals—CA (μm^2)	808 (653–967) ^A	742 (534–907) ^B
Midrib area—MA (μm^2)	53,576 (43,072–57,898) ^A	37,510 (31,284–42,227) ^B
Relation CA/MA (%)	1.57 (1.31–1.76) ^B	1.98 (1.56–2.36) ^A

Different letters in columns indicate significant differences between treatments ($p < 0.05$).

Table 2. Influence of fire treatment on the morphometric characteristics of the secretory system in the stem of *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae). Measurements represent the median and quartiles [Q1–Q3].

Stem Portion	Morphometric Characteristics	Treatment	
		Control	Fire
Primary phloem in young stem	Density of secretory canals (in 0.2 mm ²)	6 (5–7) ^B	7 (6–8) ^A
	Lumen area of the secretory canals (µm ²)	143 (106–178) ^A	150 (108–212) ^A
	Total lumen area of the secretory canals (µm ²)	788 (637–1062) ^B	1028 (797–1345) ^A
Secondary phloem in basal stem portion	Density of secretory canals (in 0.2 mm ²)	7 (5.75–7) ^A	6 (4–7) ^B
	Lumen area of the secretory canals (µm ²)	177 (158–204) ^B	238 (173–286) ^A
	Total lumen area of the secretory canals (µm ²)	1240 (920–1420) ^A	1216 (990–1601) ^A

Different letters in columns indicate significant differences between treatments ($p < 0.05$).

The lumen area of the secretory canals in the primary phloem of young stems did not differ between plants from the control and fire-treated groups; however, the total lumen of the secretory canals in the young stem portions was greater in fire-treated plants (Table 2). In the older stem portions, the lumen area of the secretory canal in the secondary phloem was greater in plants from the fire-treated group than in those from the control group (Table 2). However, the total lumen area of the secretory canals in the secondary phloem did not differ between plants from the different treatments (Table 2).

3.2. Chemical Analysis

We identified an average of 71% of the chemical constituents present in the leaves of control individuals of *P. heptaphyllum* and 54% of the constituents in the group subjected to fire using gas chromatography coupled with mass spectrometry (Table 3). For certain compounds, it was only possible to identify the molar mass.

Table 3. Chemical compounds identified in leaf extracts of *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae) plants under control and fire treatments. Measurements represent the mean $\pm$ standard deviation.

Compound	Rt (min)	MM (g/mol)	Relative Percentage Per Treatment		p-Value
			Control	Fire	
MM = 220	21.55	220	0.85 $\pm$ 0.03 ^A	0.71 $\pm$ 0.08 ^A	0.2880
MM = 278a	25.98	278	1.84 $\pm$ 0.14 ^B	3.43 $\pm$ 0.38 ^A	0.0252
MM = 278 b	26.86	278	0.27 $\pm$ 0.27 ^B	1.07 $\pm$ 0.12 ^A	0.0163
Phytol	31.30	296	1.04 $\pm$ 0.06 ^B	1.78 $\pm$ 0.17 ^A	0.0205
MM = 284	34.50	284	1.25 $\pm$ 0.68 ^A	1.05 $\pm$ 0.41 ^A	0.7920
Esqualene	42.61	410	3.77 $\pm$ 0.29 ^A	3.43 $\pm$ 0.26 ^A	0.4470
γ -tocopherol	45.86	416	1.29 $\pm$ 0.10 ^A	1.67 $\pm$ 0.33 ^A	0.4720
Vitamin E	47.27	430	34.19 $\pm$ 0.39 ^A	20.79 $\pm$ 1.67 ^B	0.0009
Sitosterol	51.20	414	19.71 $\pm$ 1.33 ^A	15.40 $\pm$ 1.22 ^A	0.0661
β -amyirin	51.97	426	3.25 $\pm$ 0.54 ^A	2.87 $\pm$ 0.27 ^A	0.4860
α -amyirin	53.21	426	7.99 $\pm$ 0.60 ^A	8.49 $\pm$ 0.81 ^A	0.7020
Total identified (%)	-	-	71.25	54.41	-

Different capital letters in columns indicate significant differences between treatments ($p < 0.05$). Rt: retention time (min); MM: molar mass (g/mol).

The main constituents identified in the leaves of *P. heptaphyllum* through ethyl acetate extraction were vitamin E, sitosterol, α -amyirin, squalene, and β -amyirin. Most of the compounds did not differ between the conditions analyzed; however, three compounds

showed significant increases in individuals subjected to fire: MM = 278a, phytol, and MM = 278b. Vitamin E was the only compound that decreased under fire exposure (Table 3).

4. Discussion

Our results showed that resin secretory canals formed in the leaves and stem of *P. heptaphyllum* plants independently of the experimental conditions. The presence of phloem-associated secretory canals is a constitutive feature in this species [27,28]. However, differences in the abundance and size of secretory canals in leaves and stems (Tables 1 and 2) and in the chemical profile of leaves (Table 3) were observed between plants from the different treatments.

The influence of environmental factors on the secretory system has been documented in some species, with reports on the influence of light, temperature [30], water availability [31], nutrients [36], and fire [37,38], among others, on the structural and functional aspects of the secretory structures. Studies on fires are scarce and appear restricted to a few species, especially those from temperate zones [37,38]. Low-intensity fires seem to induce the formation of resin secretory canals in *Pinus ponderosa* [37]. *Pinus palustris* trees subjected to fire had smaller secretory canals than trees that did not experience fire [38]. In the present study with *P. heptaphyllum*, we observed that young organs that originated after fire exposure exhibited a more developed secretory system than plants from the control group. The percentage of the area occupied by secretory canals in the leaf midribs (Table 1) and the density and size of secretory canals in the primary phloem of the young stems (Table 2) were higher in plants exposed to the fire. It is worth noting that this proportional increase in canal area may be, at least in part, associated with an allometric effect caused by postfire stress. In this case, the reduction in midrib size observed in fire-treated individuals may have led to an apparent increase in canal area relative to tissue size, without necessarily indicating an absolute increase in canal formation. Although it was not possible to obtain direct measurements of leaf and leaflet length due to sample processing for anatomical analysis, we acknowledge this limitation and suggest that further studies should include morphological data to better understand these proportional changes.

Fire can induce first- and second-order injuries in trees, which can lead to physiological impairments in carbon and water relationships, consequently limiting plant functioning and growth [39]. This could explain the smaller midribs observed in the fire-treated *P. heptaphyllum* plants. In general, the availability and distribution of metabolites in the plant body are essential for the formation of new shoots [40] and influence the development of the secretory system [29]. Considering that the recovery of trees after fire events depends on environmental conditions and species-specific traits [39], different plant species are expected to respond differently to fire in terms of secretory system development. We observed a more developed secretory system in the leaves and young stems that originated after the fire treatment in *P. heptaphyllum*. Previous studies reported the induction of a greater development of the secretory system in plants as a response to environmental stress factors [30,41]. The relatively rapid increase in the production of plant phytohormones, such as ethylene and methyl jasmonate, following injury events can lead to the activation of defense-related genes by increasing the formation of secretory structures [41–43]. Thus, the expected increase in the production and release of these hormones in response to fire-induced stress may explain the greater development of the secretory system in young organs of *P. heptaphyllum* after fire events.

By contrast, the density of secretory canals in the secondary phloem in the older stem portions of *P. heptaphyllum* was higher in control plants than in fire-treated plants (Table 2). In fact, damage to cambium activity is expected to occur after fire events [39,44], decreasing meristematic activity or leading to cambial cell necrosis, which could affect

the production of phloem cells, including secretory canals, in the secondary phloem of *P. heptaphyllum*. However, in the present study, the fire conditions applied did not cause necrosis of the cambium, as new cells and secretory structures continued to form, albeit in smaller quantities. Although the density of secretory canals in the secondary phloem of *P. heptaphyllum* stem was lower in fire-treated plants than in control plants, the lumen area of the canals was greater in fire-treated plants (Table 2). Lytic enzymes are involved in cell wall changes during secretory canal growth and have been detected in the secretory system in *P. heptaphyllum* [27]. In this species, the three-dimensional growth of the secretory canals, including the enlargement of the lumen, appears to involve the action of cellulases [27], whose increased activity is proven to be stimulated by the action of ethylene [45], a plant growth regulator for which increased levels are a natural phenomenon in response to adverse environmental conditions [46,47], such as the stressful conditions of fire.

Since the development of secretory canals seems to be directly related to the amount of secretions produced and accumulated [48], it is expected that the greater development of the secretory system in young organs of fire-treated *P. heptaphyllum* plants leads to a greater amount of secretions produced and accumulated compared to control plants. The greater development of the secretory system in the leaves and young stem portions of *P. heptaphyllum* formed after the fire treatment may be related to the direction of energy resources to these portions of the plant body that resprouted after being burned. In fact, studies have shown that there is a greater allocation of resources for aboveground biomass formation in plants having buds positioned at or below ground level and protected inside bark [49], as occurs in *P. heptaphyllum* (personal observation). So, in the case of *P. heptaphyllum*, an important bulk of these resources could be invested in the development of resin canals, which have a very important ecological role in the interaction of these new organs with external environmental factors.

The stress caused by fire can trigger a cascade of complex mechanisms in the plant body [39]. Biotic attacks have been reported as common second-order effects of fire in trees [39], and the postfire mortality of surviving trees is often associated with insect attacks and microbial infections [39,50–52]. The improved secretory system of *P. heptaphyllum* after a fire event could represent a mechanism for improved protection against herbivores and pathogens during this crucial stage of plant reestablishment. In fact, the defensive role against biotic agents has been reported for the resin produced by *P. heptaphyllum* [26,53].

The amount and composition of chemicals produced by plants vary in response to external factors [13,29,54,55]. The major constituents identified in the leaf extracts of *P. heptaphyllum* in this study were vitamin E, sitosterol, α -amyrin, squalene, and β -amyrin (Table 3). Vitamin E, the name given to a group of eight tocopherols and tocotrienols [56], was the most abundant compound found in the leaves of both control and fire-treated plants of *P. heptaphyllum*. Studies demonstrated that levels of vitamin E, particularly α -tocopherol, increase in response to various biotic and abiotic stresses [57,58]. However, vitamin E was the only compound that decreased in the control group compared to the fire exposure group (Table 3). This was possibly due to the degree of fire stimulus since, in cases of more severe disturbances, levels of α -tocopherol, for example, tend to decrease [57]. Tocopherols confer tolerance to various abiotic stresses owing to their antioxidant activity, and a decrease in the levels of these compounds under stressful conditions may demonstrate a plant's sensitivity to the stress to which it is subjected [59]. By contrast, we detected increases in the concentrations of three compounds in fire-treated individuals of *P. heptaphyllum*, namely M = 278a, M = 278b, and phytol (Table 3), which is a constituent of chlorophyll and a precursor of tocopherols [60]. Certain stress factors, such as high and low temperatures, drought, and frost, influence the amount of phytol and compounds related to it in different plants [61]. Therefore, fire stimulus may have induced an increase in phytol levels in

the leaves of *P. heptaphyllum*. The physiological role of phytol in plant defense remains unknown, but it has been proven to have antimicrobial activity [60].

5. Conclusions

Our data proved that fire influences the development of the secretory system in the leaves and stems of *P. heptaphyllum* and point to differential responses in the primary and secondary phloem of aerial vegetative organs. While the secretory canals occupied a greater total area of the primary phloem in leaves and young stems of plants subjected to fire, in older stem portions the fire did not induce sufficient changes in the secretory system to culminate in changes in the proportional area occupied by the resin canals in the secondary phloem. Thus, we suggest that fire can increase the development of the secretory system in the youngest parts of the plant, favoring the production of secretions in the leaves and stems in primary growth. These data may present added economic value since they can guide strategies for sustainable exploitation of the secretions of this species.

Besides corroborating information that points to the plasticity of the secretory system in this species, our results contribute to the understanding of the influence of fire on the secretory system of Cerrado plants, providing data that help to understand the process of interaction between species and the environment in which they are found. The results obtained in this study are particularly important when considering the progressive increase in fires resulting from climate change caused by human activities. Knowledge of the plant secretory system's response to fires can support the understanding of plant interactions with climate disturbances and has added value when considering the ecological and economic importance of the secretions produced by *P. heptaphyllum*.

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References

1. Furley, P.A. The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian cerrados. *Glob. Ecol. Biogeogr.* **1999**, *8*, 223–241. [\[CrossRef\]](#)
2. Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; Da Fonseca, G.A.; Kent, J. Biodiversity hotspots for conservation priorities. *Nature* **2000**, *403*, 853–858. [\[CrossRef\]](#)
3. Coutinho, L.M. Fire in the ecology of the Brazilian cerrado. In *Fire in the Tropical Biota: Ecosystem Processes and Global Challenges*; Springer: Berlin/Heidelberg, Germany, 1990; pp. 82–105.
4. Arruda, F.V.d.; Sousa, D.G.d.; Teresa, F.B.; Prado, V.H.M.d.; Cunha, H.F.d.; Izzo, T.J. Trends and gaps of the scientific literature about the effects of fire on Brazilian Cerrado. *Biota Neotrop.* **2018**, *18*, e20170426. [\[CrossRef\]](#)
5. Simon, M.F.; Grether, R.; de Queiroz, L.P.; Skema, C.; Pennington, R.T.; Hughes, C.E. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 20359–20364. [\[CrossRef\]](#)
6. Rizzini, C.; Heringer, E. Estudo sobre os sistemas subterrâneos difusos de plantas campestres. *An. Acad. Bras. Ciênc.* **1966**, *38*, 85–112.
7. Durigan, G. Zero-fire: Not possible nor desirable in the Cerrado of Brazil. *Flora* **2020**, *268*, 151612. [\[CrossRef\]](#)
8. Miranda, H.S.; Sato, M.N.; Neto, W.N.; Aires, F.S. Fires in the Cerrado, the Brazilian savanna. In *Tropical Fire Ecology: Climate Change, Land Use, and Ecosystem Dynamics*; Cochrane, M.A., Ed.; Springer: Berlin/Heidelberg, Germany, 2009; pp. 427–450.
9. Dantas, D.; Souza, M.J.; Vieira, A.; Oliveira, M.; Pereira, I.; Machado, E.; Souza, C.M.; Rocha, W. Soil influences on tree species distribution in a rupestrian cerrado area. *FLORAM* **2018**, *25*, e20170605. [\[CrossRef\]](#)
10. Silva-Moraes, H.; Cordeiro, I.; Figueiredo, N. Flora and floristic affinities of the Cerrados of Maranhão State, Brazil. *Edinb. J. Bot.* **2019**, *76*, 1–21. [\[CrossRef\]](#)
11. Calixto Junior, J.T.; de Moura, J.C.; Lisboa, M.A.N.; Cruz, G.V.; Gonçalves, B.L.M.; Barreto, E.d.S.; Barros, L.M.; Drumond, M.A.; Mendonça, A.C.A.M.; Rocha, L.S.G. Phytosociology, diversity and floristic similarity of a Cerrado fragment on Southern Ceará state, Brazilian Semiarid. *Sci. For.* **2021**, *49*, e3459. [\[CrossRef\]](#)
12. Silva, G.O.; da Silva, R.C.; Souza, F.B.; Aguiar, B.A.C.; Lopes, V.C.; de Souza, P.B. Dynamics of a woody community in a Cerrado sensu stricto area in space and time. *Floresta* **2022**, *52*, 342–350. [\[CrossRef\]](#)
13. Langenheim, J.H. *Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany*; Timber Press: Portland, OR, USA; Cambridge, UK, 2003.
14. Daly, D.; Harley, M.; Martínez-Habibe, M.; Weeks, A. Burseraceae. In *Flowering Plants. Eudicots*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 76–104.
15. Daly, D.C.; Perdiz, R.O.; Fine, P.V.; Damasco, G.; Martinez-Habibe, M.C.; Calvillo-Canadell, L. A review of Neotropical Burseraceae. *Braz. J. Bot.* **2022**, *45*, 103–137. [\[CrossRef\]](#)
16. Cândido, J.; Lima, D.; Teixeira, P.R.; Souza, P. Florística do estrato arbustivo-arbóreo de uma área de Cerrado sensu stricto, Gurupi, Tocantins. *Encicl. Biosf.* **2016**, *13*, 1744. [\[CrossRef\]](#)
17. Solórzano, A.; Pinto, J.R.R.; Felfili, J.M.; Hay, J.D.V. Perfil florístico e estrutural do componente lenhoso em seis áreas de cerrado ao longo do bioma Cerrado. *Acta Bot. Bras.* **2012**, *26*, 328–341. [\[CrossRef\]](#)
18. Maia, R.M.; Barbosa, P.R.; Cruz, F.G.; Roque, N.F.; Fascio, M. Triterpenos da resina de *Protium heptaphyllum* March (Bourseraceae): Caracterização em misturas binárias. *Quim. Nova* **2000**, *23*, 623–626. [\[CrossRef\]](#)
19. Bandeira, P.N.; Pessoa, O.D.L.; Trevisan, M.T.S.; Lemos, T.L.G. Metabólitos secundários de *Protium heptaphyllum* March. *Quim. Nova* **2002**, *25*, 1078–1080. [\[CrossRef\]](#)
20. Marques, D.D.; Sartori, R.A.; Lemos, T.L.G.; Machado, L.L.; Souza, J.S.N.d.; Monte, F.J.Q. Chemical composition of the essential oils from two subspecies of *Protium heptaphyllum*. *Acta Amazon.* **2010**, *40*, 227–230. [\[CrossRef\]](#)
21. Corrêa, M.P. *Dicionário das plantas úteis do Brasil e das exóticas cultivadas: HL*; Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal: Rio de Janeiro, Brazil, 1984; Volume 4.
22. Oliveira, F.A.; Vieira-Júnior, G.M.; Chaves, M.H.; Almeida, F.R.; Florêncio, M.G.; Lima, R.C., Jr.; Silva, R.M.; Santos, F.A.; Rao, V.S. Gastroprotective and anti-inflammatory effects of resin from *Protium heptaphyllum* in mice and rats. *Pharmacol. Res.* **2004**, *49*, 105–111. [\[CrossRef\]](#)
23. Bandeira, P.; Lemos, T.; Santos, H. Atividade antimicrobiana e antioxidante do óleo essencial de *Protium heptaphyllum*. In *Proceedings of the 46° Congresso Brasileiro de Química*; Resumos; ABQ: Salvador, Brazil, 2006.
24. Rao, V.S.; Maia, J.L.; Oliveira, F.A.; Lemos, T.L.; Chaves, M.H.; Santos, F.A. Composition and antinociceptive activity of the essential oil from *Protium heptaphyllum* resin. *Nat. Prod. Commun.* **2007**, *2*, 1199–1202. [\[CrossRef\]](#)
25. Santos, F.A.; Frota, J.T.; Arruda, B.R.; de Melo, T.S.; da Silva, A.A.d.C.A.; Brito, G.A.d.C.; Chaves, M.H.; Rao, V.S. Antihyperglycemic and hypolipidemic effects of α , β -amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. *Lipids Health Dis.* **2012**, *11*, 1–8. [\[CrossRef\]](#)
26. Becerra, J.X.; Venable, D.; Evans, P.; Bowers, W. Interactions between chemical and mechanical defenses in the plant genus *Bursera* and their implications for herbivores. *Am. Zool.* **2001**, *41*, 865–876.

27. Palermo, F.H.; Rodrigues, M.I.d.A.; de Nicolai, J.; Machado, S.R.; Rodrigues, T.M. Resin secretory canals in *Protium heptaphyllum* (Aubl.) Marchand. (Burseraceae): A tridimensional branched and anastomosed system. *Protoplasma* **2018**, *255*, 899–910. [[CrossRef](#)] [[PubMed](#)]
28. Palermo, F.H.; de Nicolai, J.; Seixas, D.P.; de Melo Silva, S.C.; Rodrigues, T.M. Distribuição, morfologia e histoquímica do sistema secretor em raízes de *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae). *Holos Environ.* **2017**, *17*, 37–44.
29. Lin, J.; Sampson, D.; Ceulemans, R. The effect of crown position and tree age on resin-canal density in Scots pine (*Pinus sylvestris* L.) needles. *Can. J. Bot.* **2001**, *79*, 1257–1261.
30. Rodrigues, T.; Buarque, P.; Coneglian, A.; Reis, D. Light and temperature induce variations in the density and ultrastructure of the secretory spaces in the diesel-tree (*Copaifera langsdorffii* Desf.-Leguminosae). *Trees* **2014**, *28*, 613–623. [[CrossRef](#)]
31. Rodrigues, M.I.d.A.; Santos, E.M.G.; Nicolai, J.d.; Rodrigues, T.M. Leaf traits and herbivory in a resin-producing plant species growing in floodable and non-floodable areas of the pre-Amazonian white-sand forest. *Rodriguésia* **2024**, *75*, e01382023. [[CrossRef](#)]
32. Rissi, M.N.; Baeza, M.J.; Gorgone-Barbosa, E.; Zupo, T.; Fidelis, A. Does season affect fire behaviour in the Cerrado? *Int. J. Wildland Fire* **2017**, *26*, 427–433. [[CrossRef](#)]
33. Whelan, R.J. *The Ecology of Fire*; Cambridge University Press: Cambridge, UK, 1995.
34. Johansen, D. *Plant Micro Technique*, 1st ed.; MC Graw Hill Co. Inc.: New York, NY, USA; London, UK, 1940.
35. Sakai, W.S. Simple method for differential staining of paraffin embedded plant material using toluidine blue O. *Stain Technol.* **1973**, *48*, 247–249. [[CrossRef](#)]
36. Moreira, X.; Zas, R.; Solla, A.; Sampedro, L. Differentiation of persistent anatomical defensive structures is costly and determined by nutrient availability and genetic growth-defense constraints. *Tree Physiol.* **2015**, *35*, 112–123. [[CrossRef](#)] [[PubMed](#)]
37. Hood, S.; Sala, A.; Heyerdahl, E.K.; Boutin, M. Low-severity fire increases tree defense against bark beetle attacks. *Ecology* **2015**, *96*, 1846–1855. [[CrossRef](#)]
38. Slack, A.W.; Zeibig-Kichas, N.E.; Kane, J.M.; Varner, J.M. Contingent resistance in longleaf pine (*Pinus palustris*) growth and defense 10 years following smoldering fires. *For. Ecol. Manag.* **2016**, *364*, 130–138. [[CrossRef](#)]
39. Bär, A.; Michaletz, S.T.; Mayr, S. Fire effects on tree physiology. *New Phytol.* **2019**, *223*, 1728–1741. [[CrossRef](#)] [[PubMed](#)]
40. Clarke, P.J.; Lawes, M.J.; Murphy, B.P.; Russell-Smith, J.; Nano, C.E.; Bradstock, R.; Enright, N.J.; Fontaine, J.B.; Gosper, C.R.; Radford, I. A synthesis of postfire recovery traits of woody plants in Australian ecosystems. *Sci. Total Environ.* **2015**, *534*, 31–42. [[CrossRef](#)]
41. Tulik, M.; Jura-Morawiec, J. An arrangement of secretory cells involved in the formation and storage of resin in tracheid-based secondary xylem of arborescent plants. *Front. Plant Sci.* **2023**, *14*, 1268643. [[CrossRef](#)]
42. McKay, S.A.B.; Hunter, W.L.; Godard, K.-A.; Wang, S.X.; Martin, D.M.; Bohlmann, J.; Plant, A.L. Insect attack and wounding induce traumatic resin duct development and gene expression of (–)-pinene synthase in *Sitka spruce*. *Plant Physiol.* **2003**, *133*, 368–378. [[CrossRef](#)] [[PubMed](#)]
43. Schmidt, A.; Gershenzon, J. Cloning and characterization of two different types of geranyl diphosphate synthases from Norway spruce (*Picea abies*). *Phytochemistry* **2008**, *69*, 49–57. [[CrossRef](#)] [[PubMed](#)]
44. Partelli-Feltrin, R.; Smith, A.M.; Adams, H.D.; Thompson, R.A.; Kolden, C.A.; Yedinak, K.M.; Johnson, D.M. Death from hunger or thirst? Phloem death, rather than xylem hydraulic failure, as a driver of fire-induced conifer mortality. *New Phytol.* **2023**, *237*, 1154–1163. [[CrossRef](#)]
45. Ferrarese, L.; Trainotti, L.; Moretto, P.; de Laureto, P.P.; Rascio, N.; Casadoro, G. Differential ethylene-inducible expression of cellulase in pepper plants. *Plant Mol. Biol.* **1995**, *29*, 735–747. [[CrossRef](#)]
46. Abeles, F.B.; Morgan, P.W.; Saltveit, M.E., Jr. *Ethylene in Plant Biology*; Academic Press: Cambridge, MA, USA, 2012.
47. Achard, P.; Baghour, M.; Chapple, A.; Hedden, P.; Van Der Straeten, D.; Genschik, P.; Moritz, T.; Harberd, N.P. The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 6484–6489. [[CrossRef](#)]
48. Rodríguez-García, A.; Martín, J.A.; López, R.; Mutke, S.; Pinillos, F.; Gil, L. Influence of climate variables on resin yield and secretory structures in tapped *Pinus pinaster* Ait. in central Spain. *Agric. For. Meteorol.* **2015**, *202*, 83–93. [[CrossRef](#)]
49. Clarke, P.J.; Lawes, M.; Midgley, J.J.; Lamont, B.; Ojeda, F.; Burrows, G.; Enright, N.; Knox, K. Resprouting as a key functional trait: How buds, protection and resources drive persistence after fire. *New Phytol.* **2013**, *197*, 19–35. [[CrossRef](#)]
50. Breece, C.; Kolb, T.; Dickson, B.; McMillin, J.; Clancy, K. Prescribed fire effects on bark beetle activity and tree mortality in southwestern ponderosa pine forests. *For. Ecol. Manag.* **2008**, *255*, 119–128. [[CrossRef](#)]
51. Conedera, M.; Lucini, L.; Valese, E.; Ascoli, D.; Pezzatti, G.B. Fire resistance and vegetative recruitment ability of different deciduous trees species after low-to moderate-intensity surface fires in southern Switzerland. In *Proceedings of the VI International Conference on Forest Fire Research*; ADAI: Coimbra, Portugal, 2010; p. 12.
52. Westlind, D.J.; Kelsey, R.G. Predicting post-fire attack of red turpentine or western pine beetle on ponderosa pine and its impact on mortality probability in Pacific Northwest forests. *For. Ecol. Manag.* **2019**, *434*, 181–192. [[CrossRef](#)]

53. Asteggiano, A.; Occhipinti, A.; Capuzzo, A.; Mecarelli, E.; Aigotti, R.; Medana, C. Quali–quantitative characterization of volatile and non-volatile compounds in *Protium heptaphyllum* (Aubl.) Marchand resin by GC–MS validated method, GC–FID and HPLC–HRMS2. *Molecules* **2021**, *26*, 1447. [[CrossRef](#)]
54. Yang, L.; Wen, K.-S.; Ruan, X.; Zhao, Y.-X.; Wei, F.; Wang, Q. Response of plant secondary metabolites to environmental factors. *Molecules* **2018**, *23*, 762. [[CrossRef](#)]
55. Li, Y.; Kong, D.; Fu, Y.; Sussman, M.R.; Wu, H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiol. Biochem.* **2020**, *148*, 80–89. [[CrossRef](#)] [[PubMed](#)]
56. Schneider, C. Chemistry and biology of vitamin E. *Mol. Nutr. Food Res.* **2005**, *49*, 7–30. [[CrossRef](#)] [[PubMed](#)]
57. Munné-Bosch, S. The role of α -tocopherol in plant stress tolerance. *J. Plant Physiol.* **2005**, *162*, 743–748. [[CrossRef](#)]
58. Niu, Y.; Zhang, Q.; Wang, J.; Li, Y.; Wang, X.; Bao, Y. Vitamin E synthesis and response in plants. *Front. Plant Sci.* **2022**, *13*, 994058. [[CrossRef](#)]
59. Hasanuzzaman, M.; Nahar, K.; Fujita, M. Role of tocopherol (vitamin E) in plants: Abiotic stress tolerance and beyond. In *Emerging Technologies and Management of Crop Stress Tolerance*; Elsevier: Amsterdam, The Netherlands, 2014; pp. 267–289.
60. Fujimoto, T.; Abe, H.; Mizukubo, T.; Seo, S. Phytol, a constituent of chlorophyll, induces root-knot nematode resistance in *Arabidopsis* via the ethylene signaling pathway. *Mol. Plant-Microbe Interact.* **2021**, *34*, 279–285. [[CrossRef](#)]
61. Ekeberg, D.; Jablonska, A.M.; Ogner, G. Phytol as a possible indicator of ozone stress by *Picea abies*. *Environ. Pollut.* **1995**, *89*, 55–58. [[CrossRef](#)]

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