

Article

Toward Safer Resin Tapping: Assessing Alternative Chemical Stimulants for *Pinus pinaster*

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Abstract: The use of chemical stimulants in resin tapping is essential for prolonging the resin flow and enhancing production. Traditional stimulants, primarily composed of sulfuric acid, pose concerns related to workplace safety, environmental impact, and tree health. In this study, we compared alternative stimulant pastes containing ethrel, salicylic acid, and citric acid with the traditional Spanish and Brazilian stimulant pastes with higher contents of sulfuric acid. We tapped *Pinus pinaster* seedlings with five different stimulants, using untreated and mechanically wounded plants as controls. The resin yield, tree growth, and physiological parameters were compared. The pines stimulated with citric acid released ca. 50% more resin, while ethrel and salicylic acid yielded similar amounts to the traditional paste, suggesting their potential as viable alternatives. Although all stimulants reduced the seedling growth, no significant differences were observed in the midday water potential or stomatal conductance. The internal resin accumulation and resin canal density were strongly correlated with the total resin production, and more-acidic pastes tended to cause xylem damage and resin retention. Our findings suggest that moderate acidity is sufficient to trigger resin biosynthesis and release, and that safer, less corrosive formulations, like citric acid, may provide viable, safer, and more sustainable alternatives to conventional stimulants. While the results from the seedlings provide a rapid and cost-effective screening tool, anatomical and physiological differences from mature trees should be considered when extrapolating findings to operational settings.

Keywords: maritime pine; resin tapping; resin yield; stimulant pastes; sulfuric acid; salicylic acid; ethrel; citric acid



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1. Introduction

Resin is a natural exudate, primarily composed of terpenes and resin acids, and serves as one of the major defense mechanisms of conifers in response to abiotic and biotic stressors [1,2]. Resin is produced by the epithelial cells surrounding the resin canals and it is stored in their lumen. If mechanical damage occurs, resin is released from the canals and solidifies upon exposure to air as volatile terpenes evaporate, which increases the concentration of higher-molecular-weight compounds, and thus, forms a physical and chemical barrier against external agents [3]. Besides its ecological function, resin is a valuable non-timber forest product with extensive applications, including adhesives, coatings,

pharmaceuticals, and bio-based chemicals [4]. Resin tapping has played a historically significant role in forest-based economies, particularly in regions dominated by species such as *Pinus elliottii*, *Pinus pinaster*, and *Pinus merkusii* [5]. The growing demand for sustainable and renewable raw materials and its potential to integrate into sustainable forest management frameworks has led to a renewed focus on resin tapping as a viable economic and ecological practice [6,7].

Resin tapping involves inducing controlled incisions into the bark of living pine trees to expose resin ducts and promote resin exudation [8]. Although resin tapping dates to prehistoric times, it was not until the early 20th century when chemical stimulants were applied to the fresh wounds to prolong resin secretion by delaying wound closure and stimulating resin biosynthesis [9], leading to the development of less invasive and more efficient tapping methods [5]. Early stimulants were based on liquid sulfuric acid. Due to safety concerns, the first solid pastes combined sulfuric acid with kaolinite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$) and calcium chloride (CaCl_2), later replaced by gypsum [10]. This traditional paste has been shown to increase the resin flow by prolonging wound activity and remains widely used, although concerns regarding their long-term impact on tree health, workplace safety, and unwanted chemical residues persist [11].

In the last two decades, new stimulant formulations have been tested. Stimulants primarily contain an acidic compound that accelerates the resin flow and reduces crystallization, along with a carrier material that ensures adhesion to the wound [4]. At the anatomical level, the acid could facilitate the resin flow and release by affecting the turgor pressure of the epithelial cells lining the resin canal, increasing their size and facilitating resin release [5,12–15]. The resin flow is also enhanced because its acids do not crystallize [6]. Additionally, the acid induces the synthesis of defense proteins against pathogens and, due to its oxidative capacity, interacts with plant tissue, leading to the generation of reactive oxygen species [16–18]. This results in an extended wound area and prolonged resin flow and induces the formation of traumatic resin canals [6,19,20]. Thus, resin canal traits have been commonly used as proxies for tree carbon allocation to resin-based defenses and resin production [12,21–23]. New stimulant formulations include ethrel (2-chloroethylphosphonic acid), an ethylene precursor that increases radial growth and promotes the formation of resin canals [24–26]. Other active ingredients, such as plant hormones involved in defense, including salicylic acid, jasmonates [25,27–30], and citric acid [31], and adjuvants, including iron, potassium, and copper [26,32], have also been tested, yielding promising results. The efficacy of stimulant pastes varies based on the tree species, climatic conditions, and application frequency [11,27].

Resin tapping and stimulant pastes can impact the tree growth and health status [33,34]. Trees allocate substantial metabolic resources to resin biosynthesis, which can lead to reduced radial growth [35]. The wound activity varies based on the tapping intensity and stimulant application [33], whereas excessive resin extraction can reduce the tree lifespan and timber quality [8] and increase the susceptibility to droughts [36]. The increased susceptibility to various stressors in tapped trees warrants special attention in the current context of climate change. In particular, *Pinus pinaster*, the only pine currently tapped for resin in the Iberian Peninsula, has proven to be especially sensitive to recent drought periods, with mortality episodes being reported throughout its distribution range [37].

The objective of this study was the early selection of new stimulants with a lower concentration of sulfuric acid or an alternative formula for resin production in *Pinus pinaster*. To achieve this, stimulant pastes with different active ingredients were tested on *Pinus pinaster* seedlings to compare their effects on resin production and plant physiological status. Few studies have compared the anatomical and physiological impacts of stimulants with different levels of acidity on *P. pinaster*, and even fewer have assessed alternative,

safer stimulants under controlled conditions using young seedlings. The development of early screening methods to evaluate stimulant efficacy and plant health impacts can significantly accelerate the identification of safer formulations. Thus, our study addressed a key knowledge gap by comparing the resin yield and plant responses to both traditional and alternative pastes with reduced or no sulfuric acid content. While seedling-based assays do not fully replicate the anatomical complexity of mature trees, they offer a practical first step to identify promising formulations under controlled conditions. We hypothesized that (i) the resin production of *P. pinaster* is directly related to the acidity of the stimulant paste; (ii) the combined effect of wounding and chemical stimulants negatively affects the plant growth and wood quality, and (iii) plants stimulated with less sulfuric acid and alternative active compounds can yield similar resin production with less tissue damage and growth reduction than the traditional stimulant pastes.

2. Materials and Methods

2.1. Plant Material and Treatments

Pinus pinaster seedlings were grown over three years in 3.5 L plastic pots filled with 70% peat and 30% sand (*v:v*) and fertilized annually with slow-release fertilizer (Osmocote Plus Standard, 15:9:12 (N:P:K); 12–14-month release, ICL, Barcelona, Spain). The seedlings were watered at full capacity during the whole experiment. On 9 July 2020, 126 seedlings were selected for the resin-tapping experiment and the basal diameter and height were measured so that trees with similar sizes were evenly distributed between the treatments. The seedlings were arranged in parallel rows in a north–south orientation, which alternated treatments to minimize the environmental differences at the facilities in Universidad Politécnica de Madrid (School of Forestry Engineering, Spain; Lat. 40.452°; Lon. −3.724°). Every three weeks, starting at 10 cm from the pot surface, a circular wound was produced with a 1 cm diameter punch in the stem of all the plants, except for the control treatment, which removed the bark and the cambium and avoided damaging the xylem [31]. The hole was filled with the stimulant paste to ensure the same volume of stimulant in each seedling. The resin from the exposed xylem and the surroundings tissues was collected in a test tube placed and sealed with Parafilm until the end of the experiment. All the tubes and Parafilm tape were weighed before starting the experiment. This process was repeated three times. Every new wound was produced 5 cm above the previous wound in a spiral direction, starting at 5 cm from the base of the trunk and progressing upward clockwise (Figure 1).

Seven treatments were tested. Two treatments were used as controls: (i) control: plants without tapping and stimulant; (ii) wounded: plants tapped without the application of any stimulant. The other five treatments consisted of the application of different resin-tapping stimulant pastes according to the following formulas (percentages are expressed as mass proportions): (iii) sulfuric or traditional paste: 34.2% sulfuric acid 98% pure, 35.5% distilled water and 30.3% plaster; (iv) ethrel: 2.8% ethephon (60% *v/v*), 14% sulfuric acid 98% pure, 1.7% polysorbate, 1% cetyl alcohol, 60.2% distilled water, 4% vaseline, 5.5% silica, and 10.8% sawdust; (v) salicylic: 1% salicylic acid, 25% sulfuric acid 98% pure, 5% propylene glycol, 19% wheat straw, and 50% distilled water; (vi) citric: 40% citric acid, 26% calcium bentonite, 0.5% propylene glycol, and 33.5% distilled water; and (vii) pretta or Brazilian paste: 50.4% sulfuric acid 98% pure, 16.2% crushed rice husk, and 33.4% distilled water). Each treatment was applied to 18 seedlings.

We considered that the acidity in an aqueous solution of the stimulant pastes mainly depends on the quantity of sulfuric acid or citric acid in their formula (Table 1) and was calculated as the maximum capacity of release protons in an aqueous media to the theoretical concentration of the hydronium ion (H_3O^+) per gram of paste (Table 1).



Figure 1. Figure detail of the microtapping incision made using a punch tool (a); close-up of the incision (b); application of the stimulant paste (c); incision with the applied paste (d); sealing with the Parafilm (e); and third incision (f).

Table 1. Percentage of citric acid or sulfuric acid, moles of H_3O^+ per liter of stimulant paste, and pH of the stimulant pastes used in the experiment.

Stimulant Paste	Acid (%)	$[\text{H}_3\text{O}^+]$ En Moles/Litro	pH
Citric	40%	0.039	1.4
Ethrel	13.5%	0.189	0.72
Salicylic	24.5%	0.6125	0.21
Sulfuric	33.5%	1.139	−0.06
Pretta	49.4%	2.47	−0.39

2.2. Growth and Physiological Measurements

The basal diameter and height were measured at the beginning and at the end of the experiment. Growth was determined as the difference between the final and initial values. The relative growth rate was calculated as $(\ln(\text{Hf}) - \ln(\text{Hi})) / (\text{tf} - \text{ti})$, where Hf is the final height or final diameter, Hi is the height or diameter at the beginning of the experiment, and $(\text{tf} - \text{ti})$ is the duration of the tapping experiment. Six individuals of each treatment were randomly selected to measure the midday needle water potential (Ψ_{md}) and stomatal conductance (g_{sw}). Measurements were carried out on the upper part of the plant. For the Ψ_{md} measurements, needles were kept in plastic bags at 4 °C until measured in the laboratory with a pressure chamber (model 1000, PMS Instrument Co., Albany, OR, USA) within two hours. The stomatal conductance was measured with a porometer (model SC-1, Decagon Devices Inc., Pullman, WA, USA) on three dwarf shoots per seedling. This process was repeated four times during the experiment: before each tapping wound and at the end of the experiment.

2.3. Resin Yield and Dry Weight

At the end of September, all the test tubes were collected and weighted. The net weight of the released resin was obtained gravimetrically as the difference between the initial and final weights of the test tubes (released resin, RR). Additionally, the internal resin was extracted by modifying the protocol described in [38]. Briefly, a 20 cm long stem piece (10 cm above and below the wound) was cut into small transversal discs (ca. 5 mm width) with a razorblade. The disks were transferred into preweighed test tubes and immediately frozen at $-80\text{ }^{\circ}\text{C}$ until analyzed. For the resin extraction, hexane was added to the tubes until the disks of wood were completely covered. Then, the tubes were immersed in an ultrasonic bath at $20\text{ }^{\circ}\text{C}$ for 15 min and left for 24 h at room temperature under a fume hood. The extract was filtered through grade GF/F filters and collected inside preweighed glass test tubes. The whole extraction step was repeated. The glass test tubes were left inside the fume hood at room temperature until the solvent was evaporated to dryness (ca. one week). The residue was weighed (internal resin, IR). The addition of RR and IR was defined as the total resin content (TR). Finally, after the anatomical measurements (see below), the disks were dried in an oven at $103\text{ }^{\circ}\text{C}$ until a constant dry weight. For the data analysis, the values of total internal resin, total released resin, and total resin of every plant were referred to on a dry weight basis.

2.4. Resin Duct Density

Before determining the dry weight, the axial resin canal density on stem disks above the wounds (except for the control treatment) of six seedlings per treatment was measured with a stereo microscope with an integrated camera (Leica S9 I, Leica Camera AG, Wetzlar, Germany). Images were processed with ImageJ ver. 1.54 [39], where a surface of 8 mm^2 was defined in the last growth ring. The axial resin canal density (RCD) was assessed as the number of resin canals included within this area. Wound healing and the presence of chemical burns were also considered.

2.5. Statistical Analysis

The effect of the stimulants in the resin production and growth was tested by one-way ANOVA. The effect of the stimulants in physiological traits was analyzed using a linear mixed-model effect (LME) after checking the normality of the data. Ψ_{md} or g_s was used as the response variable; the date of measurement, the stimulant, and their interaction as explanatory variables; and the ID of the plant as a random effect. Statistical analyses were performed using a mixed-effects model with the date of measurement, the stimulant and their interaction as explanatory variables, and the ID of the plant as a random effect. When significant differences were found, post hoc comparisons were obtained with pairwise tests computed separately for each date and for each treatment across days. Finally, Pearson correlation coefficients between RR, IR, and TR and the physiological and growth variables were calculated. Data analyses were performed with statistical software R (ver. 4.0.0).

3. Results

3.1. Growth and Physiological Traits

At the beginning of the experiment, the mean height ($\pm\text{se}$) of the plants was $105.0 \pm 3.5\text{ cm}$ and the mean diameter was $13.0 \pm 0.8\text{ mm}$, with no significant differences between treatments. After three months, we observed higher growth and relative growth rates in the control plants than in the tapped plants, with no significant differences between stimulants. The control seedlings grew an average of $8 \pm 1.1\text{ cm}$ (0.2 month^{-1}), whereas the tapped seedlings grew $3.7 \pm 1.0\text{ cm}$ (0.01 month^{-1}) (Figure 2a). The diameter growth and diameter relative growth rates tended to be higher in the plants stimulated with *ethrel* ($2.8 \pm 0.3\text{ mm}$;

0.07 month^{-1}), intermediate with *sulfuric* and *pretta* ($1.6 \pm 0.3 \text{ mm}$; 0.04 month^{-1}), and the lowest in the control plants (Figure 2b).

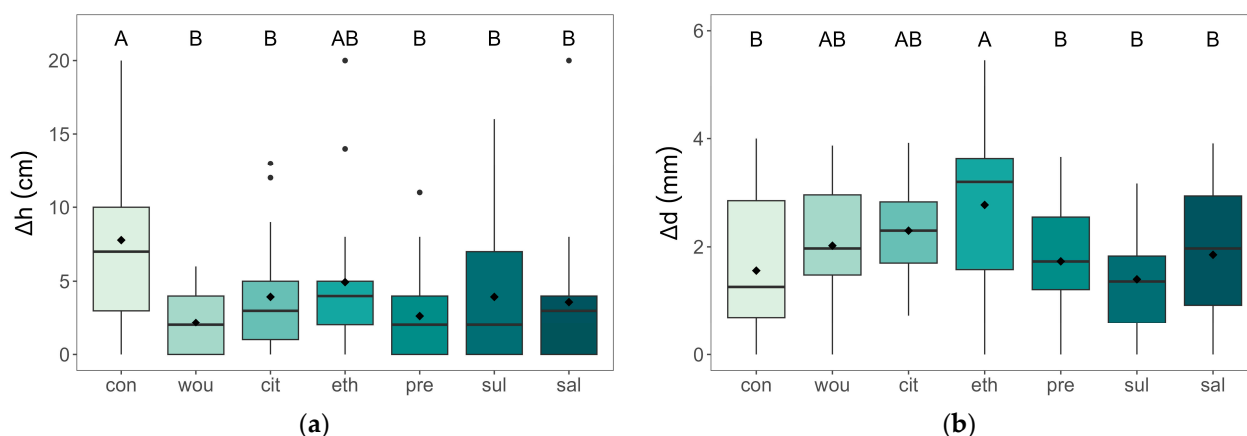


Figure 2. Boxplots of changes in height (a) and basal diameter (b) of *Pinus pinaster* seedlings between the beginning of the tapping experiment (09/07) and before the harvest (25/09). Con: control, no wound or stimulant; wou: wounded, tapped plants without stimulant; and the rest are tapped plants stimulated with cit: citric acid; eth: ethrel; pre: pretta paste; sul: sulfuric acid; and sal: salicylic acid. The straight horizontal black line within each box indicates the median. Different letters represent significant differences between treatments ($p < 0.05$).

The average values of Ψ_{md} were higher than -1.4 MPa throughout the experiment, suggesting no significant impact on the plant water status. The lowest values were measured at the end of July in wounded plants, at the end of August in plants stimulated with citric acid, and at the end of September in plants stimulated with ethrel and salicylic acid (Figure 3a). The control plants and plants stimulated with sulfuric acid and pretta maintained a similar Ψ_{md} during the whole experiment (Figure 3a). Stomatal conductance (g_{sw}) showed a similar trend in all the tapped treatments, with only slight differences in the mean values per date. The lowest values were shown at the beginning of the experiment ($196 \pm 12 \text{ mmol s}^{-1} \text{ m}^{-2}$), and the highest at the end of July, just before the second wound ($341 \pm 12 \text{ mmol s}^{-1} \text{ m}^{-2}$). Then, g_{sw} progressively decreased until it reached $253 \pm 10 \text{ mmol s}^{-1} \text{ m}^{-2}$ before the harvest (Figure 3b).

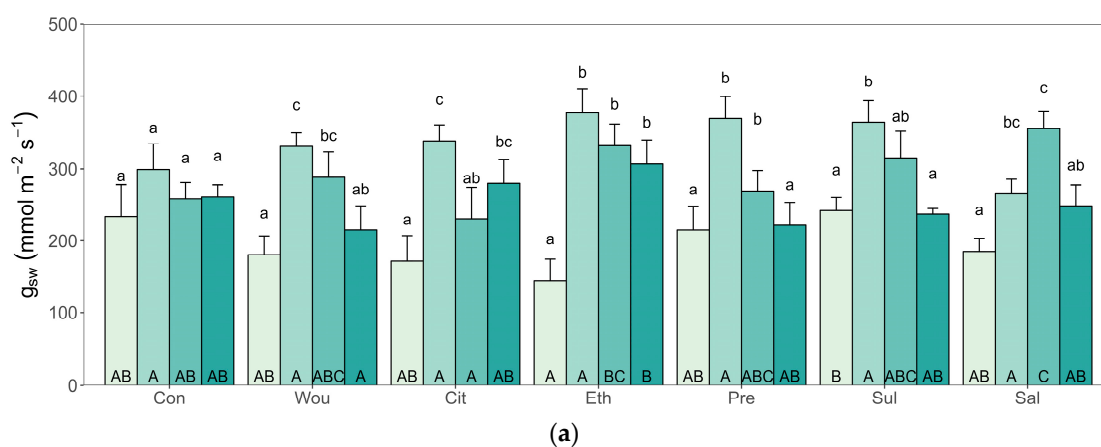


Figure 3. Cont.

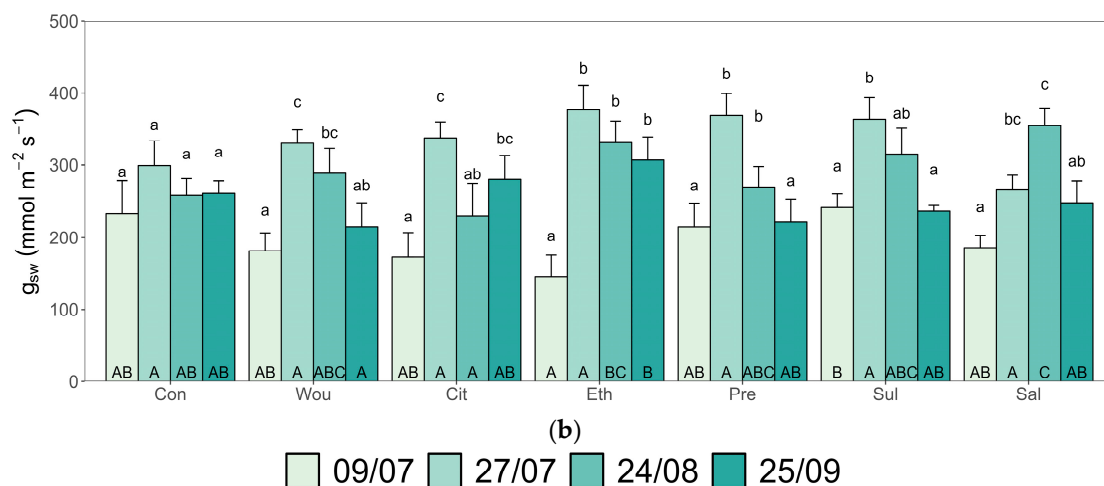


Figure 3. Mean water potential at midday (Ψ_{md}) (a) and mean stomatal conductance (g_{sw}) (b) of *Pinus pinaster* seedlings before the tapping (09/07) and on three dates (27/07, 24/08, 25/09) after the tapping and applying different chemical stimulants. Con: control, no wound or stimulant; Wou: wounded, tapped plants without stimulant; Cit: citric acid; Eth: ethrel, tapped plants stimulated with ethrel; Pre: pretta, tapped plants stimulated with pretta paste; Sul: sulfuric, tapped plants stimulated with the traditional stimulant paste based on sulfuric acid; and Sal: salicylic, tapped plants stimulated with salicylic acid. Bars represent the standard errors. Different lowercase letters represent differences between the dates within a given treatment ($p < 0.05$). Different uppercase letters represent significant differences between the treatments on the same date ($p < 0.05$).

3.2. Resin Yield

The released resin (RR) was the highest in plants stimulated with citric acid ($0.019 \pm 0.003 \text{ g g}^{-1}$), showing a production 50% higher than with the other stimulants ($0.012 \pm 0.002 \text{ g g}^{-1}$) and ca. 2.5 times higher than wounding. The RR increased from the first to the third wound, except in plants with citric acid, which showed a similar production of resin regardless of the number of wounds (Figure 4a). The proportion of internal resin (IR) ranged between 60% in plants stimulated with citric acid and more than 80% in the other treatments (Figure 4b). The plants stimulated with citric acid showed similar values of IR than the wounded plants (Figure 4b). The highest resin yield (TR) was observed in the pines tapped with stimulants with sulfuric acid in the formula (Eth, Pre, Sul, and Sal; Figure 4b), and was five times higher than in the control plants. The total resin yield of plants with citric acid was 30% lower than treatments with sulfuric acid but higher than the control and wounded plants (Figure 4b).

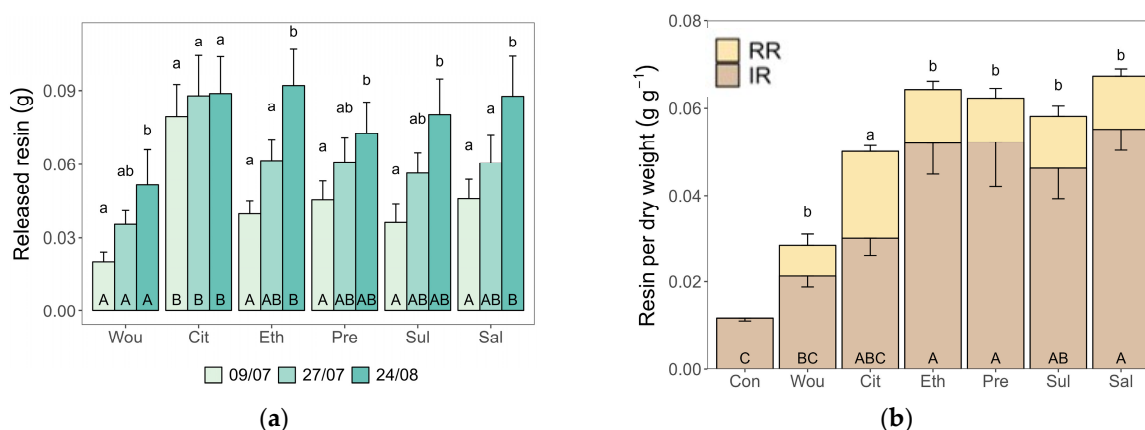


Figure 4. Released resin at each wound date (09/07, 27/07, 24/08) after tapping and applying different chemical stimulants (a) and internal (light brown) and released (light yellow) resin yields at

the end of the experiment (b). Con: control, no wound or stimulant; Wou: wounded, tapped plants without a stimulant; Cit: citric, tapped plants stimulated with citric acid; Eth: ethrel, tapped plants stimulated with ethrel; Pre: pretta, tapped plants stimulated with pretta paste; Sul: sulfuric, tapped plants stimulated with the traditional stimulant paste based on sulfuric acid; Sal: salicylic, tapped plants stimulated with salicylic acid. Bars represent the standard errors. In (a), different lowercase letters represent differences between dates within a given treatment ($p < 0.05$) and different uppercase letters represent significant differences between the treatments on the same date ($p < 0.05$). In (b), different lowercase letters represent significant differences in the released resin between the treatments ($p < 0.05$) and different uppercase letters represent differences in the internal resin between the treatments ($p < 0.05$).

3.3. Resin Canal Density

As occurred with resin production, the highest resin canal density was measured in the treatments with stimulants (Figure 5a). The control and wounded plants exhibited the lowest density, with no significant statistical difference between them. A chemical burn was observed in the xylem of some individuals treated with stimulants with sulfuric acid in their formulas (Figure A1a). Furthermore, the plants treated with stimulants containing lower concentrations of sulfuric acid or citric acid exhibited faster healing of the tapping wounds (Figure A1b).

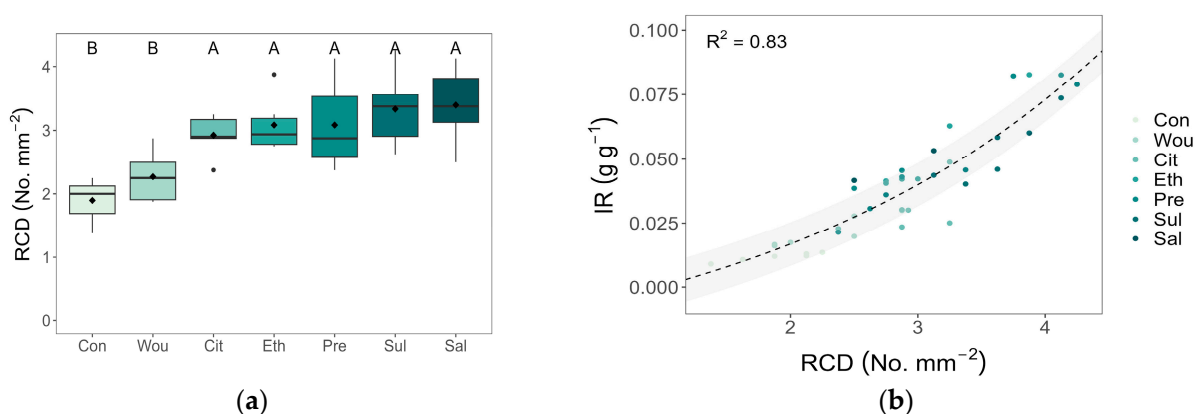


Figure 5. The resin canal density (RCD) (a) and relationship between the resin canal density (RCD) and internal resin (IR) of *Pinus pinaster* seedlings at the end of the experiment (b). Con: control, no wound or stimulant; Wou: wounded, tapped plants without a stimulant; Cit: citric, tapped plants stimulated with citric acid; Eth: ethrel, tapped plants stimulated with ethrel; Pre: pretta, tapped plants stimulated with pretta paste; Sul: sulfuric, tapped plants stimulated with the traditional stimulant paste based on sulfuric acid; and Sal: salicylic, tapped plants stimulated with salicylic acid. See the Materials and Methods Section for the complete formulas of the stimulant pastes. Bars represent the standard errors. Different letters represent significant differences between the treatments ($p < 0.05$).

3.4. Correlations

The resin yield was slightly correlated with the plant basal diameter ($r = 0.40$) but not with the plant height or relative growth rate (Figure A2). The total resin yield was more determined by the IR ($r = 0.96$) than by the RR ($r = 0.58$), which were also positively correlated, i.e., plants that released more resin also stored more resin in the xylem. The density of resin canals was positively correlated with the TR ($r = 0.92$), RR ($r = 0.47$), and IR ($r = 0.90$) (Figure 5b).

4. Discussion

The results of our resin microtapping experiment suggest that the use of new stimulants in resin tapping, as alternatives to the traditional stimulant paste, appears to be

feasible. Whereas sulfuric acid-based formulations did enhance the resin yield, moderate- or even low-acid alternatives produced similar results with less physiological damage. Seedlings stimulated with ethrel and salicylic acid, with lower concentrations of sulfuric acid, showed similar resin yields than those stimulated with the traditional stimulant paste. Our findings point to the direction that the resin yield is not strictly proportional to the acid strength but requires a minimum acidity threshold to trigger resin production and release. We also observed that high concentrations of sulfuric acid negatively affect seedling development and stem tissues, with pronounced xylem burns. Moreover, the growth was reduced in the tapped seedlings compared with the untreated controls, indicating a trade-off between the growth and defense under resin induction (Figure 2). This supports the notion that mechanical wounding and the action of chemical stimulants divert carbon resources from primary growth to the synthesis of defensive compounds. Notably, the slightly higher diameter growth observed in the ethrel-treated plants may reflect ethylene-mediated stimulation of cambial activity, as has been reported for similar formulations. Reducing the concentration of sulfuric acid is highly desirable to mitigate risks to both the environment and human health [31] and to minimize potential long-term detriments to wood quality through cambial necrosis and discoloration [6].

We observed a gradual increase in the released resin over time, mirroring patterns in mature stands (Figure 4; [40]). The resin production following the first tapping was primarily associated with the release of pre-existing resin stored within constitutive resin canals, mainly due to the synergistic effects of wounding and the application of stimulants [39]. Later, a complex signaling network mediated by phytohormones, such as ethylene, jasmonate, and salicylic acid, and reinforced by reactive oxygen species triggered the formation of traumatic resin canals and further resin biosynthesis [18,41–43]. The specific mechanism by which sulfuric acid potentiates these signaling cascades remains poorly understood [15]. In our experiment, a significant amount of the newly synthesized resin remained trapped within the stem (IR). This was likely due to xylem sealing caused by chemical burns (Figure A1); the rapid wound-healing ability of young seedlings [44]; and variations in the healing capacity based on the applied stimulant [45], which prevented further resin exudation. Unfortunately, we did not perform detailed histological or microscopic analyses to assess the potential occlusion, suberization, or other anatomical changes in the resin ducts or xylem tissue. Future studies using histological staining and imaging techniques are needed to clarify whether the strong correlation between the low pH and internal resin accumulation is mediated by the physical blockage of resin transport pathways.

Remarkably, the citric acid stimulant, which was the least acidic, did not damage the xylem and resulted in the highest resin release (Figure 4), as found before in another microtapping experiment with the same species [31], probably due to reduced resin crystallization. These findings suggest that a weak acid with a buffering capacity may inhibit the solidification of the released resin, thereby facilitating resin release. Conversely, the sulfuric acid-based stimulants resulted in higher internal resin accumulation, which was likely caused by chemical burns and rapid xylem occlusion. These contrasting patterns point to fundamentally different modes of action, where weaker acids may favor exudation, while stronger acids may induce biosynthesis but also limit resin release. The seedlings treated with ethrel and salicylic acid also favored resin accumulation (Figure 4). Both phytohormones promote resin canal formation and activate the plant's defense system [24,41]. Although with the current stimulant paste formula, we were unable to extract the resin effectively, other experiments showed that the resin yield was maximized with ethrel and salicylic acid in *P. pinaster*, *P. radiata*, and *P. elliotii* [5,11,27,46,47] and that salicylic acid maintained resin flow rates for longer [11]. Thus, the resin yield was not solely dependent

on the acid concentration. Instead, a minimum level of acidity was required to increase the resin release and biosynthesis. Moreover, combining various resin chemical elicitors may activate multiple pathways of resin induction [42,48,49] and improve the yield. Moreover, we acknowledge that the complexity of stimulant formulations—including multiple adjuvants and carriers, such as sawdust or polysorbate—makes it difficult to disentangle the specific contribution of each component. While our experiment focused on testing complete pastes, future studies should consider simplified or factorial designs to isolate the individual effects of acidic compounds versus inert materials and also study the pH buffering capacity of plant tissues in field conditions.

While biochemical processes underpin resin production, the physical impact of resin tapping further influences the seedling growth dynamics and anatomical defenses. Successive tapping incisions negatively impacted the tree growth of the seedlings and mature pines and reduced their recovery capacity after stress [34,50,51]. In some previous studies, stem wounding reduced the hydraulic conductance, thereby limiting the water availability for transpiration and affecting the stomatal conductance (g_s), though without altering the water potential (Ψ) [52] or drought recovery [53]. However, our study did not detect significant differences in the g_s or Ψ_{md} between treatments, despite temporal variations observed throughout the study period. These fluctuations may be attributed to environmental factors, including the temperature and vapor pressure deficit during summer, as well as leaf ontogeny, while the small wound did not affect the plant water relations [54]. The limited variations observed in these two physiological traits between the treatments may reflect the buffered nature of the experimental conditions and well-watered pots. Under natural field environments, greater fluctuations in temperature, soil moisture, and vapor pressure deficit could amplify the treatment effects on plant water status, particularly during summer [40].

Induced anatomical defenses, such as the formation of new resin canals, are metabolically costly and inherently slower than the synthesis of chemical defenses [55–57]. Trade-offs between carbon allocation to growth or induced defenses have been observed in pines, where a higher resin duct density was related to lower growth rates during tapping [58], and also between growth and constitutive resin canals [33], although in other studies, there was no evidence of this trade-off between growth and constitutive defense [21,59]. In our experiment, we found a strong link between the density of resin ducts and wounding when combined with the action of the stimulant. Additionally, we observed a positive correlation between the resin duct density and both the IR and TR (Figure 5), reinforcing the anatomical basis of induced oleoresin production [8]. The stimulants, particularly those containing active elicitors, promoted greater resin duct formation, which, in turn, increased the tree's capacity to store and exude resin. This confirms that the resin canal density can serve as a reliable proxy for evaluating stimulant efficacy and predicting the resin output potential. Furthermore, the interconnected network of axial and radial resin canals in seedlings is considerably simpler than in mature trees, suggesting that the effects of stimulants may be more pronounced in older individuals [30]. To enhance the extrapolation of seedling-based results to mature trees, future work should aim to develop size-scaling models or allometric relationships linking the plant dimensions, resin canal anatomy, and resin yield across developmental stages.

Resin production in tapped trees was affected not only by the stimulant applied but also by the plant's basal diameter. This relationship between the tree size and resin yield has been well-documented in previous experiments with seedlings [31] and field trials with mature trees, where more vigorous individuals, with denser crowns and higher basal area increments, generally produce greater amounts of resin [21,60,61]. Vigorous trees are likely able to mobilize carbon reserves more efficiently, either by forming a greater number of resin canals or by increasing the resin synthesis within pre-existing canals [62–64]. Anatomical

studies of the xylem structure during tree development have further demonstrated that tree vigor strongly influences the number of constitutive resin canals formed, with years with higher growth correlating with increased resin canal formation [23,58]. In our study, a moderate positive correlation ($r = 0.40$) between the basal diameter and total resin yield supports this pattern, suggesting that plant size and vigor can serve as early indicators of resin production potential. Additionally, plants with denser resin canal networks are both more efficient at storing and potentially at mobilizing resin in response to wounding and chemical signals. Given the promising performance and lower corrosiveness of citric acid-based stimulants, further formulation refinements will be necessary to ensure their practical applicability in operational settings. Enhancing adhesion to the wound surface through hydrophobic carriers, increasing resistance to wash-off under rain or irrigation, or developing slow-release delivery systems (e.g., via encapsulation or gels) could help maintain stimulant activity over longer periods. These improvements would make citric acid pastes more viable for field use while preserving their safety and efficacy advantages.

5. Conclusions

Our findings show that the resin yield was not strictly proportional with the acidity of the stimulant pastes, but that a minimum level of acidity was sufficient to induce oleoresin production and canal formation. Seedlings stimulated with citric acid released the highest amount of resin, about 50% more than with other stimulants, while ethrel and salicylic acid yielded amounts similar to those of the traditional sulfuric acid-based paste. Despite the effectiveness of stronger acids, they caused more tissue damage and greater internal resin retention, likely due to xylem sealing or necrosis. In contrast, citric acid caused no visible xylem burns and favored resin exudation. The strong correlation between the resin canal density and total resin yield ($r = 0.92$) and between the basal diameter and resin yield ($r = 0.40$) reinforced the anatomical basis of induced resin production. Importantly, our study demonstrated that low-acidity formulations—particularly citric acid—can provide safer and potentially more sustainable alternatives for resin tapping. Therefore, a field trial using this stimulant is recommended. Furthermore, our use of young *Pinus pinaster* seedlings under controlled conditions highlights the feasibility of early screening systems for stimulant evaluation [4,30,31,33]. While the results must be cautiously extrapolated to field conditions due to anatomical and physiological differences with mature trees, this approach offers a time- and cost-efficient tool to identify promising formulations prior to large-scale trials [30].

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Abbreviations

The following abbreviations are used in this manuscript:

Con	Control (plants without wounds or stimulant)
Wou	Wounded (plants tapped without stimulant)
Eth	Ethrel (stimulant paste containing ethephon)
Pre	Pretta paste (Brazilian stimulant paste)
Sul	Sulfuric acid-based stimulant paste
Sal	Salicylic acid-based stimulant paste
Cit	Citric acid-based stimulant paste
Hf	Height or diameter at the end of the experiment
Hi	Height or diameter at the beginning of the experiment
tf – ti	Duration of the tapping experiment
Ψ_{md}	Midday needle water potential
g_{sw}	Stomatal conductance
RR	Released resin
IR	Internal resin
TR	Total resin content (RR + IR)
RCD	Resin canal density
ANOVA	Analysis of variance
LME	Linear mixed-effects model
SE	Standard error

Appendix A

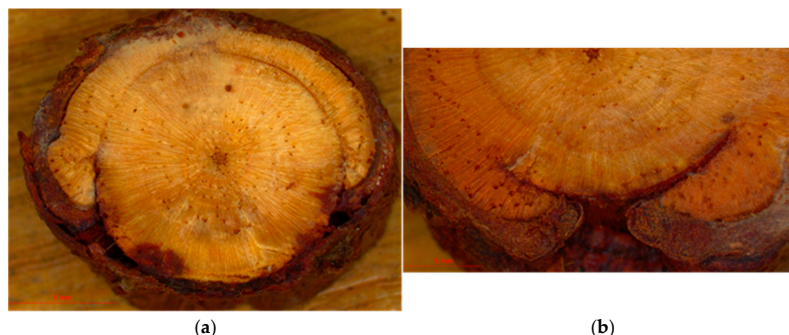


Figure A1. Details of xylem with necrosis in the cambium and chemical burn in the xylem in a *P. pinaster* seedling stimulated with pretta (a). Details of the xylem of a seedling stimulated with citric acid. Note the better healing capacity and absence of a chemical burn (b).

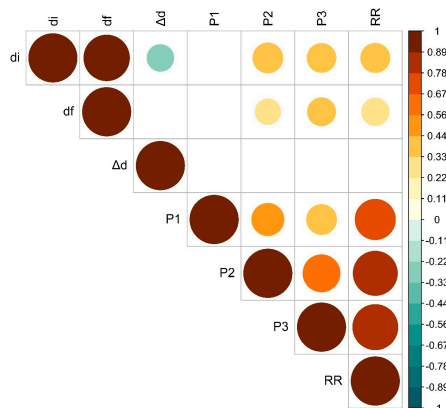


Figure A2. Correlation matrix between the plant initial diameter (di), plant final diameter (df), diameter growth during the experiment (Δd), released resin at each wound date (P1: 09/07, P2: 27/07, P3: 24/08), and total released resin (RR).

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