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Original Article

Effect of Ethephon on the Formation of Traumatic Resin Ducts in *Pinus merkusii* Seedlings

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What is already known on this topic?

- *Previous studies have applied ethepon to various other pine species.*
- *Previous studies have applied ethepon to Pinus merkusii at different concentrations, but have not addressed its wood anatomy.*

What this study adds on this topic?

- *Provides information related to the anatomical response of Pinus merkusii wood after ethepon application.*
- *This study focuses on the traumatic resin canal, which is strongly suspected to be related to sap productivity.*
- *Results showed that in terms of wood anatomy, Pinus merkusii formed significantly more resin canals after ethepon application.*

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ABSTRACT

Pinus merkusii shows great potential as a resin-producing wood in Indonesia, with the global demand for pine resin reaching 1 million tons annually. This study investigated the effect of ethephon on the anatomical properties of *P. merkusii,* particularly the resin duct that affects resin productivity, to increase the efficiency of pine resin tapping. Ethephon was mixed with Vaseline to create concentrations of 0%, 2%, 4%, and 8% and then applied to the seedlings at 0.5 g for 3 and 6 weeks. The results showed that various ethephon concentrations significantly increased the frequency, diameter, and proportion of the traumatic resin duct, as well as ray frequency, where 8% of the ethephon concentration yielded the best results. Furthermore, the treatment duration from 3 to 6 weeks significantly increased the proportion of the traumatic resin ducts, ray frequency, and ray height, where the 6 weeks showed the best results. The results indicated that ethephon promoted the formation of traumatic resin duct formation in *P. merkusii* seedlings, which has a strong correlation with resin production and could potentially increase future resin productivity.

Keywords: Resin-producing wood, stimulant, wood anatomy, wood formation

Introduction

Pinus merkusii Jungh. et de Vries is a resin-producing wood in Indonesia with a wide range of applications and significant potential. As a nontimber product, pine resin is a major commodity in global trade. According to Salsabila and Chumaidi (2022), Indonesia, particularly Perum Perhutani (Indonesia State Forest Enterprise), contributes 10% of the world's pine resin demand, and its annual exports reach 90,000 tons. On the other hand, the strong global demand for pine resin, which reaches almost 1 million/year and continues to increase, highlights the need for innovative approaches to increase its productivity to meet world demand for pine resin. Therefore, studies on the effect of pine productivity improvement treatments on *P. merkusii* are particularly relevant.

Several methods can be used to increase the productivity of resin from pine trees, and one of the most effective is to apply stimulants. Pine tree stimulants typically contain an acidic substance that stimulates a reaction, condition, or process that induces resin production. In pine resin tapping, adding an acidic substance can reduce the crystallization, freezing, or drying of the resin that comes out due to stem injury (Sukadaryati et al., 2014). Stimulants can also enhance resin productivity by inducing the formation of resin ducts. Resin is the product of the physiological processes of a tree, and the various factors affecting these physiological processes also influence the amount of resin produced by the tree (Suharlan & Herbagung, 1983). Pine resin flows through the resin ducts when the pine stem is injured; this type of resin is categorized as oleoresin and contains resin acids and turpentine. The two types of resin ducts are normal resin ducts, which naturally occur in the life cycle of resin-producing plants, and traumatic resin ducts, which appear as a form of defense response of woody plants against any disturbance. The latter type increases resin production and flow (Derose et al., 2017).

The resin duct is formed through several mechanisms: lysigenous, when the duct develops from the disintegration of cells; schizogenous, when the duct forms through the separation of cells; and schizolysigenous, which is the combination of both mechanisms (Tsoumis, 1969). In addition to being influenced by the genetic traits of pine trees that naturally have different resin production capabilities, resin duct formation is affected by environmental and physiological factors. In terms of physiology, resin duct formation is influenced by growth hormones (Susilowati et al., 2013).

The resin duct is a prominent feature found in pine trees. Resin ducts are plentiful and evenly distributed in pine trees compared with other resin-producing plants. Normal and traumatic resin ducts (TRDs) sometimes can be found within the same growth ring (Panshin & Zeeuw, 1970). Whether normal or traumatic, an increase in the number and diameter of resin ducts can indicate the tree's level of resistance to injury and pest attack (Hood et al., 2015). Induced traumatic resin ducts, or commonly known as TRDs, are assumed to indicate the resistance level of pine against wounds, insect attacks, or pathogens and potentially aid in increasing resin production (Schmidt et al., 2011). Resin ducts can either occur naturally during wood development or be induced by external factors. In response to injury, conifers form an axial resin duct in the secondary xylem, which may be limited in number or may not appear at all (Nagy et al., 2000). Therefore, stimulants are employed to enhance the formation capability of axial resin ducts.

Ethylene is one of the hormones that play a role in resin duct formation and acts as a link between external and internal factors (Abeles et al., 1992). Stimulants essentially trigger an increase in the ethylene levels of resin-producing plants, raising the osmotic and turgor pressure and causing a rapid and prolonged surge in resin flow (Moir, 1970 in Hidayati, 2005). Ethylene can be used to stimulate resin exudation. Furthermore, resin formation can be increased by activating ethylene in the plant and creating a wound . Kasmudjo (1992) stated that stimulants can open a narrowed or blocked resin duct through acid heating, hydrolyzing the resin ducts and parenchyma cells, decreasing the pressure, and allowing the cell fluid to flow out, resulting in thin resin that flows out for a long duration. In general, the concentration of stimulants has a significant effect on the average productivity of pine resin.

Ethephon is a widely used ethylene-releasing agent in agriculture (Li et al., 2021). This organic chemical product helps regulate plant growth and plays a significant role in fruit ripening and resin flow stimulation in injured tissue. When applied, ethephon decomposes into several other substances (such as ethylene, hydrochloric acid, and phosphoric acid), takes 5–6 hours after application to work effectively, and has no immediate effect on the treated tissue (Yew, 1998). According to Mori et al. (2011), ethephon stimulates the formation of new resin ducts. Wolter and Zinkel (1984), Babu et al. (1987), and Yamamoto and Kozlowski (1987) reported an increase in the number of resin ducts in some resin-producing woods after the application of ethephon at various doses, leading to enhanced production capacity. The present study aims to investigate the effect of different ethephon concentrations and treatment durations on wood anatomical changes, particularly TRD formation, in *P. merkusii* seedlings. On the other hand, the formation of TRD as a defense response in resin-producing wood can be influenced by changes in environmental conditions, not limited in only tissue disturbance. Therefore, the use of seedlings in wood anatomical experiments enhances the ability to control environmental conditions. The optimum concentration and treatment duration that yield the best results are also determined. The results may provide useful information for the future development of pine resin stimulants in order to increase the global production of pine resin.

Material and Methods

Study Location and Field Stage

This study was carried out in a private nursery in Pule, Trenggalek, East Java, Indonesia. The average temperature of the study area ranges from 19°C to 32°C, with humidity levels between 60% and 95%. The annual rainfall (February–December 2022) is between 18 mm and 35 mm (Badan Pusat Statistik, 2022). Thirty seedlings of 2-year-old *P. merkusii* in healthy condition with similar height and diameter were chosen for this study. The seedlings were grouped based on treatment duration (3 and 6 weeks) and then subgrouped based on ethephon concentration (including control, 0%, 2%, 4%, and 8%). The concentrations were selected to maintain the acidic nature of the ethephon, preventing harm to the seedlings. Except for the control group, the seedlings were then given a wound of 1 × 1 cm size with a depth of ±0.2 cm at 10 cm above the ground. Ethephon (Ethrel, 480 g/L; from PT. Bayer Indonesia), which was diluted with Vaseline (0.815–0.880 g/mL; from PT. Brataco, Bekasi, Indonesia) into different concentrations, was applied to the wound at an amount of ±0.5 g. The wound was then covered with parafilm (Parafilm PM996, Bemis Company, Sheboygan Falls, WI HQ, United States). After 3 and 6 weeks, each group was harvested. Samples of 4 cm long cuts around the previously stimulated area were collected and then preserved in 4% glutaraldehyde.

Sample Preparation, Observation, and Measurement

The harvested samples were cut into 2 cm long parts. The sections above the wound were then sliced using a microtome (Yamatokohki, Saitama, Japan) with 20 and 15 µm thicknesses for the transverse and tangential sections, respectively. These sections were stained with safranin (WAKO Chemical Industries), dehydrated, cleared with xylol, mounted on microscope slides, fixed with resin (Entellan New, Merck Company, Darmstadt, Germany), and covered with a cover glass. The samples were then observed and photographed under a light microscope, Olympus BX51 (equipped with a digital camera DP70, Olympus Corporation). The photographs were focused on the area of the stem whose transverse and tangential sections were treated with stimulants. The captured images were then analyzed and measured using the image analysis software Fiji ImageJ. The following anatomical features were observed and measured: TRD frequency, diameter, and proportion. Furthermore, Robnett and Morey (1974) reported a change in anatomical features of mesquite and huisache after ethephon treatments; based on this previous study, other anatomical changes, including tracheid diameter (TD), wall thickness (TW), ray frequency (RF), and height (RH) were also observed.

The diameter, frequency, and proportion of TRD were measured in the transverse section. In particular, TRD diameter was measured perpendicular to the ray by determining the distance between the two outermost points of the resin duct, including the epithelial cells. The measurement was carried out for 10 randomly selected TRDs. Meanwhile, TRD frequency was calculated in a field of 1 × 1 mm with five repetitions. The count included all TRDs within the observation field. Traumatic resin duct proportion was measured in a field of 1 × 1 mm with five repetitions, using a tracing method by creating polygon lines on the edges of a TRD that appeared inside the observation field. The area of the polygon line was then divided by the area of the observation field to calculate the proportion. Other anatomical features such as TD and TW were measured in the transverse section. Tracheid diameter was obtained by measuring the distance between the two outermost points of the tracheid with 25 repetitions. Wall thickness was calculated by subtracting the tracheid diameter from the lumen diameter and then dividing the result by two with 25 repetitions. Ray frequency was measured in the transverse section by counting its presence in a 1 mm field in 15 randomly selected areas. Ray height was obtained by measuring the distance between the two outermost points of the ray cell parallel to the tree axis in the tangential section.

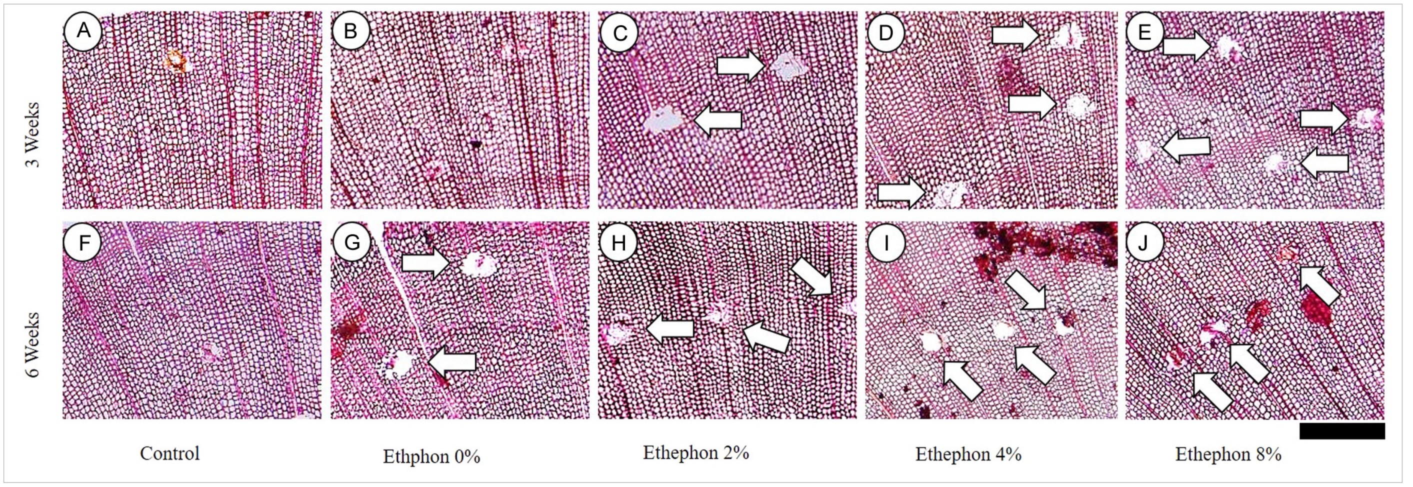

Figure 1. *Cross section of Pinus merkusii seedlings with various concentrations and treatment durations. Arrows indicate resin canals. Scale bar = 500 µm.*

The data collected for each parameter were analyzed using the Statistical Package for Social Sciences version 25.0 software (IBM Corp.; Armonk, NY, USA) with a 95% CI. Two-way analysis of variance was employed, a significant difference was calculated at $\alpha = 0.05$, and Tukey honestly significant difference (HSD) post hoc test was applied to identify which specific groups differed significantly.

Results and Discussion

The anatomical features in the transverse section of *P. merkusii* seedlings were observed after treatment with various ethephon concentrations for different durations. The results are displayed in Figure 1. An increasing TRD formation was noted from 3 weeks to 6 weeks and from the control group to 8% ethephon treatment. No TRD was formed in the control sample (Figure 1A and F). In Figure 1B, TRDs began to form in small numbers and imperfect shapes. Figure 1C, E, G, and J shows that the TRDs were fully formed, with their appearance increasing with ethephon concentration and treatment duration. Figure 2 presents the average measurements of TRDs.

Based on Figure 1, it can be seen that TRD is present in all samples except the control group, suggesting that TRDs will not appear if there are no interruptions, such as wounding or stimulation. The resin duct in the control group was considered a normal resin duct and appeared smaller in diameter compared with the TRDs in the other samples. This finding aligns with IAWA (2004), which stated that the difference

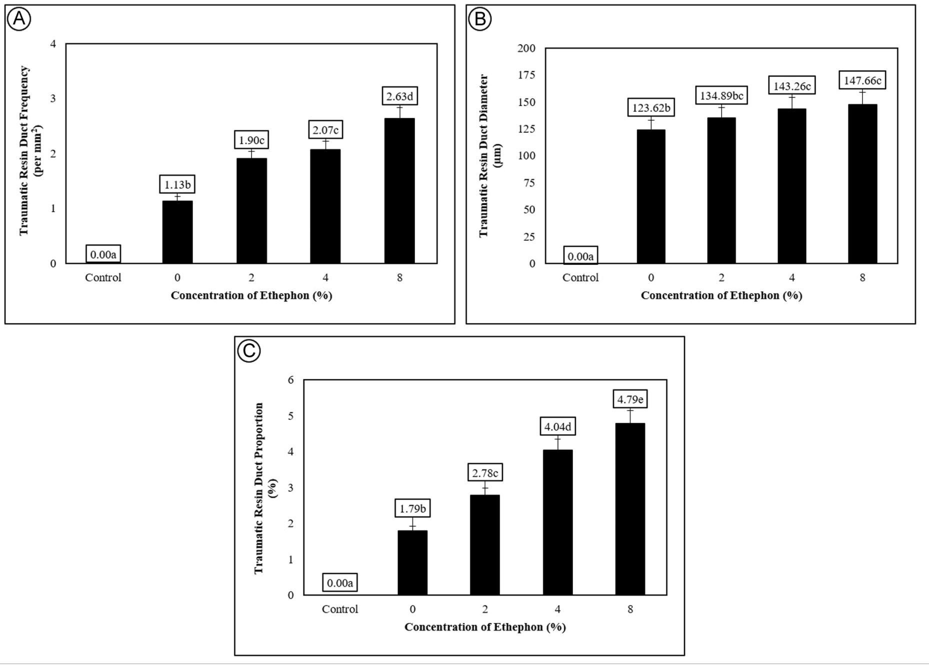

Figure 2. *Average measurement of traumatic resin duct frequency (A), traumatic resin duct diameter (B), and traumatic resin duct proportion (C). Standard error is shown by the error bars.*

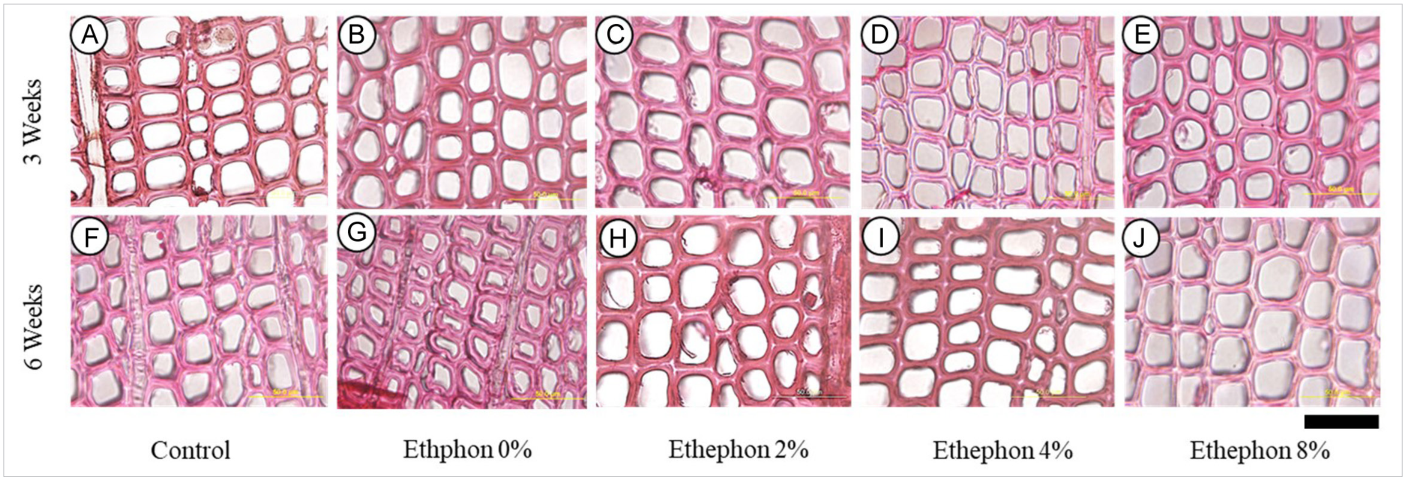

Figure 3. *Average Measurement of Ray Frequency*.

between TRD and normal resin duct is their size: TRD has a larger diameter than normal resin duct. Traumatic resin ducts may only appear when the stem is interrupted, at least by being wounded. This phenomenon was observed in the samples treated with only wounding without ethephon in this study (Figure 1B and G). It is clearly shown that wounding without ethephon resulted in the formation of TRDs, albeit in small amounts and incomplete differentiation. It is also indicated that the TRDs without stimulation took over 3 weeks to differentiate completely. When the same treatment of wounding without any stimulant was continued for 6 weeks (Figure 1G), the TRDs appeared in fully differentiated conditions in the sample. Furthermore, the other samples treated with ethephon concentrations of 2% or higher exhibited a significant increase in TRD, indicating that ethephon helps stimulate tissue growth. The ethylene content released by ethephon plays a crucial role in the increase in TRD formation.

Statistical analysis shows that ethephon concentration significantly affected the frequency, diameter, and proportion of TRD, and the treatment duration only significantly affected the proportion of TRD, as shown in Table 1. Furthermore, no interaction was found in this study. The average value of the TRD frequency after the treatment is 1.68 cell/mm^2, with the lowest value at 0.86 cell/mm^2 in the 2% ethephon concentration and gradually increased until the highest at 2.8 cell/mm^2 to the 8% ethephon concentration, as shown in Figure 2A. This finding showed a slightly lower frequency than the findings of López-Villamor et al. (2021), which found that the application of stimulants containing ethylene in 3-year-old seedlings of some pine species stimulated the frequency of resin ducts at 1.7 cell/mm^2. The TRD frequency in this study is also lower than Yamamoto and Kozlowski (1987), who reported an increase in TRD frequency of 3.9 cell/mm^2 after applying various ethephon concentrations in 1-year-old *Pinus halapensis* seedlings. Besides, the results were also lower than Praptoyo and Cabout (2013), where the ethylene releasing stimulant increased the frequency of TRD up to 2.3 cell/mm^2 in mature *P. merkusii* after 6 months of treatment duration.

Furthermore, the application of various concentrations of ethephon stimulated an increase in the average diameter of TRD until 131.2 µm; this value is much higher than the diameter of the normal resin duct of *P. merkusii* in general, reported by Susilowati et al. (2013), at 109.42 µm. The lowest diameter of TRD in this study was 123.62 µm in the control group, and the highest was 147.66 µm in the 8% concentration of ethephon, as shown in Figure 2B. This value is higher than the diameter of TRD of 3-year-old seedlings of some pine species reported by López-Villamor et al. (2021). The value is also higher than mature aged *P. merkusii* in Praptoyo and Cabout (2013) and Susilowati et al. (2013).

In general, the increase in TRD formation, both frequency and diameter, can also be caused by the acidic nature of ethephon, causing TRD expansion due to acid heating (Kasmudjo, 1992). The TRD frequency and diameter significantly increased from its first appearance in the samples with 0% ethephon concentration to those with the highest ethephon concentration. This increase in TRD frequency and diameter is correlated with the rise in the TRD proportion. The TRD proportion was determined by measuring the area of TRDs in one measurement field in the transverse section. Hence, an increase in frequency and diameter will raise the TRD proportion due to the increase in the area of TRD. The TRD proportion significantly increased from its first appearance in 0% to the highest ethephon concentration. The average value of the TRD proportion is 3.1%, with the lowest value of 1.2% in the 0% ethephon concentration and the highest of 5.19% in the highest ethephon concentration. This value is higher than the finding of Praptoyo and Cabout (2013). In addition, there is very limited information about the effect of stimulants on the TRD proportion, and further research needs to be carried out to clarify this result.

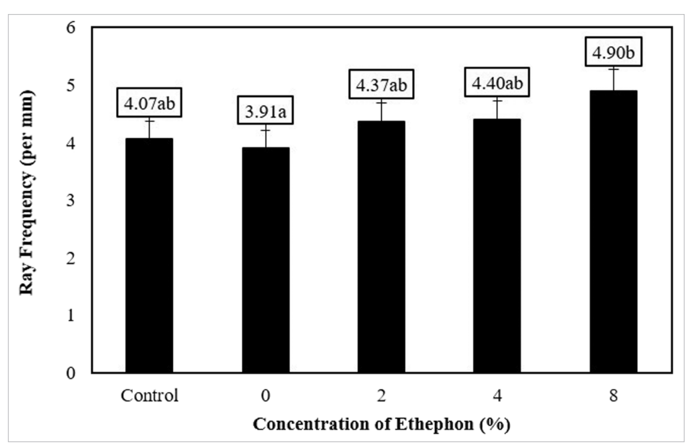

Figure 4. *Average measurement of other anatomical changes (TD (a), TW (b), RF (c), and RH (d)). Standard error is shown by the error bars.*

Table 1.
Statistical Analysis Results of Anatomical Changes of Pinus merkusii Seedlings

Parameters	Maximum	Minimum	Mean	Significance		
				$p_{Concentration}$	$p_{treatment\ duration}$	$p_{interaction}$
Traumatic resin duct						
Frequency (cell/mm^2)	2.8	0.87	1.68	.00*	.08 ns	.56 ns
Diameter (μm)	149.25	114.94	131.2	.00*	.11 ns	.34 ns
Proportion (%)	5.19	1.2	3.01	.00*	.00*	.20 ns
Other anatomical changes						
Tracheid diameter (μm)	29.15	25.02	27.22	.10 ns	.73 ns	.23 ns
Wall thickness (μm)	4.64	2.93	3.57	.10 ns	.72 ns	.21 ns
Ray frequency (cell/mm)	5.33	3.59	4.33	.03*	.00*	1.00 ns
Ray height (μm)	141.97	109.86	121.96	.07 ns	.00*	.79 ns

Note: ns = no significant difference.
*Significantly different.

Furthermore, according to Martin et al. (2002), TRDs require time to form and fully differentiate. For instance, when using a 0% concentration of ethephon, TRDs had fully formed after 3 weeks. However, after 6 weeks, the TRDs had completely differentiated and their number had increased. The samples with high ethephon concentrations showed fully differentiated TRDs in just 3 weeks. This finding indicates that ethephon may help speed up TRD formation in under 3 weeks. After 6 weeks, the frequency and diameter of TRDs increased even more.

With prolonged treatment, more resin ducts were formed, and the frequency and diameter increased (Martin et al., 2002). In line with this statement, the TRD in this study may not have fully appeared after 3 weeks of treatment. Hence, the frequencies measured tend to be low. After 6 weeks of treatment, many TRDs had completely formed and differentiated, resulting in a slight increase in TRD frequency and diameter. However, the increase was not statistically significant. This lack of significance in diameter increase may be attributed to the volatile nature of ethylene, as suggested by Ginting et al. (2015), who reported that ethylene may dissipate before completing the 6-week treatment. These results are in line with the study of Praptoyo and Cabout (2013), who discovered that treatment duration with ethylene-releasing stimulants influence the increase in TRD formation. Although the TRD frequency and diameter did not show significant increases in this study, both played an important role in expanding the measurable area of cell proportions, leading to a significant increase in TRD proportion due to the treatment duration. The TRD proportion increased from 2.82% to 3.2% in 3 and 6 weeks, respectively. The TRD proportion in this study has a positive potential correlation with resin productivity, which is supported by Derose et al. (2017), who stated that the high frequency and broad diameter of TRD will increase resin flow.

Other anatomical changes, including TD, TW, RF, and RH, were also observed. The appearance of tracheid cells is displayed in Figure 3. Both concentration and treatment duration had no effect on the TD and TW. The TD and TW measurements did not show any specific trend, and the values fluctuated with the ethephon concentration and treatment duration. Statistical analysis revealed that in terms of anatomical changes, different ethephon concentrations only significantly affected RF, and treatment duration significantly affected the RF and RH, as shown in Table 1. After the ethephon concentration treatment, the average value of the RF was 4.33 cells/mm, with the lowest value at 3.59 and the highest at 5.33 in 0% and 8% ethephon concentrations, respectively, as shown in Figure 4. The RF measurements demonstrated a significant increase from the control to the samples with the highest ethephon concentration. Besides, this significant increase was also noticed in RF and RH for the treatment duration ranging from 3 weeks to 6 weeks, with the values for RF at 3.98 and 4.67 cells/mm and RH at 115.8 and 128.34 μm, respectively. Yamamoto and Kozlowski (1987) reported an increase in RF with various ethephon concentrations and treatment durations for *Pine* spp. Furthermore, Fuchs et al. (2010) mentioned that ray cells in conifers are involved in storing and transporting nutrients. Ray cells deliver nutrients to aid in the recovery of injured or wounded parts caused by tapping (Gartner, 1995). Based on these previous findings, ethephon may not directly affect the RF. The increase in RF may be mainly due to the defensive response of *P. merkusii*. This response helps transport additional nutrients to the injured part for recovery. The increase in the frequency of ray cells allows for additional nutrients to be transported. A similar trend was found for RH, which showed a slight increase with the increasing ethephon concentration; however, the effect was not significant. This phenomenon could be attributed to the pine tree's effort to enhance nutrient transport to the injured part.

Conclusion and Recommendations

This study found that ethephon can enhance the development of TRD in *P. merkusii* seedlings. The ethephon concentrations significantly increased the frequency, proportion, and diameter of TRDs, and also RF, with the highest results found in the 8% concentration. Furthermore, the duration of ethephon treatment had a significant effect on the proportion of TRDs, RF, and RH, with the highest results in the 6 weeks of treatment duration. These findings provide valuable information about ethephon as a potential stimulant for future formula development to increase resin production and meet the global demand for pine resin.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – W.D.N., Y.F.; Design – W.D.N., Y.F.; Supervision – W.D.N.; Resources – W.D.N., Y.F.; Materials – W.D.N., Y.F.; Data Collection and/or Processing – Y.F.; Analysis and/or Interpretation – Y.F.; Literature Search – Y.F.; Writing Manuscript – Y.F.; Critical Review – W.D.N.; Other – Y.F.

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