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The Approach in Selecting the Best Genetic Resistance against Invasive Aphid for Indigenous Tropical *Pinus merkusii* Jungh. et de Vriese in Indonesia

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Abstract: *Pinus merkusii*, a natural tropical pine species of Indonesia, is cultivated as the second most important artificial forest for the industry in Java, after teak, to produce oleoresin. Its genetic improvement began in 1977 because of its critical role in raising community incomes. Meanwhile, the effort for genetic improvement in aphid (*Pineus boerneri*) resistance has just recently started since its spread was only found broadly in Java by 2004. The second-generation progeny trial for this purpose was established in 2010, with materials from the best growing 34 families of the first generation. This study aimed to obtain the best pine genotypes through screening the existing natural variations found on important characters. The reported incidence of the trigger was when the experiment was attacked significantly at 30.7% after four years, while some 67 individuals were unexpectedly still performing well after six years. The results show that blocks affect differences for all traits of diameter, aphid resistance, and oleoresin productions, and all families differ except for the west-side yield of oleoresin production. Furthermore, heritability values at individual and family levels were moderate for the diameter (h^2_i = 0.16; h^2_f = 0.53) and eastern oleoresin (h^2_i = 0.14; h^2_f = 0.42). The gain is 4.3% when 30% of families with the best diameters are retained, while the genetic gain reaches 11% for oleoresin production. As one of the important traits in the breeding program, aphid resistance has a weakly inherited trait (h^2_i =0.07; h^2_f =0.29). Interestingly, this trait shows positive moderate genetic correlations with the two essential economic values of diameter (r_g = 0.66) and oleoresin production (r_g = 0.40). Therefore, the selection of the diameter and oleoresin production will not substantially affect the resistance.

Keywords: tropical pine; *Pineus boerneri*; genetic parameters; growth; improvement; oleoresin

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1. Introduction

Pinus merkusii, a natural tropical pine species of Indonesia, is cultivated as the second most important forest for the industry in Java, after teak, to produce oleoresin. Artificial plantations of this pine are developed mainly by the company Perum Perhutani. The oleoresin is tapped in "contracts" by people whose primary livelihoods are farming near the pine regions [1]. This pine genetic improvement, due to its critical role in creating oleoresin and boosting community income, has been going on since 1977 [2]. However, the genetic improvement of pest resistance only recently started, particularly for aphid (*Pineus boerneri*), since its spread was broadly found in Java in 2004 [3–5]. This invasive

pest [6] has long been known to damage seven other pine species in many countries [7]. Given the hazard, it is necessary to screen for optimal aphid tolerance in the second-generation progeny experiment using the best growth individuals from the first-generation progeny trial.

The risks of climate change have been recorded to influence the environment greatly. As a result, the organisms contained therein, including plants and pests, should adapt to the changing environments. It was reported that the shockwaves of climate change harshly stroke Indonesia, which include extra droughts, hot temperature waves, and floods [8]. The United Nations Development Program Indonesia [9] claim that, in most regions of Java and Sumatra, the rainy seasons start 10 to 20 days later than it did between 1961 and 1990 and 1991 and 2003. These occurrences are likely to persist since specific locations in Indonesia, mainly south of the equator, may have extended dry seasons, but more intense rainy seasons with inconsistent rainfall patterns. Furthermore, elevated temperatures drive the soil to dry out, reduce groundwater resources, and downgrade the land to move into desertification [10]. Precipitation influences forest health [11], and this effect is comparable for plantations in Java, Indonesia [12].

Research into probable worldwide shifts of 76 pest species—of their distributions due to climate change—reveals that pest global distributions may increase and vary among areas significantly. It is concluded that climate change is projected to enlarge the overall pest dispersal worldwide [13]. Aphid (*Pineus boerneri*) incidence to pine plantations in Java, first identified in 2004, may be explained by the aforementioned impact of climatic changes [10] and the research of several pest species dispersals growing globally [13]. The aphid pine is exotic [14] and was first discovered in small areas in 1990 in Baturaden (Central Java) and in 1994 in Bandung [15]. It was recorded that this pest was found in Columbia in 2008, and many countries across Africa had already experienced similar attacks in the several decades before, around 1975–1990 [7,16]. These differences in the incidence years might describe worldwide pest dispersal and the close causal relationships and sequences between precipitation, tree vigor, and pest population [17–19].

The records show that controlling aphid outbreaks by fogging and drilling is costly and provides only temporary effects. Meanwhile, nature offers various insecticidal metabolites for resistance produced within the plant body [20,21]. This ability might vary genetically within species [22,23], so that variations in plant secondary metabolism become interesting targets for plant breeding [24]. Considering the unclear pattern that alters the environments, serious efforts are required to identify and screen *Pinus merkusii* genotypes for aphid resistance through its breeding program, for restoring pine plantations in Java. This procedure is expected to find genetically more stable individuals in resistance against unstable environments and become a solution to sustain company and community revenues. Therefore, this research discusses an evaluation involving a 7-year progeny trial for aphid resistance and oleoresin production grown in 2010. This investigation was prompted by the documented aphid incidences of prosecutions being opposed at a rate of 30.7 percent at four years old. However, 67 individuals were surprisingly still in good health after six years [25,26], which might become a promising potency for overcoming aphid problems in the future. This study aimed to obtain the best pine genotypes through screening the existing natural variations found on important characters revealed in the progeny trial for further development. This research analyzed differences in resistance, growth (diameter), and oleoresin production harvested from the east and the west-side across families to obtain genetic parameters of the traits.

2. Materials and Methods

2.1. Experimental Site

The progeny trial was located at 111°42′7.67″S, 7°44′6.64″ E in the village of Mendak, the district of Ponorogo, East Java province, under forest concessions controlled by KPH Lawu Ds, Perum Perhutani. Furthermore, the topography is mainly hilly, at 869 m above sea level. The daily mean temperature is 25 °C, with a minimum temperature of 18 °C, and a maximum of 31 °C. In the last five years, 2016 to 2020, annual rainfall was recorded in

the range of 2500 to around 2958 mm in year^{-1}. The soil type at the location is andosol, containing high organic matter.

2.2. The Trial Design

In 2010, a progeny experiment was created to test the pine for aphid resistance and determine which families are more prolific in oleoresin. The materials were from the best growing individuals of the first-generation progeny trial grown in Cijambu, West Java, which was initially designed and composed of complete genetic materials from natural populations in Kerinci, Tapanuli, and Aceh (Sumatra), and genetic material infusion from Sulawesi. The trial site was cleared before measuring, mapping, and inserting individual sticks for planting. After knowing the number of seedlings available, 30 × 30 × 30 cm holes were prepared and added with 2 kg of manure. The progeny trial consisted of 34 selected families from Cijambu first-generation trial in Sumedang. Family refers to an individual female parent in which the seeds, as its progeny, were collected; four tree plots per family were grown per block, with 4 × 4 m spacing at replications of eight blocks.

2.3. Data Assessment

The assessment process was undertaken in a 7-year progeny trial in April–December. The diameter of breast height (DBH) measured at 1.3 m from the ground, oleoresin production was assessed separately from the west and east sides, and plant resistance to aphids were the parameters used to evaluate this research. Oleoresin production was carried out by drilling the stem at 50 cm above the ground with a drilling diameter of 10 mm. It was measured after three days by weighing and assessing the plant resistance conducted by observing the aphid infestation using scoring systems. The scoring description is as follows: 2 = heavy (the most vulnerable) with >50% whitish points on the crown; 3 = medium with whitish points of 25–50% on the crown; 4 = light with whitish points <25% on the crown and 5 = healthy (resistant).

2.4. Data Analysis

2.4.1. Analyses of Variance

Variance analyses of trial data were based on the following linear model.

$$Y_{ijk} = \mu + B_i + F + B_i(F)_j + \varepsilon_{ijk}$$

where, Y_{ijk} is the plot mean of the jth family in the ith block; μ is overall mean; B_i is the effect of ith block; F_j is the effect of the jth family; $B_i(F)_j$ is the interaction effect of ith block and jk family; ε_{ijk} is the residual error with a mean of zero.

2.4.2. Genetic Parameter

Mixed model analyses used variance components for the genetic parameter calculation. Block was considered a fixed effect, while the individual family was treated as a random effect. The mean family variance component was used to estimate individual tree heritability ($h^2{}_i$) [27].

$$h^2{}_i = 1/r \times \sigma^2{}_f/\sigma^2{}_p$$

where, r is the coefficient of relationship; $\sigma^2{}_f$ is the variance between families; $\sigma^2{}_p$ is the phenotypic variance = ($\sigma_f{}^2 + \sigma_m{}^2$); $\sigma^2{}_m$ is the variance between plots.

$$h^2{}_{fam} = \sigma^2{}_f/(\sigma^2{}_f + \sigma^2{}_{bf}/b + \sigma^2{}_e/nb)$$

where $\sigma^2{}_f$ is the component of variance due to family; $\sigma^2{}_{bf}$ is the component of variance due to block x family interactions; $\sigma^2{}_e$ is the residual error; b is the harmonic mean number of blocks per family; n is the harmonic mean number of trees per family.

The variance component of the block (σ^2_b) is not included in the denominator of the formulae of heritability; therefore, the estimated heritability is appropriate to the selection on the block-adjusted data.

Genetic correlations (r_g) between traits were calculated according to [27]:

$$r_g = \{\sigma_{f\,(x,y)}\}/\{\sigma^2_{f\,(x).}\ \sigma_f^2{}_{(y)}\}^{1/2}$$

where, σ_f (x,y) is the covariance component at the family level of two different traits; σ_f^2 (x) is the variance component of trait x at the family level; σ_f^2 (y) is the variance component of trait y at the family level.

The expected genetic gain (denoted ΔG) in the trial was calculated as follows [28]:

$$\Delta G = i\ .\ \sigma_p^2\ .\ h_i^2$$

where i is the selection intensity; σ_p^2 is the phenotypic variance; h_i^2 is the individual heritability for the trait of interest.

3. Results

The results show that blocks affect differences for all traits, and all families differ except for the west oleoresin production (Table 1). Furthermore, the diameters representing growth and aphid resistance indicate the highest probabilities of significance between families with $p < 0.01$ and east oleoresin at $p = 0.02$. The interaction between block and family only occurs on aphid resistance.

Table 1. Analysis of variance of *P. merkusii* diameter (cm), aphid resistance, eastern and western oleoresin productions from a 7-year progeny trial.

Source of Variation	df	Sum of Square	Mean Square	F Value	Pr > F
Diameter					
Block	7	150.98	21.57	2.16	0.0366 *
Family	33	688.85	20.87	2.09	0.0005 **
Blockxfam	223	2536.29	11.37	1.14	0.1267 ns
Error	450	4494.47	9.99		
Aphid resistance					
Block	7	61.83	8.83	28.29	<0.0001 **
Family	33	19.23	0.58	1.87	0.0030 **
Blockxfam	223	114.83	0.51	1.65	<0.0001 **
Error	450	140.50	0.31		
East oleoresin					
Block	7	463.02	66.14	3.94	0.0006 **
Family	33	915.75	27.75	1.65	0.0234 *
Blockxfam	161	3422.20	21.25	1.26	0.0739 ns
Error	147	2470.42	16.80		
West oleoresin					
Block	7	1178.03	168.29	8.41	<0.0001 **
Family	33	947.29	28.70	1.43	0.0764 ns
Blockxfam	163	3832.76	23.51	1.18	0.1585 ns
Error	149	2981.67	20.01		

Remarks: ns = not significant, * = significant at level of 0.05, ** = significant at level of 0.01.

Estimations demonstrate that both heritability values at individual and family levels were moderate for diameter and eastern oleoresin (Table 2). The gain will be 4.3% when 30% of families with the best diameter are retained. Meanwhile, oleoresin production will generate a genetic gain of 11% for 30% of the reserved best families.

Table 2. Genetic parameters of *P. merkusii* traits; diameter, aphid resistance, eastern and western oleoresin productions, from a 7-year progeny trial.

Trait	Genetic Parameter						
	Mean	σ^2_f	σ^2_{rxf}	σ^2_e	CV (%)	h^2_i	h^2_f
Diameter	14.32	0.4329	0.6296	9.9167	17.44	0.16	0.53
Aphid resistance	3.51	0.2894	2.8731	19.4070	15.16	0.07	0.29
East oleoresin	6.57	0.0069	0.0559	0.3297	23.69	0.14	0.42
West oleoresin	6.82	0.6873	3.6941	15.6168	24.50	0.05	0.23

The results of the analyses on genetic correlations (Table 3) notice that there are two positive relationships between diameter and aphid resistance (r_g = 0.66) and between eastern oleoresin and aphid resistance (r_g = 0.40).

Table 3. Genetic correlations between *P. merkusii* traits from a 7-year progeny trial.

Genetic Correlations	Diameter	Aphid Resistance	Eastern Oleoresin	Western Oleoresin
Diameter	-	0.66	−0.29	−0.50
Aphid resistance		-	0.40	−0.46
Eastern oleoresin			-	1.13
Western oleoresin				-

4. Discussion

This research to define growth by diameter, which is simpler to measure than height, is based on other pine studies revealing strong genetic relationships between both features, particularly at younger ages of 4.7–8 years [29–32]. Furthermore, in *Pinus merkusii* [33], the diameter as demonstrated to be more directly linked to oleoresin production than height, making it the most important property for indirect selection of high-yielding oleoresin, as seen in *Pinus massoniana* [29].

Differences between families in three out of four traits are sufficient to represent the essential screened characters for selection of the best performers, where growth depicts plant vigor and approaching volume, aphid resistance refers to plant health, and oleoresin production describes the species' primary expected by-product for economic reasons. Similar variations of diameter and oleoresin yield among families in pine, including *Pinus merkusii*, have also been recorded [34–36]. Additionally, intraspecific diversity on resistance to insects has also been described in numerous forest tree species among different genotypes of individual trees/clones/families or provenances [37].

The research discovered an interaction between block and family on aphid resistance, demonstrating that the environment affects how trees respond to aphid assault at the microclimate level [37]. The effects might be even greater when environment changes caused by the climate changes occur. This is a regular occurrence in pine, and similar signs were found in other pest outbreaks when the environment has a significant impact [38,39]. The design for field trial has commonly been intended to minimize the environment. However, the edaphic effects in trial sites are usually considerably greater than the climatic effects [40]. Differences in moisture content of the soil should affect the soil fertility and tree growth in which those two factors might influence the surrounding microclimate, which is beneficial for the aphid.

A variation in the diameter, in a moderate heritability value with h^2_i = 0.16 and h^2_f = 0.53, shows that further genetic improvement on growth is promising through selection in this second-generation experiment. This is because the families already originated from the best growth of first-generation individuals. Therefore, the next further selection on the diameter should demonstrate the better future performance of individuals on growth. Moderate genetic control for oleoresin production in this study (h^2_i = 0.14, h^2_f = 0.42) has also been

identified in many studies at more mature age trials [30,34,41,42]. This is a good indicator for *Pinus merkusii* to obtain better resin production after proper screening. However, in aphid resistance, the genetic variation is relatively low with heritability values of $h^2_i = 0.07$, $h^2_f = 0.29$; therefore, selection based on this character may not be appropriate. This character was observed on another trial in West Java, for the same pest on *Pinus merkusii*, with many genetic materials (96 families). The result showed a specified moderate individual with low family heritability values ($h^2_i = 0.14$, $h^2_f = 0.2$) [5], confirming the response at a family level in this study. Variations of individual resistance within families might be responsible for this fact.

The expected genetic gains from selecting 30% of the best individuals/families are substantial for oleoresin-yield (11%), and this gain is advantageous, realizing that the species are grown explicitly for oleoresin production. The relatively high percentage of selection (30%) is assigned based on the limited initial number of families in the second-generation progeny trial. Subsequently, the genetic variation of selected individuals/families will decrease compared to the total initial genotypes grown in the test. The first-generation progeny trial, which originated from genetic materials for this progeny trial, was established from extensive sources with hundreds of families from throughout its natural distribution in Indonesia. Therefore, for future genetic improvement initiatives, adding genetic materials for resistance via new accessions of the best-selected families from the first generation may be an option for increasing the genetic variety of the population. It is important to note that the genetic parameters discussed may be overestimated whenever interactions of the family–sites of traits were notable, because they were only established at one location.

The result of aphid resistance analysis has shown that it is lowly inherited. Interestingly, the resistance character, as one critical criterion, exhibits positive moderate genetic correlations with the two essential economic values; medium with a diameter ($r_g = 0.66$) and oleoresin production ($r_g = 0.40$), suggesting that the selection on the diameter and oleoresin production will not give a substantial effect on the resistance. However, the environment, or the interaction of genetics and environment may have an effect because of significant positive phenotypic associations between aphid resistance and diameter ($r_p = 0.98$ **), and between resistance and oleoresin production ($r_p = 0.79$ **), have been observed in *Pinus merkusii* [26]. Therefore, these positive genetic relationships provide expected genotypes for good growth, better oleoresin production, and excellent resistance, even though resistance is not highly inherited. In other studies, better oleoresin productions also promoted resistance to pests [31,43]. Secondary plant metabolites play an essential role in numerous plant protection systems against insects.

The plant quality traits, including nutrition and accumulation of secondary metabolites, were shown to affect the deterioration of insect performance on the trees [44,45]. However, another study on *Pinus merkusii* indicated that this aphid attack did not significantly affect tree diameters, even for those classified as moderately infested trees; moreover, the aphid did not impact the resin yield. There was no indication of whether this was a temporary or a permanent situation, but the study revealed that the secondary metabolites of a monoterpene, sesquiterpene, and diterpene recorded higher for trees with 3% aphid coverage than those covered at 23%. This approved the role of metabolites for defense on *Pinus merkusii* aphid resistance [5]. Furthermore, the secondary plant chemistry exemplified in *Pinus contorta var. latifolia* was reported under substantial genetic control, and significant differences were found attributed to differences among its families [39]. Therefore, it is worthwhile to investigate genetic differences in secondary metabolites found in *Pinus merkusii* that confer resistance.

5. Conclusions

Our results show that significant variations found in the defense against aphids in tropical pines are weakly genetically controlled; however, they are closely related to other characters—diameter and oleoresin production. These two characters show moderate heritability values that are essential for facilitating further selections in pine breeding

programs. Aphid resistance in pine is influenced by the environment, which may include irregular climate changes. This study presents the best tropical pine individuals as new "insights" into defensive genotype responses against aphids, for further development.

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References

1. Nugroho, P.; Numata, S.; Aprilianto, N.A. Perceived Forest-based Ecosystem Services and Attitudes Toward Forest Rehabilitation: A Case Study in the Upstream of Central Java, Indonesia. *J. Ilmu Kehutan.* **2020**, *14*, 185–197. [CrossRef]
2. Hardiyanto, E.; Danarto, S. Ex situ Conservation of Pinus merkusii in Java, Indonesia. In *Situ and Ex Situ Conservation of Commercial Tropical Trees*; ITTO Project PD 16/96 Rev.4(F); ITTO: Yogyakarta, Indonesia, 2001; pp. 263–269.
3. Siregar, U.; Diputra, I. Diversity of Pinus merkusii Jungh. et de Vriese of Tapanuli Strain based on Microsatellite Markers. *J. Silvikultur Trop.* **2013**, *4*, 88–99. [CrossRef]
4. Iskandar, T. Penilaian Kesehatan Kebun Benih Semai Pinus Merkusii Dengan Metode Fhm (Forest Health Monitoring) Di KPH Sumedang-Health Assessment for Seedling Seed Orchard of Pinus merkusii Using FHM (Forest Health Monitoring) Method in KPH Sumedang. *J. Silvikultur Trop.* **2018**, *9*, 99–108. [CrossRef]
5. Sumantoro, P. Musyafa Serangan Hama Kutu Lilin (Pineus boerneri Annand.) pada Tanaman Uji Keturunan Pinus merkusii generasi II Umur 9 Tahun di Tampomas Sumedang. Ph.D. Thesis, Gadjah Mada University, Yogyakarta, Indonesia, 2012.
6. Invasive Species Compend. Cabi Pineus Boerneri (Pine Woolly Aphid). 2022. Available online: https://www.cabi.org/isc/datasheet/41319 (accessed on 18 January 2022).
7. Chilima, C.Z.; Leather, S.R. Within-tree and seasonal distribution of the pine woolly aphid Pineus boerneri on Pinus kesiya trees. *Agric. For. Entomol.* **2001**, *44*, 139–145. [CrossRef]
8. Measey, M. Indonesia: A Vulnerable Country in the Face of Climate Change. *Glob. Major. E-J.* **2010**, *1*, 46–56.
9. *The Other Half of Climate Change: Why Indonesia Must Adapt to Protect its Poorest People*; UNDP: Jakarta, Indonesia, 2007.
10. Syaukat, Y. The Impact Of Climate Change On Food Production And Security and Its Adaptation Programs in Indonesia. *J. ISSAAS* **2011**, *17*, 40–51.
11. Xiao, F.; Ouyang, H.; Zhang, Q.; Fu, B.; Zhang, Z. Forest ecosystem health assessment and analysis in China. *J. Geogr. Sci.* **2004**, *1*, 18–24. [CrossRef]
12. Avia, L.Q. Change in rainfall per-decades over Java Island, Indonesia. In Proceedings of the 8th International Symposium for Sustainable Humanosphere, Medan, Indonesia, 18–19 October 2018. [CrossRef]
13. Yan, Y.; Wang, Y.; Feng, C.; Wan, P.M.; Chang, K.T. Potential distributional changes of invasive crop pest species associated with global climate change. *Appl. Geogr.* **2017**, *82*, 83–92. [CrossRef]
14. Rachmatsyah, O.; Siregar, U.J.; Haneda, N.F.; Nandika, D.; Hidayat, P. Distribution of pine woolly adelgids infestation on pinus merkusii plantation in Java. *J. Manaj. Hutan Trop.* **2012**, *18*, 191–197. [CrossRef]
15. Anggraeni, I. *Penyakit Karat Tumor Pada Sengon Dan Hama Cabuk Lilin Pada Pinus*; Badan Litbang Kehutanan Kementerian Kehutanan Republik Indonesia: Bogor, Indonesia, 2012.
16. Rodas, C.A.; Serna, R.; Bolaños, M.D.; Granados, G.M.; Michael, J.; Hurley, B.P. Biology, incidence and host susceptibility of Pineus boerneri (Hemiptera: Adelgidae) in Colombian pine plantations. *South. For. A J. For. Sci.* **2015**, *77*, 165–171. [CrossRef]
17. Munster-Swendsen, M. The Effect of Precipitation on Radial Increment in Norway Spruce (Picea abies Karst) and on the Dynamics of a Lepidopteran Pest Insect. *J. Appl. Ecol.* **1984**, *24*, 563–571. [CrossRef]
18. Powers, J.S.; Sollins, P.; Harmon, M.E.; Jones, J.A. Plant-pest interactions in time and space: A Douglas-fir bark beetle outbreak as a case study. *Landsc. Ecol.* **1999**, *14*, 105–120. [CrossRef]

19. Navarro-Cerrillo, R.M.; González-Moreno, P.; Ruiz-Gómez, F.J.; Sánchez-Cuesta, R.; Gazol, A.; Camarero, J.J. AntonioGazol Drought stress and pests increase defoliation and mortality rates in vulnerable Abies pinsapo forests. *For. Ecol. Manage.* **2022**, *504*, 119824. [CrossRef]
20. Birkett, M.A.; Pickett, J.A. Prospects of genetic engineering for robust insect resistance. *Curr. Opin. Plant Biol.* **2014**, *19*, 59–67. [CrossRef]
21. Horikoshi, R.; Goto, K.; Mitomi, M.; Oyama, K.; Sunazuka, T.; Omura, S. Identification of pyripyropene A as a promising insecticidal compound in a microbial metabolite screening. *J. Antibiot.* **2017**, *70*, 272–276. [CrossRef]
22. Hu, Q.; Zhao, J.; Cui, J. The Relationships Between the Level of Lignin, a Secondary Metabolite in Soybean Plant, and Aphid Resistance in Soybeans. *Plant Prot.* **1993**, *19*, 8–9.
23. Batyrshina, Z.S.; Yaakov, B.; Shavit, R.; Singh, A.; Tzin, V. Comparative transcriptomic and metabolic analysis of wild and domesticated wheat genotypes reveals differences in chemical and physical defense responses against aphids. *BMC Plant Biol.* **2020**, *20*, 19. [CrossRef] [PubMed]
24. Verpoorte, R.; Memelink, J. Engineering secondary metabolite production in plants. *Curr. Opin. Biotechnol.* **2002**, *13*, 181–187. [CrossRef]
25. Purwanto; Handarto; Cahyono, L.R. Mulyono Evaluasi Uji Keturunan Pinus merkusii Tahan Hama Kutu Lilin. *Bul. Penelit. Hutan Lestari Produktif.* **2017**, *20*, 8–12.
26. Purwanto; Baskorowati, L.; Hendrati, R.L.; Susanto, M.; Mashudi; Setiadi, D.; Nurtjahjaningsih, I.L.G.; Pudjiono, S.; Kurniawan, A.; Wirabuana, P.Y.A.P. Evaluation of Aphid Resistance and Oleoresion Production in Indigenous Tropical Pine (Pinus merkusii Jungh. Et de Vriese). Under review. *Forests* **2022**, in press.
27. Wiliams, E.R.; Matheson, C.A.; Harwood, C. *Experimental Design and Analysis for Tree Improvement*, 2nd ed.; CSIRO Publishing: Collingwood, VIC, Australia, 2002.
28. Shelbourne, C. Genetic gains from different kinds of breeding population and seed or plant production population. *S. Afr. For. J.* **1992**, *160*, 49–65. [CrossRef]
29. Liu, Q.; Zhou, Z.; Fan, H.; Liu, Y. Genetic Variation and Correlation among Resin Yield, Growth, and Morphologic Traits of Pinus massoniana. *Silvae Genet.* **2013**, *62*, 38–44. [CrossRef]
30. Pswarayi; Barnes, R.D.; Birks, J.S.; Kanowski, P. Genetic parameter estimates for production and quality traits of Pinus elliottii Engelm. var. elliottii in Zimbabwe. *AGRIS* **1998**, *45*, 216–222.
31. Westbrook, J.W.; Resende, M.F., Jr.; Munoz, P.; Walker, A.R.; Wegrzyn, J.L.; Nelson, C.D.; Neale, D.B.; Kirst, M.; Huber, D.A.; Gezan, S.A.; et al. Association genetics of oleoresin flow in loblolly pine: Discovering genes and predicting phenotype for improved resistance to bark beetles and bioenergy potential. *New Phytol.* **2013**, *199*, 89–100. [CrossRef]
32. Woolaston, R.; Kanowski, P.; Nikles, D. Genetic Parameter Estimates for Pinus caribaea var. hondurensis in Coastal Quensland, Australia. *Silvae Genet.* **1990**, *39*, 21–28.
33. Muslimin, I. Korelasi genetik Pertumbuhan dan Produksi Getah Pada Uji Keturunan Pinus merkusii Di KPH Banyumas Barat (Genetic Correlation of Growth and Resin Yield in Progeny Test Pinus merkusii Jungh. et de Vriese at KPH Banyumas Barat). *J. Penelit Kehutan Sumatrana* **2017**, *1*, 22–34. [CrossRef]
34. Roberds, J.H.; Strom, B.L.; Hain, F.P.; Gwaze, D.P.; Mckeand, S.E.; Lott, L.H. Estimates of genetic parameters for oleoresin and growth traits in juvenile loblolly pine. *Can. J. For. Res.* **2009**, *33*, 2469–2476. [CrossRef]
35. Nugrahanto, G.; Na'iem, M.; Indrioko, S.; Faridah, E.; Widiyatno, W. Widiyatno Pemuliaan Pinus Bocor Getah: Korelasi Genetik Produksi Getah Pada Tiga Sub Galur Uji Keturunan Pinus Merkusii Di Kph Banyumas Barat. *AgriEnvi. J. Ilmu Pertan.* **2020**, *14*, 78–88.
36. Santos, W.; Cristina, D.; Souza, L.; Luiz, M.; De Moraes, T.; Aguiar, V. De Genetic variation of wood and resin production in Pinus caribaea var. hondurensis Barret & Gol- fari. *Silvae Genet.* **2016**, *65*, 31–37. [CrossRef]
37. Lieutier, F. Changing Forest Communities: Role Of Tree Resistance To Insects In Insect Invasions and Introductions. In *Invasive Forest Insects, Introduced Forest Trees, and Altered Ecosystems*; Paine, T.D., Ed.; Springer Science+Business Media B.V.: Berlin/Heidelberg, Germany, 2008; pp. 15–51.
38. Larson, E.R.; Peak, P. Influences of the biophysical environment on blister rust and mountain pine beetle, and their interactions, in whitebark pine forests Mountains. *J. Biogeogr.* **2011**, *38*, 453–470. [CrossRef]
39. Ott, D.S.; Alvin, D.Y.; Wallin, K.F. Genetic Variation of Lodgepole Pine, Pinus contorta var. latifolia, Chemical and Physical Defenses that Affect Mountain Pine Beetle, Dendroctonus ponderosae, Attack and Tree Mortality. *J. Chem. Ecol.* **2011**, *37*, 1002–1012. [CrossRef] [PubMed]
40. Zobel, B.J.; Talbert, J. *Applied Forest Tree Improvement*; Wiley: New York, NY, USA, 1984.
41. Prasetia, R. Potensi Getah Pertanaman Uji Keturunan Pinus merkusii Jungh. et de Vriese Materi Introduksi Genetik Asal Aceh di RPH Sumberjati, BKPH Sempolan, KPH Jember. Bachelor's Thesis, Gadjah Mada University, Yogyakarta, Indonesia, 2008.
42. Shimizu, J.Y.; Spir, I.H.Z. Selection of slash pine on breeding values for high resin production Seleção de Pinus elliottii pelo valor genético para alta produção de resina. *Bol. Pesqui. Florest.* **1999**, *38*, 103–117.
43. Strom, B.L.; Goyer, R.A.; Ingram, L.L., Jr.; Boyd, G.D.L.; Lott, L.H. Oleoresin characteristics of progeny of loblolly pines that escaped attack by the southern pine beetle. *For. Ecol. Manage.* **2002**, *158*, 169–178. [CrossRef]

44. Dalin, P.; Bjorkman, C. Native Insects Colonizing Introduced Tree Species—Patterns And Potential Risks. In *Invasive Forest Insects, Introduced Forest Trees, and Altered Ecosystems*; Paine, T.D., Ed.; Springer Science Business Media B.V.: Berlin/Heidelberg, Germany, 2008; pp. 63–77.
45. Schoonhoven, L.M.; Van Loon, B.; van Loon, J.J.; Dicke, M. *Insect–Plant Biology*; OUP Oxford: Oxford, UK, 2005.