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Article

Heartwood Chemistry Predicts Natural Durability in *Pinus nigra* clones: The Critical Role of Resin Acid over Stilbenes in Decay Resistance

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Abstract

The natural durability of wood, determined primarily by its chemistry, meets the growing demand for environmentally sustainable alternatives to toxic wood preservatives. This study investigated the relationship between heartwood chemical composition and decay resistance among fifty-two (52) *Pinus nigra* Arn. clones from a clonal seed orchard in Greece. Quantitative ¹H-NMR spectroscopy was employed to determine total acetone extractives (TAE), total stilbenes (TS), and total resin acids (TRA) in heartwood samples, while decay resistance was evaluated through standardized weight loss tests using *Coniophora puteana* (Schumach.) P. Karst. (1865) and *Porodaedalea pini* (Brot.) Murrill (1905). The heartwood exhibited exceptionally high extractive content (mean TAE = 304.15 mg/g_{dhw}), with resin acids (68.26%) predominating over stilbenes (22.31%). Regression analysis showed that TAE and TRA were the strongest predictors of decay resistance, explaining 33% of the variance, while stilbenes exhibited weaker and more variable associations. *Porodaedalea pini* caused significantly higher mean weight loss (11.43%) than *C. puteana* (3.55%), indicating species-specific fungal aggressiveness. Among individual resin acids, abietic acid were the most influential contributors to decay resistance. The results demonstrate that resin acids have dominant role over stilbenes in determining the natural durability of *P. nigra* heartwood and could serve as effective biochemical markers for selective breeding.

Keywords: black pine; natural durability; heartwood extractives; pinosylvins; resin acids; clones; weight loss; brown-rot fungi; white-rot fungi; selective breeding

1. Introduction

Increased public awareness of environmental issues, particularly the harmful use of toxic chemicals with considerable negative environmental impacts, leads primarily to the development of ecological preservatives and impregnants, and, on the other hand, to the cultivation and use of naturally resistant wood as an alternative to impregnated or coated wood. The idea of using wood preservatives, which are often powerful poisons, seems to counter the effort to promote the use of naturally durable wood which has sustainable character and excellent environmental profile [1]. The purpose of restricting chemical preservatives is to promote the use of naturally durable wood, thus

maintaining the lifespan of wooden constructions [2], and simultaneously eliminating costly and potentially hazardous chemical treatments that pose risks to human health and the environment.

The natural durability of wood, determined by its intrinsic resistance resistibility [3], varies between and within forest species, across different origins, and among trees of varying ages within the same species [4,5]. One of the key factors in determining the natural durability of wood is the presence of extractives, whose quality and quantity affect the wood's susceptibility to decay [4,6,7]. The significance of heartwood extractives for natural durability has long been recognized [8], and has been repeatedly discussed [9–14]. As a result, significant differences in natural durability exist between heartwood and sapwood.

Extractives consist of an enormous variety of individual compounds, including both lipophilic and hydrophilic types, and are considered nonstructural wood constituents e.g. terpenoids, steroids, fats, waxes, and phenols. These are almost entirely extracellular and low-molecular-weight compounds [15]. Notably, polyphenolic extracts, such as stilbenes, function either as chemical (mycotoxic) agents [16] or as physical barriers [17] against microorganisms, significantly influencing the quality of softwoods [18] and hardwoods [19]. Additionally, resin acids, which are components of oleoresin, are exuded in response to mechanical wounds or biotic attacks, serving as a defense mechanism in living trees [4]. This demonstrates that several types of extractives are highly heritable heartwood properties [20,21]. The formation of stilbenes, such as pinosylvins, in typical pine species is driven by the rapid activation or catalysis of multiple genes [22–25]. Similarly, the production of resin acids is regulated by genes encoding diterpene synthase and related enzymes responsible for diterpene acids synthesis [26].

The significant biodegradation wood resistance variability, partly driven by genetic factors, indicates that this trait could be improved through selection [27]. Genetic variation in natural durability has been studied in a number of softwood species such as *Pinus sylvestris* [20,21,28–32], *Pinus taeda* [33], *Pinus pinea* [34], *Picea abies* [35], *Picea glauca* [36], *Cupressus lusitanica* [16] and *Larix* spp. [18,37,38].

In our previous studies, the Peloponnesian *Pinus nigra* heartwood was found to be the richest source of stilbenes [39] as well as resin acids [40] identified to date and is considered the best natural source for the production of such bioactive extracts. These differences in biologically active compounds between the studied populations of Greek *P. nigra* and the previously reported *P. nigra* or other Pine species might be explained as a genetic adaptation of the studied population to the environmental conditions of Southern Greece forests or adaptive defense mechanisms. These results indicate a high potential for effective selection and advanced breeding of pharmaceutical and high economic value bioactive substances from *P. nigra* clones [41].

On the above-mentioned research, this work highlights the crucial role of high concentration of such substances in enhancing wood protection and natural resistance against decay fungi. More accurately, the objective of this study was to assess the relationship between the fungitoxic total acetone extractive content (TAE) and heartwood decay resistance. We particularly focused on specific stilbenes i.e. pinosylvin (P) and its mono- (PMME) and dimethyl ether (PDME) derivatives, and some abietane-, i.e. abietic acid (AA), dehydroabietic acid (DAA), neoabietic acid (NAA), palustric acid (PLA), and levopimaric acid (LPA) and pimarane-type, i.e. pimaric acid (PMA), sandaracopimaric acid (SPA), and isopimaric acid (IPA) resin acids of *Pinus nigra* L.. Black pine is silviculturally considered a very important coniferous species in Greece and other European countries, as well as in countries outside its natural range where it was planted, e.g., North and South America, Australia, and New Zealand. Black pine is regarded as one of the primary timber-producing species in Greece, and significant effort has been put into genetic improvement of the species [42]. Promoting the cultivation and use of naturally resistant Black pine wood provides an eco-friendly and sustainable alternative to chemically treated wood, extending the lifespan of wooden structures while avoiding the environmental and health risks linked to chemical preservatives.

2. Materials and Methods

2.1. Plant Material

The plant material was sampled from a 10 ha *Pinus nigra* Arn. clonal seed orchard (CSO), established in the western part of the Peloponnese in 1978. The CSO comprises fifty-two (52) clones, and a total number of 2,700 grafts, derived from intensively selected plus trees, originated from four marginal provenances (Zarouhla, Feneos, Parnonas and Taigetos) of the natural Black pine forest of the Peloponnese peninsula. Clones (one ramet/clone) were randomly assigned at 6 m X 6 m spacing within replications (single tree plot design), without blocking, with the only restriction that no grafts of the same clone were planted closer than 30 m [43].

Sampling, coring, heartwood discrimination and orientation, as well as the extraction protocol, ¹H-NMR spectra analysis and qualitative-quantitative determination of stilbenes and resin acids were extensively described in Ioannidis et al. (2017, 2019).

2.2. Sampling

In brief, the *P. nigra* heartwood samples, consisting of 12-mm diameter increment cores, were extracted, in an average sampling height of 30 cm above ground and in a north-south orientation. In total, 260 healthy individuals were sampled, covering all 52 clones participating in the CSO, regardless of their trunk and canopy form and 260 increment cores were extracted, e.g. five ramets per clone. The cores were sampled during October and November and stored in darkness at -76°C. Heartwood was separated from the rest of the core, using the benzidine discrimination method, and milled to produce ≤0.75 mm particles, which were freeze-dried, for 48h at -52°C and 0.03 mbar pressure, to ensure almost complete removal of moisture and volatile compounds. Half, in radial orientation, of each heartwood core was subjected to extraction and the other half to decay test as described below.

2.3. Extraction Protocol

In brief, resin acids were extracted with acetone from 200 mg (±0.1 mg) of freeze-dried ground heartwood. The mixture was first placed in an orbital shaker at 350 rpm (Edmund Bühler GmbH, Germany) in darkness at room temperature for 24 h, followed by its transfer to an ultrasonic bath (Semat, UK) for 1 h to complete the extraction. The liquid phase was separated by centrifuging (3075×g for 15 min) (Eppendorf 5810R, Germany) and the solvent was evaporated in a heated vacuum rotary evaporator (Buchi, Switzerland) at 40 °C to determine the weights.

2.4. ¹H-NMR Spectral Analysis

The dried extractives were dissolved in 600 µL deuterated chloroform (CDCl₃, Euriso-Top) and submitted to chemical analysis by ¹H-NMR, (Bruker DRX400, Bruker, USA), using syringaldehyde (Acros Organics, Belgium) as internal standard. Typically, 16 scans were collected into 32 K data points over a spectral width of 0-16 ppm with a relaxation delay of 1 s and an acquisition time of 1.7 s. Prior to Fourier transformation, an exponential weighting factor corresponding to a line broadening of 0.3 Hz was applied. The spectra were phase corrected and integrated automatically using Topspin software (Bruker, USA). For the peaks of interest, precise manual integration was carried out, and the resulting values were used to quantify the studied chemical by comparing the areas of the selected signals to that of the internal standard. The studied chemical were the total acetone extractive content (TAE) with a particular focus on specific stilbenes i.e. pinosylvin (P) and its mono- (PMME) and dimethyl ether (PDME) derivatives, and some abietane-, i.e. abietic acid (AA), dehydroabietic acid (DAA), neoabietic acid (NAA), palustric acid (PLA), and levopimaric acid (LPA) and pimarane-type, i.e. pimaric acid (PMA), sandaracopimaric acid (SPA), and isopimaric acid (IPA) resin acids of *Pinus nigra* L.. Concentrations are based on freeze-dried heartwood (dhw) and

expressed in mg/g_{dhw}. All measurements were conducted at the Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens.

2.5. Decay Tests

The remaining half of each non-extracted increment heartwood core, oriented radially, was further divided into two radial sections. Each section was then used to assess decay resistance against two species of fungi, *Coniophora puteana* (Schumach.) P. Karst. (1865) and *Porodaedalea pini* (Brot.) Murrill (1905). The decay tests followed a similar approach to those described by Viitanen et al. [44], Harju et al. [30], Venäläinen et al. [38] and Mohareb et al. [16] which were modified versions of the standardized EN 113 test [45], too. The performed modified decay test was based on EN 113, using the above-mentioned specimens whose dimensions were 12mm in diameter and as long as the size of the heartwood in the radial dimension. The heartwood samples for the decay tests were placed on pure cultures of *C. puteana* (strain BAM Ebw. 15) and *P. pini* (IMFE collection Pp 13) growing on agar in 12 mm Petri dishes at 37 °C. Prior to the weight loss test, the heartwood samples were not leached. The samples were placed on a stainless-steel rack to avoid contact with the fungi's culture media. The incubation time lasted 24 weeks, after which the samples were dried at 60 °C for 48 h (or until constant weight) and reweighed. The oven-dry heartwood weight loss (WL) during the experiment was used as an inverse measure of decay resistance and was expressed in relative terms. Weight loss was determined gravimetrically and calculated according to Equation (1):

$$WL = \left(\frac{m_b - m_a}{m_b} \right) \times 100 [\%] \quad (1)$$

where: m_b is the oven-dry mass before incubation (g) and m_a is the oven-dry mass after incubation (g) wood block [16,37,46–50]

2.6. Statistical Analysis

2.6.1. Simple Linear Regression Model

Firstly, a simple linear regression model (Model 1) was applied, following the approach of Venäläinen et al. (2003), Gierlinger et al. (2004a) and Harju and Venäläinen (2006), to examine whether weight loss depended on chemical heartwood characteristics- specifically, total acetone extractives, total resin acids, and total stilbenes, determined as:

$$WL = b_0 + b_1 \times C + e \quad (2)$$

where:

WL = the weight loss in relative terms (%),

b_0 = the intercept,

b_1 = the coefficient of the predictor C, independent variable

C = the total acetone extractives (TAE) or the total stilbenes (TS) or the total resin acids (TRA) or every single stilbene substance, i.e. P, PMME and PDME of the heartwood, and

e = the residuals.

2.6.2. Multiple Linear Regression Model

Subsequently, a multiple regression analysis (Model 2) was conducted to examine whether weight loss was influenced by the contents of stilbenes (TS) and resin acids (TRA), both of which are known for their protective roles in wood. The aim was to evaluate the relative contribution of each group to antimicrobial activity. The model determined as:

$$WL = b_0 + b_1 \times TS + b_2 \times TRA + e \quad (3a)$$

where:

WL = the weight loss in relative terms (%),

b_0 = the intercept,

TS = the total stilbenes content,

TRA = the total resin acids content,
b₁ = coefficient of variable TS,
b₂ = coefficients of variable TRA,
e = the residuals.

Following the above model, a new multiple regression analysis (also called Model 2) was conducted to examine whether weight loss was influenced by the contents of the different stilbenes, noted for their contribution to the defense mechanisms of wood. The aim was to evaluate the relative contribution of each stilbene substance to antimicrobial activity. The model determined as:

$$WL = b_0 + b_1S_1 + b_2S_2 + e \tag{3b}$$

where:

WL= the weight loss in relative terms (%),
b₀ = the intercept,
S₁ = one of the stilbene substance,
S₂ = other stilbene substance,
b₁ = coefficient of variable S₁,
b₂ = coefficients of variable S₂,
e = the residuals.

Finally, a multiple regression analysis (Model 3) was performed to examine whether weight loss depended on the different types of stilbenes (TS) and resin acids (TRA), with the aim of assessing the antimicrobial contribution of each individual compound, determined as:

$$WL = a_0 + b_1 \times P + b_2 \times PMME + b_3 \times PDME + e \tag{4a}$$

$$WL = a_0 + c_1 \times AA + c_2 \times DAA + c_3 \times NAA + c_4 \times PLA + c_5 \times LPA + c_6 \times PMA + c_7 \times SPA + c_8 \times IPA + e \tag{4b}$$

$$WL = a_0 + b_1 \times P + b_2 \times PMME + b_3 \times PDME + c_1 \times AA + c_2 \times DAA + c_3 \times NAA + c_4 \times PLA + c_5 \times LPA + c_6 \times PMA + c_7 \times SPA + c_8 \times IPA + e \tag{4c}$$

where:

WL= the weight loss in relative terms (%),
a₀ = the intercept,
b₁....b₃ = coefficients of variables of different stilbenes, i.e. P, PMME and PDME,
c₁....c₈ = coefficients of variable of different resin acids, i.e. AA, DAA, NAA, PLA, LPA, PMA, SPA and IPA, and
e = the residuals.

where:

TAE = total acetone extractive, P = pinosylvin, PMME = pinosylvin monomethyl ether, PDME = pinosylvin dimethyl ether, AA = abietic acid, DAA = dehydroabietic acid, NAA = neoabietic acid, PLA = palustric acid, LPA = levopimaric acid, PMA = pimaric acid, SPA = sandaracopimaric acid and IPA = isopimaric acid.

Descriptive statistics and simple and multiple regressions were performed on the percentage (%) of weight loss. Weight loss data were subjected to appropriate log or arcsine transformation before statistical analysis to meet the assumptions of parametric tests and were re-transformed to be added in tables and graphs.

The residuals were assessed for normality and homoscedasticity, and no noteworthy deviations from the underlying assumptions were observed. In multiple linear regression models, multicollinearity was assessed through estimating the variance inflation factor (VIF), a quantitative measure of multicollinearity. Model selection for regression analysis was based on the two most well-known statistical model selection rules, namely AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion), a quantitative measure to evaluate and compare different models [52]. The effect size for ANOVA/regression models were estimated using Cohen's f² index [53]. All statistical analysis was performed using SPSS v.20 software for Windows (IBM SPSS Statistics 2011, IBM Corp., Armonk, NY, USA). In all statistical hypothesis testing procedures, the significance level was predetermined at $\alpha = 0.05$ ($p \leq 0.05$).

3. Results

The heartwood of the studied trees exhibited an exceptionally high acetone extractive content (TAE = 304.15 mg/g_{dhw}), which contained significant amounts of stilbenes (TS = 59.92 mg/g_{dhw}) [39] and resin acid (TRA = 219.98 mg/g_{dhw}) [40] quantities (Table 1).

The total acetone extractives of the heartwood samples amounted to an average of 304.15 mg/g_{dhw} [range 81.79 - 480.28 mg/g_{dhw}], (Table 1). The total stilbenes per dry heartwood weight amounted to an average of 59.92 mg/g_{dhw} [range 10.99 - 128.22 mg/g_{dhw}] (Table 1). The total resin acid (TRA) content amounted to an average of 219.98 mg/g_{dhw} [range 32.20 - 456.56 mg/g_{dhw}], (Table 1).

Table 1. The descriptive statistics for the total acetone extractives (TAE), the total stilbenes (TS) and the total resin acids (TRA) of the *Pinus nigra* L. heartwood samples (n=260).

Content (mg/g _{dhw})	Min.	Median	Max.	Mean	Std. Err.	Std. Dev.
Total Acetone Extractives (TAE)	81.79	310.32	480.28	304.15	5.8257	96.0163
Total Stilbenes (TS)	10.99	56.66	128.22	59.92	1.3411	21.7846
Total Resin Acids (TRA)	32.20	212.80	456.56	219.98	6.6369	102.3413

The resin acid fraction constituted 68.26% of the total extractive content, while stilbenes were present in substantial quantities accounting for 22.31% of the total extractive acetone extractives (Table 2). The remaining 9.43% corresponds to other substances present in the TAE, i.e. flavonoids, fatty acids, minor unidentified resin acids, other phenols, waxes, sterols, unsaponifiables, and others.

Table 2. The descriptive statistics for the proportions of the total stilbenes (TS) and the total resin acids (TRA) in the total acetone extractives (TAE) content of the *Pinus nigra* L. heartwood samples (n=260).

(%)	Min.	Median	Max.	Mean	Std. Err.	Std. Dev.
TS/TAE	4.85	19.95	58.15	22.31	0.7120	11.4808
TRA/TAE	14.47	70.96	97.73	68.26	1.0282	16.5791
Other substances	0	6.72	58.16	9.43	0.7007	11.2987

Significant amounts of pinosylvin and its derivatives were found in the heartwood of black pine trees from the Peloponnese (Table 3). Pinosylvin monomethyl ether (PMME) was the main component of stilbenes with an average of 40.32 mg/g_{dhw}, followed by pinosylvin (P) (average 17.07 mg/g_{dhw}) and pinosylvin dimethyl ether (PDME) (2.54 mg/g_{dhw}).

Table 3. The descriptive statistics for the pinosylvin (P) and its mono- (PMME) and dimethyl ether (PDME) derivatives of the *Pinus nigra* L. heartwood samples (n=260).

Content (mg/g _{dhw})	Min.	Median	Max.	Mean	Std. Err.	Std. Dev.
Pinosylvin (P)	1.19	16.11	40.23	17.07	0.4129	6.7582
Pinosylvin Monomethyl Ether (PMME)	8.94	37.88	94.28	40.32	0.9606	15.5495
Pinosylvin Dimethyl Ether (PDME)	0.21	2.29	7.91	2.54	0.0738	1.2194

Abietic acid was the most abundant acid (76.77 mg/g_{dhw}), followed by palustric acid (47.94 mg/g_{dhw}) and neoabietic acid (39.34 mg/g_{dhw}), all of which are classified as abietane-type resin acids. The next most abundant acid was pimaric acid, with a mean concentration of 22.54 mg/g_{dhw}. Dehydroabietic acid was found at moderate levels, while the rest, levopimaric acid, isopimaric acid, and sandaracopimaric acid, were observed in lower concentrations (Table 4).

Table 4. The descriptive statistics for the studied abietane-, i.e. abietic acid (AA), dehydroabietic acid (DAA), neoabietic acid (NAA), palustric acid (PLA), and levopimaric acid (LPA) and pimarane-type, i.e. pimaric acid (PMA), sandaracopimaric acid (SPA), and isopimaric acid (IPA) resin acids of the *Pinus nigra* L. heartwood samples (n=260).

Resin type	Content (mg/g _{dhw})	Min.	Median	Max.	Mean	Std. Err.	Std. Dev.
Abietane	Abietic (AA)	7.00	75.20	181.75	76.77	2.3102	37.2505
	Dehydroabietic (DAA)	2.56	10.53	38.59	11.69	0.3531	5.6933
	Neoabietic (NAA)	2.91	38.04	101.82	39.34	1.3073	21.0799
	Palustric (PLA)	9.76	46.10	105.22	47.94	1.4325	23.0990
	Levopimaric (LPA)	0.08	3.33	64.91	8.07	0.7055	11.3754
Pimarane	Pimaric (PMA)	2.20	21.54	59.42	22.54	0.6998	11.2838
	Sandaracopimaric (SPA)	0.16	2.55	6.67	2.72	0.0922	1.4858
	Isopimaric (IPA)	0.50	9.69	34.09	10.91	0.0405	6.5335

The evaluation of *Pinus nigra* heartwood weight loss demonstrated notable differences in decay intensity between the two fungal species examined (Table 5). *P. pini* induced a substantially higher mean weight loss of 11.43 %, with values ranging from 1.78 % to 48.45 %, indicating considerable variability in its degradative activity (SD = 9.11 %). In contrast, *C. puteana* caused a lower mean weight loss of 3.55%, with a narrower range of 1.01–12.59 % and a smaller standard deviation (SD = 1.73 %), suggesting more uniform but less aggressive decay. The markedly greater mean and variability associated with *P. pini* confirm its stronger and less consistent deteriorative effect on *P. nigra* heartwood compared with *C. puteana* under the tested conditions.

Table 5. The descriptive statistics for the *Pinus nigra* L. heartwood weight loss, expressed in relative terms (%) for tested fungi.

Fungus	Min.	Median	Max.	Mean	Std. Err.	Std. Dev.
<i>Porodaedalea pini</i>	1.78	8.86	48.45	11.43	0.5649	9.1086
<i>Coniophora puteana</i>	1.01	3.21	12.59	3.55	0.1071	1.7265

3.1. Simple Regression Model

The values of the dependent variable was modeled on the logarithmic scale to satisfy the assumptions of linearity and homoscedasticity and to linearize the relationship with the independent variables. The simple regression model (Equation (2)) statistically significantly predicts the outcome variable for most of the independent chemical variables for both tested fungi (Tables 6 and 7). Among the predictor variables tested in Equation (2), for *P. pini*, TAE accounted for the greatest proportion of variance in weight loss, significantly ($p < 0.001$) predicted the explanatory variable ($R^2_{adj} = 0.322$, $f^2 = 0.480$), followed by TRA ($p < 0.001$, $R^2_{adj} = 0.234$, $f^2 = 0.310$). In contrast, PDME explained only a negligible fraction of the variance ($p = 0.015$, $R^2_{adj} = 0.019$, $f^2 = 0.023$), indicating it is a relatively weak predictor (Table 6). In contrast, when TS was included as the predictor variable, the model failed to explain a significant proportion of the variance in weight loss ($p = 0.193$, $R^2_{adj} = 0.007$, $f^2 = 0.007$). Similarly, when P and PMME were included as independent variables, the model did not significantly predict weight loss ($p = 0.209$, $R^2_{adj} = 0.006$, $f^2 = 0.006$, and $p = 0.169$, $R^2_{adj} = 0.007$, $f^2 = 0.007$, respectively).

Table 6. The results of regression model (Equation (2)) testing the predictive ability of total acetone extractives (TAE), total resin acids (TRA), and pinosylvil dimethyl ester (PDME) on weight loss by *Porodaedalea pini* (Brot.) Murrill (1905).

Model 1: Equation (2)			Statistics					
Predictor variable	<i>p</i>	Constant	b	R ²	R ² _{adj}	f ²	AIC	BIC
TAE	<0.001	1.571	-0.002	0.324	0.322	0.48	108.403	111.956
TRA	<0.001	1.288	-0.002	0.237	0.234	0.31	139.961	143.514
PDME	0.014	0.814	0.044	0.023	0.019	0.023	194.8	198.353

b: regression coefficients, R2adj: adjusted coefficient of determination, f²: Cohen's effect size, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion.

The regression analyses summarized in Table 7 assess the influence of total stilbenes (TS), pinosylvin (P), and pinosylvin monomethyl ester (PMME) on the weight loss of wood caused by *C. puteana*. All three predictor variables showed negative regression coefficients, indicating that increasing concentrations of these stilbene compounds were associated with reduced fungal degradation. Among them, pinosylvin (P) demonstrated the strongest predictive ability, as reflected by its lowest *p*-value (<0.001) and highest adjusted coefficient of determination (R^2_{adj} = 0.055). In contrast, TS and PMME exhibited weaker associations, with higher *p*-values (0.002 and 0.009, respectively), with weight loss with lower R^2_{adj} values (0.033 and 0.025, respectively). Although the adjusted R^2 and Cohen's f^2 values suggest that the overall effect sizes are small, the statistical significance of the models indicates that stilbenes, particularly pinosylvin, contribute meaningfully to wood resistance against *C. puteana*. The negative regression coefficients across all models suggest an inverse relationship between stilbene content and fungal degradation. The AIC and BIC values further support the relative adequacy of the pinosylvin model compared to those of TS and PMME, reinforcing its role as a key chemical indicator of decay resistance.

Table 7. The Results of regression model (Equation (2)) testing the predictive ability of total stilbenes (TS), pinosylvin (P), and pinosylvin monomethyl ester (PMME) on weight loss by *Coniophora puteana* (Schumack.:Fr.) P. Karst. (1865).

Model 1: Equation (2)				Statistics				
Predictor variable	<i>p</i>	Constant	b	R^2	R^2_{adj}	f^2	AIC	BIC
TS	<0.001	0.609	-0.002	0.037	0.033	0.039	-119.661	-116.108
P	<0.001	0.626	-0.007	0.059	0.055	0.062	-127.864	-124.311
PMME	0.009	0.587	-0.002	0.026	0.022	0.026	-117.229	-113.676

b: regression coefficients, R^2_{adj} : adjusted coefficient of determination, f^2 : Cohen's effect size, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion.

3.2. Multiple Regression Model

Regression models based on Equation (3) revealed differences in the predictive ability of the tested variables on weight loss (Tables 8 and 9). Regarding *P. pini*, the combined model including total stilbenes (TS) and total resin acids (TRA) explained the greatest proportion of variance (R^2_{adj} =0.289, f^2 =0.417), with a statistically significant effect (p < 0.001). In contrast, models including stilbenes, i.e. pinosylvin (P) with pinosylvin dimethyl ether (PDME) (R^2_{adj} =0.035, f^2 =0.044) or pinosylvin monomethyl ether (PMME) with pinosylvin dimethyl ether PDME (R^2_{adj} =0.064, f^2 =0.077) accounted for only a small fraction of the variance, despite statistical significance (p = 0.004 and p < 0.001, respectively). Overall, TS combined with TRA outperformed the other predictor sets, whereas models including P, PMME, or PDME alone or in combination provided only weak explanatory power.

Table 8. The Results of regression model (Equation (3)) testing the predictive ability of total stilbenes (TS) and total resin acids (TRA) on weight loss by *Porodaedalea pini* (Brot.) Murrill (1905).

Model 2: Equation (3)			Statistics						
Predictors	<i>p</i>	Constant	TS	TRA	R^2	R^2_{adj}	f^2	AIC	BIC
TS+TRA	<0.001	1.621	-0.004	-0.002	0.294	0.289	0.417	131.903	135.452
		VIF	1.199	1.199					
Predictors	<i>p</i>	Constant	P	PDME	R^2_{adj}	R^2_{adj}	f^2	AIC	BIC
P+PDME	0.004	0.896	-0.009	0.069	0.042	0.035	0.044	198.973	202.522
		VIF	1.389	1.389					
Predictors	<i>p</i>	Constant	PMME	PDME	R^2	R^2_{adj}	f^2	AIC	BIC

PMME+PDME	<0.001	0.929	-0.008	0.122	0.071	0.064	0.077	192.206	195.755
		VIF	2.453	2.453					

b: regression coefficients, R²_{adj}: adjusted coefficient of determination, f²: Cohen's effect size, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion.

The regression analyses (Equation (3)) evaluating the combined effects of total stilbenes (TS), total resin acids (TRA), pinosylvin (P), pinosylvin monomethyl ether (PMME), and pinosylvin dimethyl ether (PDME) on weight loss caused by *C. puteana* is presented on Table 9. All regression models yielded negative coefficients for stilbenes, indicating that higher concentrations of these compounds were associated with reduced fungal degradation. Among the models, the combination of pinosylvin and its dimethyl ester (P+PDME) demonstrated the strongest predictive performance, with the lowest *p-value* (<0.001), highest adjusted coefficient of determination (R²_{adj} = 0.058), and the largest Cohen's f² (0.070). The P+PMME predictive also showed a significant relationship (*p* < 0.001, R²_{adj} = 0.052), while the TS+TRA and PMME+PDME models exhibited weaker associations (R²_{adj} = 0.036 and 0.028, respectively). The variance inflation factor (VIF) values for all predictors were close to or below 2.5, indicating no serious multicollinearity issues. Although the overall R²_{adj} values were modest, the data suggest that combinations of pinosylvin derivatives, particularly P and PDME, are more effective predictors of resistance to *P. pini*-induced weight loss than total stilbenes or resin acids alone. These findings emphasize the importance of specific stilbene derivatives in the defense mechanisms of wood against brown-rot decay of *C. puteana*.

Table 9. The Results of regression model (Equation (3)) test the predictive ability of total stilbenes (TS), total resin acids (TRA) and pinosylvins on weight loss by *Coniophora puteana* (Schumack.:Fr.) P. Karst. (1865).

Model 2: Equation (3)			Statistics						
Predictors	<i>p</i>	Constant	TS	TRA	R ²	R ² _{adj}	f ²	AIC	BIC
TS+TRA	0.003	0.662	-0.002	-0.0002	0.043	0.036	0.045	-105.129	-101.58
		VIF	1.199	1.199					
Predictors	<i>p</i>	Constant	P	PMME	R ²	R ² _{adj}	f ²	AIC	BIC
P+PMME	<0.001	0.623	-0.007	0	0.059	0.052	0.063	-115.978	-112.429
			1.946	1.946					
Predictors	<i>p</i>	Constant	P	PDME	R ²	R ² _{adj}	f ²	AIC	BIC
P+PDME	<0.001	0.612	-0.008	0.015	0.065	0.058	0.07	-122.507	-118.958
		VIF	1.389	1.389					
Predictors	<i>p</i>	Constant	PMME	PDME	R ²	R ² _{adj}	f ²	AIC	BIC
PMME+PDME	0.009	0.583	-0.003	0.024	0.035	0.028	0.037	-113.289	-109.74
		VIF	2.453	2.453					

b: regression coefficients, R²_{adj}: adjusted coefficient of determination, f²: Cohen's effect size, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion.

The regression model results predicting weight loss based on Equation (4) are shown in Tables 10 and 11. Regarding *P. pini*, the model incorporating only stilbenes (P, PMME, PDME) was statistically significant (*p* < 0.001) but exhibited very low explanatory power (R²_{adj} = 0.061). In contrast, the model containing only resin acids (AA, DAA, NAA, PLA, LPA, PMA, SPA, IPA) was statistically significant (*p* < 0.001) and accounted for a substantially greater proportion of variance (R²_{adj} = 0.266). The combined model, including all stilbenes and resin acids, was the most significant (*p* < 0.001) and provided the best overall fit to the data, explaining the highest amount of variance (R²_{adj} = 0.331). However, the slight improvement in AIC and BIC values from the resin acid model to the combined

model indicates that the resin acids are the primary contributors of predictive power, with the stilbenes adding little additional explanatory value. Notably, high Variance Inflation Factor (VIF) values observed for several predictors (e.g., DAA and PLA) indicate potential multicollinearity within the resin acid and combined models (Table 10).

Table 10. The results of regression model (Equation (4)) testing the predictive ability of studied stilbenes and studied resin acids on weight loss by *Porodaedalea pini* (Brot.) Murrill (1905).

Model 3: Equation (4)					Statistics														
Predictors	<i>p</i>	Consta nt	P	PMM E	PDM E										R ²	R ² _{adj}	f ²	AIC	BIC
P+PMME+P DME	<0.001	0.939	-0.002	-0.007	0.121										0.072	0.06 1	0.07 7	201.05 2	204.59 7
		VIF	1.946	3.438	2.454														
Predictors	<i>p</i>	Consta nt	AA	DAA	NAA	PLA	LPA	PMA	SPA	IPA					R ²	R ² _{adj}	f ²	AIC	BIC
AA+DAA+ NAA																			
+PLA+LPA	<0.001	1.299	-0.005	-0.001	-0.001	0.002	-0.001	-0.001	-0.005	0.01					0.289	0.26 6	0.40 6	183.67 4	187.19 9
+PMA																			
+SPA+IPA																			
		VIF	7.493	16.483	3.213	9.146	2.555	5.803	2.978	6.338									
Predictors	<i>p</i>	Consta nt	P	PMM E	PDM E	AA	DAA	NAA	PLA	LPA	PMA	SPA	IPA	R ²	R ² _{adj}	f ²	AIC	BIC	
P+PMME+P DME																			
+AA+DAA+ NAA	<0.001	1.557	-0.001	-0.009	0.06	-0.005	-0.001	0.003	0.001	-0.002	-0.001	-0.004	0.002	0.359	0.33 1	0.56 1	183.79 1	187.30 5	
+PLA+LPA																			
+PMA																			
+SPA+IPA																			
		VIF	2.018	3.521	2.695	7.573	16.50 1	3.392	9.345	2.611	5.932	2.98	6.397						

b: regression coefficients, R²_{adj}: adjusted coefficient of determination, f²: Cohen's effect size, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion, VIF: Variance Inflation Factor.

The results of regression models (Equation (4)) used to evaluate the predictive ability of selected stilbenes and resin acids on weight loss by *C. puteana* are presented on Table 11. Across all models, individual predictors displayed small regression coefficients, indicating limited influence on weight loss, although some, such as IPA (0.026) and PDME (0.0237), had slightly higher contributions. The variance inflation factors (VIFs) suggest minimal multicollinearity in the stilbene-only model, while the resin acid and combined models exhibited higher VIFs for certain predictors, particularly DAA (16.483) and PMME (3.521), indicating potential multicollinearity concerns. Adjusted R² values were low for all models (0.031–0.094), suggesting that the predictors explained only a small portion of the variability in weight loss. Model comparison using AIC and BIC further supports modest model performance, with the combined model showing the best fit (AIC = -45.276, BIC = -41.762). Overall, the results indicate that while some stilbenes and resin acids may influence weight loss, their predictive power individually and collectively remains limited.

Table 11. The results of regression model (Equation (4)) testing the predictive ability of studied stilbenes and studied resin acids on weight loss by *Coniophora puteana* (Schumack.:Fr.) P. Karst. (1865).

Model 3: Equation (4)						Statistics					
Predictors	p	Consta nt	P	PMM E	PDME	R ²	R ² _{adj}	f ²	AIC	BIC	

P+PMME+P DME	<0.001	0.62	-0.007	-0.001	0.0238											0.068	0.057	0.077	201.05	204.59
		VIF	1.946	3.438	2.454														2	7
Predictors	p	Consta nt	AA	DAA	NAA	PLA	LPA	PMA	SPA	IPA						R ²	R ² _{adj}	f ²	AIC	BIC
AA+DAA+ NAA																				
+PLA+LPA+ PMA +SPA+IPA	0.041	0.477	0.001	-0.009	-0.004	0.004	0.001	0.002	0.002	0.026						0.061	0.031	0.065	-54.617	-51.092
		VIF	7.493	16.483	3.213	9.146	2.555	5.803	2.978	6.338										
Predictors	p	Consta nt	P	PMM E	PDME	AA	DAA	NAA	PLA	LPA	PMA	SPA	IPA			R ²	R ² _{adj}	f ²	AIC	BIC
P+PMME+P DME																				
+AA+DAA+ NAA	<0.001	0.63	-0.007	-0.001	0.018	0.001	-0.008	-0.002	0.003	0.001	0.001	0.002	0.025	0.133	0.094	0.153	-45.28	-41.76		
+PLA+LPA+ PMA +SPA+IPA																				
		VIF	2.018	3.521	2.695	7.573	16.501	3.392	9.345	2.611	5.932	2.98	6.397							

b: regression coefficients, R²_{adj}: adjusted coefficient of determination, f²: Cohen's effect size, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion, VIF: Variance Inflation Factor.

4. Discussion

Natural durability of wood is largely governed by the presence and activity of extractives in the heartwood. These secondary metabolites provide chemical defense against fungal colonization and contribute to the long-term stability of wood in service [4,6,7,46,54–56]. Evidence for their central role comes from studies showing that removal of extractives renders otherwise durable wood susceptible to decay [7,57], while addition of heartwood extractives to non-durable species can significantly increase decay resistance [57–59].

Among the compounds in pine heartwood, stilbenes and resin acids have received particular attention. The antifungal action of heartwood extractives is linked to multiple mechanisms while the relative contributions of different extractive groups remain unresolved. These include the toxicity of resin components, antioxidant activity, and hydrophobic effects that reduce water penetration [60,61]. Extractives do not act solely through bulk accumulation but rather through the biological efficacy of individual compounds [47].

Phenolics have been consistently linked with decay resistance due to both antifungal properties and their role in lowering wood hygroscopicity [31,51]. Particularly, stilbenes, i.e. pinosylvin and its derivatives, exhibit strong antifungal activity across multiple species but are also vulnerable to oxidative degradation reducing their long-term effectiveness [62–64]. Pinosylvin alone is insufficient to fully explain decay resistance [50,65]. Resin acids, by contrast, primarily contribute through water repellency rather than direct toxicity [66–69]. Notably, beyond their general water-repellent function, certain abietane-type resin acids, particularly abietic acid, have been correlated with increased durability [70]. Micales et al. (1994) found that abietane-type resin acids exhibited greater fungitoxicity than pimarane-type acids in agar medium cultures.

Decay resistance varies greatly across tree species, fungal taxa (reflecting differences in their ability to overcome heartwood defenses) and experimental conditions. Scots pine (*Pinus sylvestris*) heartwood typically demonstrates greater resistance than sapwood, a difference attributed to higher levels of stilbenes and resin acids [62,63,72]. For example, Brischke et al. (2022) observed that sapwood of Scots pine experienced much greater weight loss than heartwood when exposed to decay fungi. Belt et al. (2022) reported that *Coniophora puteana* caused limited weight loss in pine heartwood, while *Rhodonia placenta* (syn. *Postia placenta*) was far more destructive, with losses ranging from 5.8 - 36.4 %. This difference was attributed to their differential capacity to degrade stilbenes: *R. placenta* showed much higher efficiency than *Coniophora puteana* [63,73,74]. Extended variation in *Pinus*

sylvestris L. heartwood weight loss was reported by Leinonen et al. (2008). They found a strong negative correlation ($r = -0.85$, $p < 0.0001$) between heartwood extractive concentration and weight loss (ranged between 0.87 - 49.28 % with an average of 22.43 %) caused by *Coniophora puteana*, confirming the protective role of extractives. Harju et al. (2003) found higher phenolic concentrations in decay-resistant Scots pine heartwood compared to susceptible trees. The same research group once more reported a strong, negative, and statistically significant correlation (-0.82 , $p < 0.001$) between weight loss of *Pinus sylvestris* L. heartwood subjected to *Coniophora puteana* and the concentration of total phenolics during the decay tests [51]. Heijari et al. (2005) found similar high correlations between the total phenolics concentration in the heartwood of Scots pine and weight loss in the decay test. Gref et al. (2000) found weight losses for the *Pinus sylvestris* L. heartwood caused by *Phanerochaete chrysosporium* and *Rhodonia placenta* (syn. *Postia placenta*) ranged up to 3.2 % (avg 2.9 %) and 8.6 % (avg 6.3 %), respectively.

Mixed results were also found by De Angelis et al. (2018) who reported that *Trametes versicolor* (L.) Lloyd and two brown rot fungi *Fibroporia vaillantii* (DC.) Parmasto and *Gloeophyllum trabeum* (Pers.) Murrill were able to degrade 0.5 %, 8.6 % and 1.0 % of *Pinus pinea* heartwood, respectively. Similarly, Hassan et al. (2024) observed in *Pinus elliottii* and *Pinus caribaea* that blocks with higher resin contents exhibited far lower weight losses (6 %) than those with low resin (21 %) when exposed to *Fomitopsis ostreiformis*. Weight loss was reduced (0 - 3 %) as resin level in wood increased. Similar variation was reported for *Pinus leucodermis*, where heartwood with four times the extractive content of sapwood exhibited correspondingly close weight loss values, i.e. 30.65 % and of 34.68 %, respectively, against *Coniophora puteana* [79].

Other species also show strong links between extractive content and decay resistance. In Norway spruce, *Trametes versicolor* caused modest losses (~10 %), whereas *Coniophora puteana* and *Rhodonia placenta* (syn. *Postia placenta*) caused up to 34 % weight loss. Extractives were found to inhibit brown rot of *Poria placenta* in *Cupressus lusitanica* [16], while in western red cedar (*Thuja plicata*), variability in decay resistance near the pith was directly linked to the presence of chemoprotective compounds [80]. Windeisen et al. (2002) found that larch heartwood with high extractive levels lost ~30% mass under fungal exposure, compared to 55-60% for low-extractive heartwood. Gierlinger et al. (2004a) reported strong positive and significant correlations (up to 0.87, $p < 0.01$) between phenolic content and decay resistance in various larch species. In Siberian larch, Venäläinen et al. (2006) likewise showed significant negative correlations ($r = -0.677$, $p = 0.006$) between phenolic concentration and weight loss of 20%, 28%, and 17%, respectively caused by brown-rot fungi *Coniophora puteana*, *Rhodonia placenta* (syn. *Poria placenta*), and *Gloeophyllum trabeum* respectively. However, significant phenotypic variation exists: some cores showed no weight loss despite fungal exposure, while others were highly susceptible [81].

Ultimately, decay resistance emerges from a complex interplay of extractive chemistry, distribution, wood structure, and fungal physiology. Despite abundant supporting evidence, the precise role of extractives remains debated. Some studies show weak or inconsistent correlations between extractive concentration and decay resistance [4,80]. For example, certain larch species produce high extractive content without improved durability [82]. Moreover, extractives may act synergistically, with multiple low-toxicity compounds working together to protect wood [13,83]. The micro-distribution of extractives within wood tissue is also critical but difficult to measure. Taylor et al. (2002) emphasized that uneven distribution, rather than absolute content, may explain observed differences in decay performance. Even within a single tree, differences between inner and outer heartwood or between samples can yield widely different outcomes [72,81]. Furthermore, extractive efficiency varies not only among tree species but also within species due to genetic differences, environmental influences, and age-related changes [4,28,84,85]. Moreover, variability in decay resistance occurs not only among fungi but also within the same fungal group, depending on their enzymatic ability to metabolize extractives [78]. Such complexity highlights the potential for conflicting results and the limitations of observed correlations. As Hart and Shrimpton (1979)

cautioned, findings should be interpreted carefully, since fungal-extractive interactions are species-specific and context-dependent.

5. Conclusions

This study demonstrates that the natural decay resistance of *Pinus nigra* heartwood is strongly influenced by its chemical composition, particularly the content and composition of extractives, but also by the specific fungi species involved. Their diverse decay capabilities involved in the decay process and can significantly alter the degree and pattern of wood degradation. Between *Porodaedalea pini* and *Coniophora puteana* tested here, the former caused significantly greater weight loss, highlighting that fungal identity dictates, in an extent, degradation severity. The exceptionally high levels of resin acids and stilbenes identified in the sampled clones highlight the defensive potential of this species against wood-decaying fungi. Statistical analyses identified that the acetone extractive content was the strongest single predictor. Multiple regression analyses unequivocally demonstrated that resin acids are the principal chemical drivers associated with reduced weight loss. Within this group, abietane-type compounds, particularly abietic acid, had the greatest impact on this response variable, highlighting their importance in decay resistance. In comparison, stilbenes, despite their abundance, offered limited improvement to the explanatory strength of the model. Variation in extractive content and decay resistance among clones indicates a significant genetic component, coupled with the high heritability of these traits, underscoring the potential for selective breeding to enhance durability traits in black pine.

The cultivation and utilization of *P. nigra* wood, a naturally durable raw material, presents a sustainable alternative to hazardous wood preservatives, increasing timber service life, especially in outdoor applications. Promoting the use of naturally resistant wood enhances the lifespan of wooden products, reduces environmental and health risks, and minimizes the forest products sector’s footprint within a climate-smart forestry framework.

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Abbreviations

Abbreviations used in this manuscript:

¹ H-NMR	Proton Nuclear Magnetic Resonance
WL	Weight Loss
TAE	Total Acetone Extractive
P	Pinosylvin
PMME	Pinosylvin Mono Methyl Ether
PDME	Pinosylvin Di-Methyl Ether
AA	Abietic Acid
DAA	Dehydro-Abietic Acid
NAA	Neoabietic Acid

PLA	Palustric Acid
LPA	Levopimaric Acid
PMA	Pimaric Acid
SPA	Sandaracopimaric Acid
IPA	Isopimaric Acid

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