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Leaf Trichome Density Rather than Phenolic Compounds Determines the Resistance of Hazelnut Cultivars to *Erysiphe corylacearum*

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Abstract

Erysiphe corylacearum, a newly emerging pathogen of hazelnut (*Corylus avellana* L.), has rapidly spread across Europe, causing severe outbreaks and threatening the sustainability of hazelnut production. This study investigated the biochemical and morphological traits associated with cultivar susceptibility to *E. corylacearum*. Eight cultivars representing a range of resistance levels were analyzed for leaf phenolic composition and trichome density. A total of 22 phenolic compounds were identified, with myricetin-3-*O*-rhamnoside, 1,7-bis-(4-hydroxyphenyl)-4,6-heptadien-3-one-hexoside, hirsutenone-hexoside, and quercetin-3-*O*-rhamnoside as dominant metabolites; however, no correlation was found between constitutive phenolic content/profile and disease resistance. In contrast, leaf trichome density showed a strong negative association with susceptibility, suggesting that denser trichomes act as a physical barrier to infection. Our results indicate that trichome density is a key morphological trait conferring resistance to *E. corylacearum* and could serve as a practical marker for breeding and selection of more resistant hazelnut cultivars.

Keywords: phenolic compounds; HPLC-MS; identification; *Corylus avellana*; disease resistance

1. Introduction

Powdery mildew diseases, caused by obligate biotrophic fungi of the genus *Erysiphe*, are among the most widespread foliar pathogens in woody crops worldwide. These fungi thrive under a wide range of environmental conditions and are well known for reducing photosynthetic efficiency, weakening host vigor, and ultimately diminishing yield and product quality [1]. Hazelnut (*Corylus avellana* L.), one of the most important nut crops in Europe and globally, has traditionally been considered relatively unaffected by powdery mildew compared to other fruit and nut species. Until recently, hazelnuts were mainly infected by *Phyllactinia guttata*, but its occurrence was late in the season and mild, thus not significantly affecting yield or overall plant health [2].

This situation changed with the emergence of a novel pathogen, *Erysiphe corylacearum*, first detected on hazelnut in Slovenia in 2020. It has spread from Turkey to almost all European countries (Italy, Ukraine, Romania, Austria, Switzerland, Germany, Slovakia, Hungary, etc.) [3]. Since its introduction, the fungus has spread rapidly in both natural forest stands and cultivated orchards, showing the potential for epidemic development under favorable conditions.

Initially, outbreaks of *E. corylacearum* were regarded as sporadic and of limited economic importance. However, in recent years, severe epidemics have been observed in

Check for updates

Academic Editor: Seiya Saito

Received: 14 April 2026
Revised: 29 April 2026
Accepted: 2 May 2026
Published: 5 May 2026

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commercial orchards, with particularly damaging effects on the predominant Slovenian cultivar 'Istrska dolgoplodna', abbreviated as 'ID'. This cultivar is the most widely grown in the country, representing the main variety across all production regions. The presence of *E. corylacearum* has made its continued cultivation highly problematic, especially because no effective fungicides are currently registered for control. Even if control products were available, regular treatments would be required, leading to high production costs that would make Slovenian hazelnuts less competitive compared to cultivars grown in other countries. In such circumstances, growers may even abandon orchards of this cultivar. While other cultivars are also affected, the infections observed so far have been less severe and, in many cultivars, are not yet considered economically damaging. Nevertheless, susceptibility appears to be highly cultivar-dependent.

Infected trees show premature defoliation, reduced vegetative growth, and significant reductions in nut yield and kernel quality [2,3]. These developments have raised concerns that the pathogen could endanger the long-term sustainability of hazelnut production, particularly in highly susceptible cultivars.

Chemical management options for powdery mildew in hazelnut are extremely limited. Moreover, the widespread occurrence of *E. corylacearum* in wild hazel populations provides a constant source of inoculum, making eradication impossible and disease control in orchards highly challenging [2]. Under these conditions, the development and use of resistant cultivars is the most promising and environmentally sustainable strategy.

In woody perennials, resistance to foliar pathogens is often linked to constitutive or inducible defense mechanisms. Elevated concentrations of phenolic compounds, such as flavonoids, tannins, and phenolic acids, are frequently associated with restricted fungal colonization, while structural traits, including cuticle thickness, trichome density, and stomatal characteristics, can further reduce infection [4,5]. Specifically, trichomes act as a physical barrier that prevents spore contact with the epidermis, alters the leaf microenvironment, and inhibits spore germination [1]. Hazelnut leaves are rich in specialized metabolites, such as diarylheptanoids and diverse polyphenols [6], but their direct role in resistance to *E. corylacearum* has not been investigated yet.

The present study builds on our previous, still unpublished work assessing the susceptibility of 49 hazelnut cultivars maintained at the Experimental Field for Nut Crops in Maribor, Slovenia, where a visual assessment of the 49 hazelnut cultivars has been performed, and those cultivars were characterized based on disease susceptibility. Based on those field resistance ratings, we selected eight cultivars representing the full spectrum from highly susceptible to resistant for detailed phytochemical and morphological analyses. By quantifying individual phenolic profiles and comparing them with observed infection levels as well as morphological traits (e.g., trichome density), we aimed to determine whether specific phenolic compounds underpin cultivar resistance or whether anatomical characteristics play a decisive role. The outcomes of this study will contribute to breeding and orchard management strategies to ensure the resilience of hazelnut production under the increasing threat of *E. corylacearum*. In addition, the findings will assist extension specialists and advisors in recommending appropriate cultivars for growers, considering the risks associated with this emerging disease.

2. Materials and Methods

2.1. Plant Material

The study was carried out at the Experimental Field for Nut Crops in Maribor, Slovenia (46°34′01″ N; 15°37′51″ E; 275 m a.s.l.). In a previous screening, 49 hazelnut (*Corylus avellana*) cultivars were evaluated for their response to *E. corylacearum* at two assessment dates, 1 July and 15 August (2023 and 2024). Disease severity was visually scored using a

rating scale (Table 1), which allowed classification of cultivars into resistance groups. To ensure reliable evaluation, no fungicides targeting *E. corylacearum* were applied in years prior to the trial (2023 and 2024) or during the experimental year (2025), enabling natural infection pressure. All plants were maintained under standard phytosanitary treatments in their 28th growing year (2025). The orchard is located on flat terrain and is managed using a bush growth system with a spacing of 4.5 × 5.5 m. All plants were uniform and in good health.

Table 1. Disease severity scale (score 1–10) and corresponding descriptions used for visual assessment of *Erysiphe corylacearum* infection on leaves of different hazelnut cultivars.

Score	% of Leaf Surface Affected	Description
1	0%	No visible symptoms
2	1–5%	Very light infection, few isolated patches; very few leaves are affected (less than 5%)
3	6–10%	Light infection, sparse colonies, minimal coalescence; few leaves affected (less than 10%)
4	11–20%	Moderate infection, visible colonies; less than 20% are affected
5	21–30%	Patchy coverage with coalescing colonies, some leaf distortion; less than 30% are affected
6	31–45%	Significant presence on upper and lower leaf surfaces, beginning of widespread colonization; approximately 50% of leaves are affected
7	46–60%	Severe infection with clear leaf damage; more than 65% of leaves are affected
8	61–75%	Severe infection; large areas covered; signs of leaf necrosis possible; more than 80% of leaves are affected
9	76–90%	Very severe infection; most of the leaf is covered; visible damage across the canopy; all leaves are affected
10	>90%	Nearly the entire leaf surface is affected; leaf deformation and premature senescence are likely; all leaves are affected

For the present investigation, leaf samples were collected on 29 May 2025, before any visible symptoms of powdery mildew were observed, thus avoiding the interference of the pathogen in subsequent analyses. Eight cultivars representing a broad resistance spectrum were selected based on the earlier field evaluation (Table 2). These included one highly susceptible cultivar ('Istrska dolgoplodna' or 'ID'), two susceptible cultivars ('TGDL' and 'Gunslebert'), three moderately susceptible cultivars ('Clark', 'Fertile de Coutard', and 'Riccio di Talanico'), and two moderately resistant cultivars ('N-650' and 'Butler'). None of the tested cultivars exhibited complete immunity to *E. corylacearum*; thus, resistant cultivars were not observed. For each cultivar, four sampling plants were used, providing four biological replicates. For trichome analysis, only the third fully developed leaf was collected. For phenolic content determination, both younger and older leaves were sampled. Younger leaves corresponded to the first and second fully developed leaves on each shoot, while older leaves corresponded to the third and fourth fully developed leaves.

Table 2. Resistance grades of hazelnut cultivars to *Erysiphe corylacearum* based on visual disease severity assessments.

Resistance Grade	Description	Cultivars
1—Resistant	No visible symptoms	None
2—Moderately resistant	Mild symptoms; minor leaf coverage	N-650, Butler
3—Moderately susceptible	Moderate symptoms; patchy leaf coverage	Clark, Fertile de Coutard, Riccio di Talanico
4—Susceptible	Severe symptoms; extensive leaf coverage and damage	TGDL, Gunslebert
5—Highly susceptible	Very severe symptoms; >75% leaf surface affected	Istrska dolgoplodna (ID)

Immediately after collection, half of the leaves were carefully stored in paper bags and put in a cooler, and half of the leaves were frozen in liquid nitrogen on site and transported in a nitrogen-cooled container to the Department of Agronomy, Biotechnical Faculty, University of Ljubljana (Slovenia). The frozen leaf samples were lyophilized and then used for subsequent biochemical analyses, whereas fresh leaves were examined to assess leaf trichome density. This was done on a Stereo Zoom Microscope OZP 558 equipped with a tablet microscope camera ODC 241 (KERN & SOHN GmbH; Balingen, Germany).

2.2. Extraction of Phenolic Compounds

Phenolic compounds were isolated from hazelnut leaves following a modified version of the method described by Medic et al. [7]. For extraction, 1 g of lyophilized hazelnut leaf tissue was combined with 3 mL of 80% methanol containing 1% formic acid (v/v) in bi-distilled water, corresponding to a 1:3 w/v ratio. The mixture was maintained in an ice-water bath for 60 minutes, followed by sonication in an ultrasonic bath (Sonis 4; Iskra Pio, Sentjernej, Slovenia). After sonication, the samples were centrifuged at 10,000× g for 10 min at 4 °C, and the resulting supernatants were filtered through 0.2 µm polyamide filters (Chromafil AO-20/25; Macherey-Nagel, Düren, Germany) prior to further analyses.

2.3. Analysis of Phenolic Compounds

Phenolic compounds were characterized using a tandem mass spectrometer (MS/MS; LCQ Deca XP MAX; Thermo Scientific, Waltham, MA, USA) operated in negative ion mode to facilitate the identification of a wide range of phenolic molecules. Detailed instrumental parameters for phenolic compound identification were applied as previously described [8]. Quantification was carried out on a Vanquish UHPLC system equipped with a diode array detector, with detection wavelengths set at 350 nm for flavonols and 280 nm for other phenolic compounds, following established protocols [7,8].

Where available, external standards were used for both identification and quantification. Compounds lacking commercial standards were identified based on their MS fragmentation patterns in comparison with the literature data, and their concentrations were estimated using structurally related compounds. The phenolic content is reported in mg/kg, and the sum of all identified phenolic compounds is presented as the total analyzed phenolic content (TAPC), expressed in mg/kg of DW (dry weight).

2.4. Methodology for Determination of Trichome Density

Trichome density on leaf lamina and leaf veins was initially assessed visually using the UPOV descriptors. However, these descriptors provide only a basic three-grade scale for the hairiness of the lower leaf surface (weak, medium, and strong). To obtain a more accurate evaluation of leaf hairiness (trichome density), we employed microscopic examination (Stereo Zoom Microscope OZP 558 equipped with a tablet microscope camera ODC 241). Trichome density was assessed as in other plants on the upper epidermis of the lower part of the third fully developed leaf, in the middle proportion of the leaf 1 cm from the main leaf vein for the leaf lamina, and in the middle proportion of the leaf for the leaf vein. Based on these observations, we developed a new 9-grade scale, which we propose for use in this and future descriptions of trichome density of the upper epidermis of leaf lamina and leaf veins in hazelnut. As visual rating systems have been shown to correlate with direct trichome counts in several other plant species [9,10], both for leaf lamina and leaf vein trichome density, we adopted a visual scale from cotton, where a 9-grade visual assessment is standard for trichome density determination [10]. The visual rating system was confirmed with trichome counting. A 0.1 mm × 0.5 mm patch of leaf lamina surface was carefully measured with a digital caliper, then carefully dissected with a scalpel. Three leaves that represented a biological repetition were used. For the trichome density of leaf

veins, 0.1 mm × 0.1 mm pieces were cut out to try to perform the counting of the trichomes. For the assessment of trichome density on hazelnut leaf lamina, the visual scoring was accompanied by the numerical numbering of trichome counts per 0.05 mm^2 of leaf surface, while for the assessment of leaf vein trichome density, only a visual scoring approach was presented, as individual trichomes were interconnected and impossible to distinguish by AI-assisted tools or naked eye measurements. This limitation likely explains the lack of published data on trichome density in hazelnut to date. Trichome density was visually assessed on both the leaf lamina and the main leaf vein using a nine-grade scale. For the upper epidermis of leaf lamina, numbers represented: 1 = glabrous (0–10 trichomes per 0.05 mm^2), 2 = extremely sparse (10–25 trichomes per 0.05 mm^2), 3 = very sparse (25–50 trichomes per 0.05 mm^2), 4 = sparse (50–75 trichomes per 0.05 mm^2), 5 = moderately sparse (75–100 trichomes per 0.05 mm^2), 6 = moderate (100–150 trichomes per 0.05 mm^2), 7 = moderately dense (150–200 trichomes per 0.05 mm^2), 8 = dense (200–300 trichomes per 0.05 mm^2), and 9 = very dense (300 or more trichomes per 0.05 mm^2). For leaf veins, due to the overlapping of trichomes, the counting was not possible; therefore, the numbers represented only visual assessments: 1 = glabrous, 2 = extremely sparse, 3 = very sparse, 4 = sparse, 5 = moderately sparse, 6 = moderate, 7 = moderately dense, 8 = dense, and 9 = very dense. The visual trichome density scoring system was quantitatively validated by comparing cultivar mean visual scores with mean trichome counts (per 0.05 mm^2 of leaf lamina). The relationship between the two measures was assessed using Spearman's rank correlation and linear regression analysis, revealing a strong and statistically significant positive association ($\rho = 0.98$, $p < 0.001$), with the regression model explaining 95% of the observed variation ($R^2 = 0.95$), thereby confirming the reliability of the visual assessment method. Examples of trichome density on leaf lamina and leaf veins for the tested cultivars are presented in Figures 1 and 2. The cross-sectioned leaf lamina best illustrates trichome density for each cultivar; the trichome density on leaf veins is also shown.

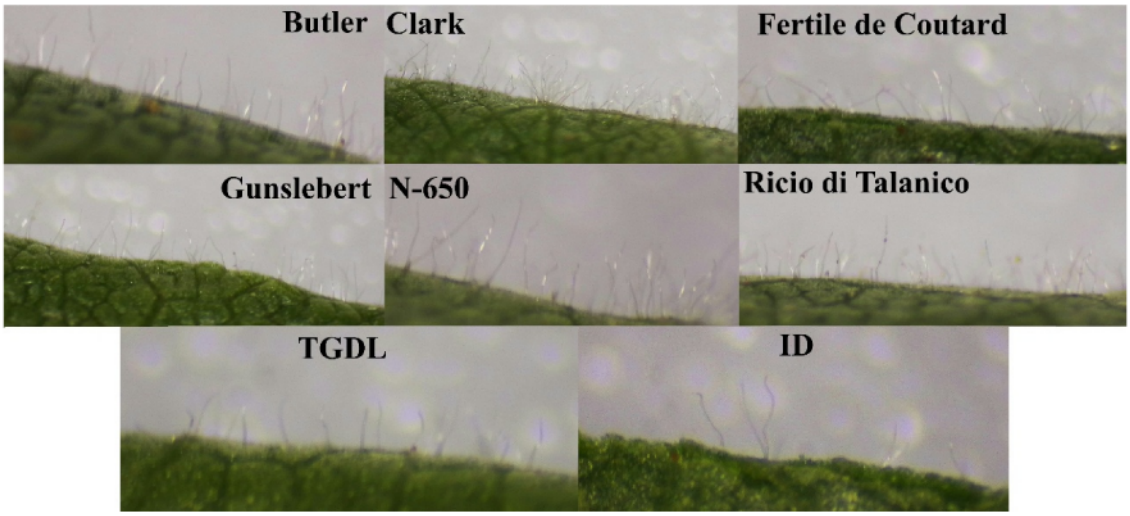

Figure 1. Visual presentation of leaf trichome density across the studied cultivars.

2.5. Chemicals

The standards and chemicals used for the analysis were procured as follows: acetonitrile, formic acid, methanol, neochlorogenic acid (trans-3-caffeoylquinic acid), cryptochlorogenic acid (4-caffeoylquinic acid), chlorogenic acid (trans-5-caffeoylquinic acid), quercetin-3-*O*-rhamnoside, quercetin-3-*O*-galactoside, and kaempferol-3-*O*-galactoside were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); *p*-coumaric acid and quercetin-3-*O*-glucoside were sourced from Fluka Chemie GmbH (Buchs, Switzerland); and myricetin-

3-*O*-rhamnoside, quercetin-3-*O*-arabinofuranoside, quercetin-3-*O*-arabinopyranoside were purchased from Apin Chemicals, Abingdon, UK.

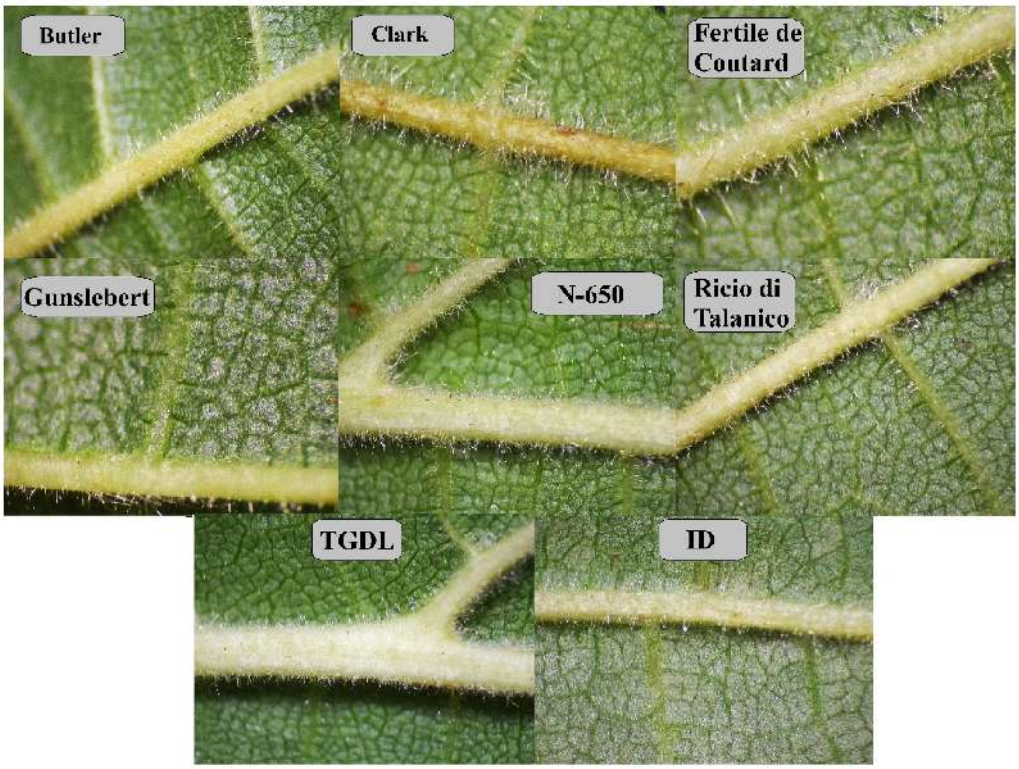

Figure 2. Visual presentation of leaf vein trichome density across the studied cultivars.

2.6. Statistical Analysis

Data were collected using Microsoft Excel 2016 and statistically analyzed with R Commander (package Rcmdr, version 4.3.3). A two-way analysis of variance (ANOVA) was performed, followed by Tukey's post hoc test for pairwise multiple comparisons to determine significant differences between groups. Multivariate analysis was conducted using Principal Component Analysis (PCA) within R Commander, with the *factoextra* package utilized for enhanced visualization and interpretation of the PCA results. Correlation analysis was conducted using the Spearman rank correlation coefficient, which assesses monotonic relationships between variables without assuming normality. The correlation matrix and corresponding *p*-values were computed in R using the rcorr() function from the Hmisc package, with the argument type = 'spearman'. This function returns both the matrix of correlation coefficients (res$r) and the matrix of associated *p*-values (res$P). The results were visualized using the corrplot package, where the strength and direction of correlations were represented by color gradients, and the numerical values of the coefficients were displayed within the matrix. Statistical significance was indicated using asterisk symbols based on predefined thresholds ($p < 0.05$, $p < 0.01$, $p < 0.001$), and it was determined at a 95% confidence level, with *p*-values of less than 0.05 considered significant.

3. Results and Discussion

3.1. Identification of Phenolic Compounds

Using information from the literature and available reference standards, a total of 22 phenolic compounds were tentatively detected in hazelnut leaves. Identification was based on their deprotonated molecular ions ($[M - H]^-$) and characteristic fragmentation patterns observed across MS^2 and MS^4 spectra, as summarized in Table 3, along with the standards to which they are expressed. The characterization and identification of the

expressed compounds have been previously reported in detail [11–13], with all detected compounds identified in hazelnut or in the leaf tissue of other plant species.

Table 3. Tentative identification of the 22 phenolic compounds in different Corylus avelana cultivars and the standards to which they are expressed.

	Compound	Rt (min)	$[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	MS^3 (*m*/*z*)	MS^4 (*m*/*z*)	Expressed As
1	Neochlorogenic acid (3-caffeoylquinic acid)	8.41	353	191 (100), 179 (43), 135 (7), 173 (3)			Neochlorogenic acid
2	Chlorogenic acid (5-caffeoylquinic acid)	10.81	353	191 (100), 179 (3)			Chlorogenic acid
3	Cryptochlorogenic acid (4-caffeoylquinic acid)	12.10	353	191 (100), 179 (4)			Cryptochlorogenic acid
4	Caftaric acid [caffeoyl tartaric acid]	13.14	311	149 (100), 179 (91), 135 (6)	131 (100), 103 (57), 87 (38)		Neochlorogenic acid
5	Caffeoylshikimic acid	13.71	335	179 (100), 135 (16)	135 (100)		Neochlorogenic acid
6	Myricetin-3-*O*-galactoside	15.22	479	316 (100), 317 (89), 179 (4), 151 (1)			Myricetin-3-*O*-rhamnoside
7	Myricetin-3-*O*-glucoside	15.48	479	316 (100), 317 (99), 179 (5), 151 (1)			Myricetin-3-*O*-rhamnoside
8	Coutaric acid [trans-*p*-Coumaroyltartaric acid]	16.41	295	**163** (100), **149** (47), 131 (7)	**163** -> 119 (100); 131 (100); **149** -> 103 (58), 87 (40)		*p*-Coumaric acid
9	Myricetin-3-*O*-pentoside	16.41	449	316 (100), 317 (99), 179 (4), 151 (1)			Myricetin-3-*O*-rhamnoside
10	Hirsutenone-hexoside	17.18	507	327 (100)	205 (100), 179 (34), 147 (20), 121 (10)	147 (100), 121 (29), 83 (5), 109 (2)	Quercetin-3-*O*-galactoside
11	Myricetin-3-*O*-rhamnoside	17.59	463	316 (100), 317 (60), 179 (4)			Myricetin-3-*O*-rhamnoside
12	Quercetin-3-*O*-galactoside	17.99	463	301 (100), 300 (21), 179 (3)	179 (100), 151 (64)		Quercetin-3-*O*-galactoside
13	Quercetin-3-*O*-glucoside	18.34	463	301 (100), 300 (15), 179 (2)	179 (100), 151 (63)		Quercetin-3-*O*-glucoside
14	Oregonin	18.68	477	327 (100)	205 (100), 179 (34), 147 (20), 121 (11), 217 (2)	121 (100), 147 (60), 83 (17), 109 (10)	Quercetin-3-*O*-galactoside
15	1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-hept-6-en-3-one derivative	19.71	491	311 (100)	205 (100), 189 (65), 121 (16), 217 (15), 147 (14)	147 (100), 121 (30), 83 (5), 109 (2)	Quercetin-3-*O*-galactoside
16	Quercetin-3-*O*-arabinopyranoside	20.26	433	301 (100), 300 (3)	179 (100), 151 (64)		Quercetin-3-*O*-arabinopyranoside
17	Quercetin-3-*O*-arabinofuranoside	20.41	433	301 (100), 300 (2)	179 (100), 151 (64)		Quercetin-3-*O*-arabinofuranoside
18	Quercetin-3-*O*-rhamnoside	20.77	447	301 (100), 300 (21), 179 (1)	179 (100), 151 (64)		Quercetin-3-*O*-rhamnoside
19	1-(4-Hydroxyphenyl)-7-(3,4-dihydroxyphenyl)-heptan-3-one-5-*O*-pentoside	21.15	461	311 (100)	205 (100), 189 (75), 217 (21), 121 (17), 147 (16)	147 (100), 121 (30), 83 (5), 109 (2)	Quercetin-3-*O*-galactoside
20	1,7-bis-(4-hydroxyphenyl)-4,6-heptadien-3-one-hexoside	21.86	473	293 (100)	291 (100), 251 (39), 275 (17), 249 (11), 197 (7)		Quercetin-3-*O*-galactoside
21	Kaempferol-3-*O*-rhamnoside	22.82	431	285 (100), 284 (34), 327 (5), 255 (2)	257 (100), 241 (40), 229 (40), 213 (23), 197 (20), 163 (19)		Kaempferol-3-*O*-galactoside
22	3-hydroxy-1,7-bis- (3,4-dihydroxyphenyl)-hepten hexoside	23.41	491	329 (100)	207 (100), 219 (24), 109 (7)	136 (100), 122 (88), 109 (46), 149 (13), 109 (9)	Quercetin-3-*O*-galactoside

Bold numbers, fragments that were further fragmented; Rt, retention time; $[M-H]^-$, pseudomolecular ion identified in negative ion mode; -> indicating the fragmented ion from the MS^2 to MS^3; (), relative abundance of fragment ions; MS^2, fragment ions obtained from pseudomolecular ion; MS^3, fragment ions obtained from the most abundant pseudomolecular ion of MS^2 fragmentation; MS^4, fragment ions obtained from the most abundant pseudomolecular ion of MS^3 fragmentation.

3.2. Quantification of Phenolic Compounds

The quantified phenolic compounds in young and old leaves of the studied cultivars are presented in Table S1 and Table S2, respectively. The most abundant phenolic compound was myricetin-3-*O*-rhamnoside, followed by hirsutenone-hexoside, 1,7-bis-(4-hydroxyphenyl)-4,6-heptadien-3-one-hexoside, and quercetin-3-*O*-rhamnoside, which is consistent with previous reports [6]. Two of these compounds are classified as flavonols, which are commonly associated with plant defense mechanisms [5,14–16], while 1,7-bis-(4-hydroxyphenyl)-4,6-heptadien-3-one-hexoside and hirsutenone-hexoside are diarylheptanoid-type phenolic compounds. Diarylheptanoids have not been studied as extensively as other phenolics; however, their conjugated carbon skeleton and

multiple hydroxyl groups enable efficient reactive oxygen species (ROS) scavenging and membrane-disruptive effects on pathogens. In addition, these compounds may participate in modulating defense signaling and reinforcing structural barriers, thereby enhancing the plant's overall resilience to biotic and abiotic stresses [17].

Furthermore, a PCA was conducted, as shown in Figure 3, where it can be seen that the compounds hirsutenone-hexoside, myricetin-3-*O*-rhamnoside, 1,7-bis-(4-hydroxyphenyl)-4,6-heptadien-3-one-hexoside, and quercetin-3-*O*-rhamnoside were primarily responsible for the separation among cultivars, both in young and old leaves. Cultivars 'Butler' and 'Clark', both of American origin, displayed higher contents of hirsutenone-hexoside, while 'Fertile de Coutard' showed the highest content of 1,7-bis-(4-hydroxyphenyl)-4,6-heptadien-3-one-hexoside. A similar trend was observed for both young and old leaves. When considering total phenolic groups, 'Riccio di Talanico' and 'Gunslebert' contained the lowest levels of phenolic compounds, whereas 'Butler' and 'TGDL' exhibited the highest total phenolic content. However, when comparing the phenolic content and phenolic profile of the studied cultivars, no relationship was observed between phenolic content and disease resistance. In the past, higher phenolic content was often associated with increased disease resistance [14]; however, recent reports show that the initial phenolic content in the plant is the result of the plant's current stress status and does not correlate with or have an effect on the plant's resistance. Rather, the resistance is associated with the plant's time to recognize the pest or disease and start the metabolite production. If the plant responds fast, it quickly contains the infection and stops it, while if the plant takes a lot of time to synthesize the phenolic compounds, the infection spreads more, and those plants or cultivars are considered less resistant [5]. Therefore, we can confirm that the plants' phenolic content or profile did not have an effect on hazelnut resistance to *E. corylacearum*. The absence of a relationship between constitutive phenolic content and resistance to *E. corylacearum* may be explained by the nature of plant defense against biotrophic pathogens. Resistance is often determined not by basal levels of secondary metabolites but by the plant's ability to rapidly recognize the pathogen and activate inducible defense responses, including increased phenolic biosynthesis, reactive oxygen species production, and cell wall reinforcement. Cultivars with efficient pathogen recognition and rapid activation of these defenses may effectively limit infection despite relatively low constitutive phenolic levels. In contrast, delayed or weaker responses may allow pathogen establishment, resulting in higher susceptibility regardless of basal phenolic content. This likely explains the lack of correlation observed in the present study, as phenolic compounds were measured prior to infection and thus reflect constitutive rather than inducible defense capacity. It should be noted that phenolic compounds were analyzed at a single time point (May 2025) in symptom-free leaves, whereas disease severity assessments were conducted over the 2023–2024 growing season. As phenolic composition is known to vary with leaf developmental stage, seasonal progression, and environmental conditions, this temporal separation represents a limitation of the study. The present results, therefore, reflect constitutive phenolic profiles rather than dynamic or inducible responses to pathogen infection.

1, neochlorogenic acid (3- caffeoylquinic acid); 2, chlorogenic acid (5- caffeoylquinic acid); 3, myricetin-3-*O*-galactoside; 4, myricetin-3-*O*-glucoside; 5, myricetin-3-*O*-pentoside; 6, myricetin-3-*O*-rhamnoside; 7, quercetin-3-*O*-galactoside; 8, quercetin-3-*O*-glucoside; 9, quercetin-3-*O*-arabinopyranoside; 10, quercetin-3-*O*-arabinofuranoside; 11, quercetin-3-*O*-rhamnoside; 12, kaempferol-3-*O*-rhamnoside; 13, cryptochlorogenic acid (4- caffeoylquinic acid); 14, caftaric acid [caffeoyl tartaric acid]; 15, caffeoylshikimic acid; 16, coutaric acid [trans-*p*-coumaroyltartaric acid]; 17, hirsutenone-hexoside; 18, oregonin; 19, 1-(4-hydroxyphenyl)-7-(3,4-dihydroxyphenyl)-heptan-3-one-5-*O*-pentoside; 20, 1,7-bis-(4-hydroxyphenyl)-4,6-heptadien-3-one-hexoside; 21, 3-hydroxy-1,7-bis- (3,4-dihydroxyphenyl)-hepten hexoside; 22, 1-(3,4-dihydroxyphenyl)-7-(4-

hydroxyphenyl)-hept-6-en-3-one derivative; 23, total hydroxycinamic acids; 24, total flavonols; and 25, total diarylheptanoid-type phenolics; TAPC (total analyzed phenolic compounds).

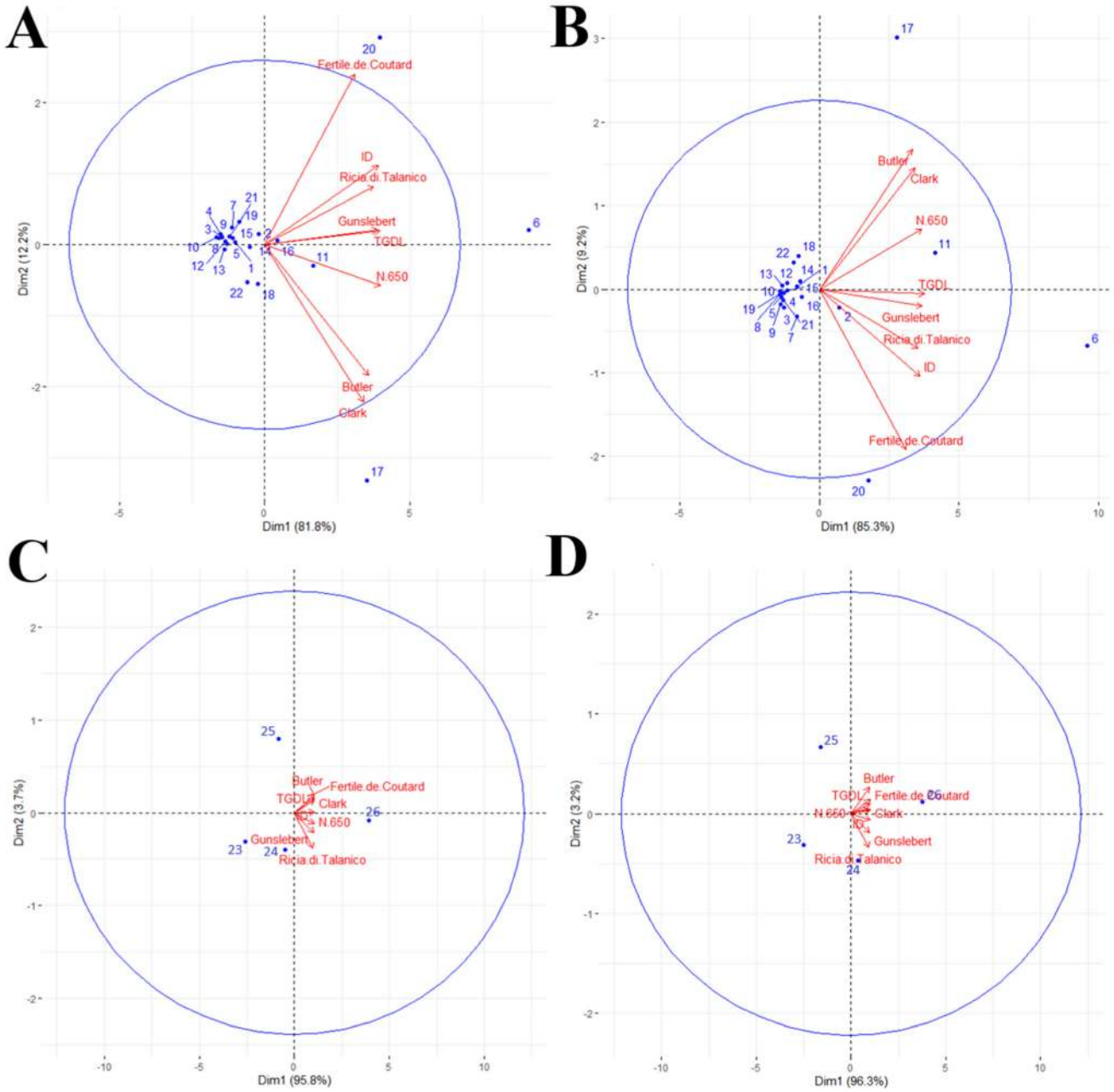

Figure 3. Principal component analysis (PCA) showing: (**A**) different phenolic content in relation to different cultivars in young leaves; (**B**) different phenolic content in relation to different cultivars in older leaves; (**C**) different total phenolic group content in relation to different cultivars in young leaves; and (**D**) different total phenolic group content in relation to different cultivars in older leaves.

3.3. Determination of Trichome Density

Looking at Figure 4, we can observe the leaf and leaf-vein trichome density of the studied cultivars represented in the proposed visual/numerical grading system. The most susceptible cultivar 'ID' had the lowest leaf trichome density, followed by the second most susceptible cultivar 'TGDL', and the third, Gunslebert. There was no significant difference in trichome density among 'Butler', 'Fertile de Coutard', and 'Riccio di Talanico', whose resistance to *E. corylacearum* was classified as moderately susceptible to moderately resistant. In contrast, 'N-650' and 'Clark' exhibited the highest trichome densities and were

also considered moderately susceptible to moderately resistant. A similar pattern was observed for leaf-vein trichome density: ID', 'Gunslebert', 'TGDL', and 'Riccio di Talanico' displayed the lowest vein trichome densities, while 'N-650' and 'Clark' showed the highest. The lowest values were observed in 'ID' (30.3 ± 3.3), followed by 'TGDL' (51.7 ± 6.1) and 'Gunslebert' (65.3 ± 6.7). Intermediate values were recorded for 'Butler' (146.7 ± 5.6) and 'Fertile de Coutard' (148.0 ± 9.3), while higher trichome densities were found in 'Riccio di Talanico' (164.7 ± 8.4) and 'N-650' (187.0 ± 8.0). The highest trichome density was observed in 'Clark' (207.7 ± 2.4). Values represent mean ± standard error (n = 3 biological replicates).

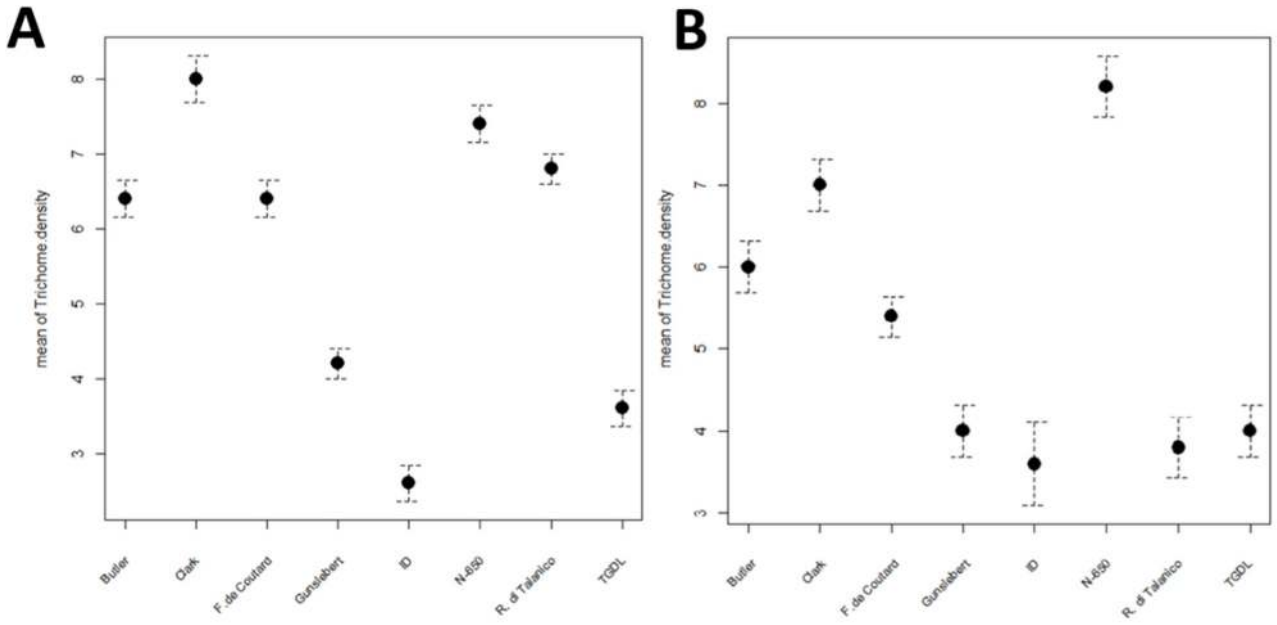

Figure 4. (**A**) Mean leaf trichome density and (**B**) mean leaf vein trichome density in the studied *Corylus avellana* cultivars. Each point represents the mean ± standard error.

Looking at Figure 5, we can see the correlations between disease resistance, trichome density, and different groups of phenolic compounds. The data confirms our hypothesis, and we can observe significant negative correlations between resistance grade and trichome density on leaves (p = 0.031) and leaf veins (p = 0.022). In contrast, phenolic compounds did not show statistically significant relationships with resistance.

Since *E. corylacearum* primarily affects the leaf lamina [2], trichome density on the leaf surface appears more important than that on the veins. Our results support this finding, indicating that leaf trichome density is directly linked to cultivar resistance to *E. corylacearum*. While phenolic compounds are often involved in plant resistance to diseases and pests [5], the mechanisms of *E. corylacearum* infection and cultivar resistance have not been found to correlate with phenolic composition. Instead, it appears that the morphological characteristics of the hazelnut leaf, particularly trichome density, are most strongly associated with resistance to this disease.

Our results showing a strong association between leaf trichome density and cultivar resistance to *E. corylacearum* align well with findings from other plant–powdery mildew systems. For example, in mulberry (*Morus* spp.), resistant germplasm exhibited about 20% higher trichome density and lower stomatal density/index than susceptible germplasm, and trichome density was strongly and negatively correlated with disease severity [18]. Similarly, in black gram (*Vigna mungo*), resistant lines displayed higher trichome density along with greater leaf thickness and lower stomatal density [19], and in mung bean (*Vigna radiata*), trichome density was also associated with resistance to *Erysiphe polygoni* [20].

These studies support our conclusion that trichome density correlates with resistance to *E. corylacearum* in hazelnut.

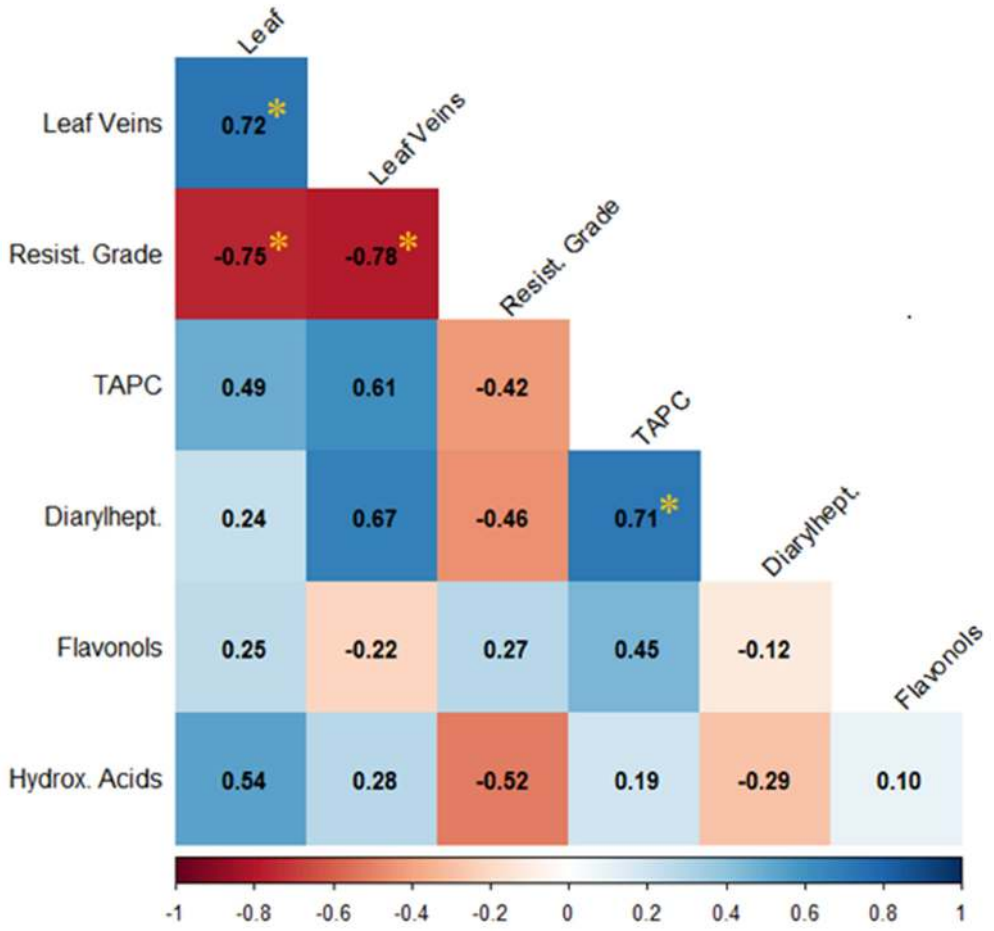

Figure 5. Spearman rank correlation coefficients (ρ) among phenolic compounds, trichome density, and resistance grade in hazelnut cultivars. Statistical significance was indicated using asterisk symbols based on predefined threshold (* $p < 0.05$).

Dense trichomes across the leaf lamina likely serve as an effective physical barrier, impeding spore deposition, reducing direct contact between spores and the leaf surface, altering the microclimate (humidity and leaf wetness), and possibly interfering with penetration or early hyphal establishment. In contrast, trichome traits of the veins appear less important, likely because powdery mildew colonization primarily occurs on the blade (intercostal) areas [2], which have a greater exposed surface area. Unlike some pathosystems in which chemical defenses (e.g., phenolics or salicylic acid-related pathways) play a major role, our results suggest that in hazelnut *E. corylacearum* interactions, morphological traits such as trichome density are more closely linked to cultivar susceptibility.

For future breeding and disease management, these results suggest that screening for trichome density on the leaf lamina could serve as a practical phenotypic marker for resistance. Further research should expand these findings by including a broader range of cultivars and environments, as well as investigating additional micromorphological traits such as stomatal density, cuticle characteristics, surface roughness, and leaf thickness to determine whether combinations of these features confer stronger resistance. From a breeding perspective, the strong association between trichome density and reduced disease severity suggests that this trait has considerable potential as a practical selection criterion. Unlike biochemical analyses, which require specialized equipment and are time-consuming, trichome density can be assessed rapidly and non-destructively using simple visual or mi-

croscopic methods. This makes it particularly suitable for large-scale screening in breeding programs. In hybrid breeding, trichome density could be used at multiple stages. First, it may serve as a criterion for selecting parental genotypes with favorable leaf surface characteristics. Second, it can be applied during early seedling evaluation, allowing for the elimination of highly susceptible individuals before field planting. This is especially valuable in perennial crops such as hazelnut, where breeding cycles are long, and field evaluations are resource-intensive. However, given that trichome density alone does not fully explain resistance, it should be integrated with other traits, including disease assessments under field conditions and potentially other morphological or physiological markers. Such a multi-trait selection approach would increase the reliability of selecting genotypes with improved resistance to *E. corylacearum*. In addition, assessing phenolic responses during pathogen development and conducting controlled inoculation experiments would provide deeper insight into inducible defense mechanisms and reduce environmental variability. This study, therefore, provides a foundation for future work aimed at elucidating the molecular and physiological mechanisms underlying resistance, including the regulation of trichome development and other defense-related pathways.

The cultivar 'Istrska dolgoplodna' (ID) represents the most widely cultivated traditional hazelnut variety in Slovenia and has historically been a key component of national hazelnut production due to its good adaptation to local environmental conditions and acceptable nut quality. However, our results confirm that this cultivar is highly susceptible to *E. corylacearum*, exhibiting the highest disease severity among all tested genotypes. Under increasing disease pressure, its continued use in new plantings may therefore pose significant phytosanitary and economic risks. In contrast, cultivars such as 'N-650', 'Butler', and 'Clark' showed lower disease severity and may represent more suitable alternatives for future orchard establishment. These cultivars could provide a more sustainable option under current disease conditions, although their full agronomic performance (yield, nut quality, and long-term adaptation) should also be considered in cultivar selection decisions.

4. Conclusions

This study provides initial evidence that morphological traits, particularly trichome density, are more strongly associated with hazelnut susceptibility to *E. corylacearum* than constitutive phenolic profiles under the conditions tested. Among the analyzed cultivars, those with higher leaf trichome density consistently exhibited lower infection levels, whereas no relationship was found between resistance and phenolic composition. These findings emphasize the importance of leaf surface micromorphology, particularly trichome density, as a primary barrier against *E. corylacearum* colonization. Trichome density may therefore serve as a useful phenotypic marker in breeding programs aimed at improving disease resistance. In addition, further studies integrating advanced microscopy and genomic tools should explore other leaf surface characteristics, such as stomatal patterning and cuticle structure, to better understand the complex mechanisms underlying resistance. Developing cultivars with favorable morphological traits offers a sustainable and cost-effective strategy for managing powdery mildew in hazelnut orchards. From an applied perspective, trichome density represents a promising, low-cost, and easily measurable trait that can be incorporated into hazelnut breeding programs. Its use as an early selection marker could significantly accelerate the identification of more tolerant genotypes and reduce the need for extensive field-based disease evaluations, thereby improving the efficiency of resistance breeding.

https://doi.org/10.3390/horticulturae12050565

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae12050565/s1. Table S1. Phenolic compounds found in young leaves of different *Corylus avelana* cultivars. Table S2. Phenolic compounds found in older leaves of different *Corylus avelana* cultivars. Table S3. Corresponding *p*-values for Spearman rank correlations among phenolic compounds, trichome density, and resistance grade.

Author Contributions: Conceptualization, A.M. and P.K.; methodology, A.M. and M.C.G.; software, A.M. and M.C.G.; validation, A.M. and P.K.; formal analysis, A.M.; investigation, A.M. and P.K.; resources, A.M.; data curation, A.M. and P.K.; writing—original draft preparation, A.M.; writing—review and editing, A.M., P.K. and M.C.G.; visualization, A.M.; supervision, P.K.; project administration, A.M.; funding acquisition, A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study is a part of program P4-0013-0481 funded by the Slovenian Research and Innovation Agency (ARIS).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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