



Intrinsic and extrinsic factors affecting the infection rate of *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. on *Quercus robur* L. seedlings

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Abstract

Oak powdery mildew, caused by *Erysiphe alphitoides*, is a widespread disease that affects oaks. We aimed to understand the complex interactions between extrinsic (environmental) and intrinsic (provenance) factors that influence the infection rate of *E. alphitoides* on *Quercus robur* seedlings. In a common garden controlled water experiment, we investigated the natural infection rate of *E. alphitoides* considering factors such as oak provenance, soil moisture availability, and the seedlings' location in the microenvironment. We examined infection rates on first- and second-flush leaves, assessed oak gas exchange responses (net photosynthesis, transpiration, and stomatal conductance) to powdery mildew infections, and evaluated stomatal size and density as a potential pathway for pathogen entry. Our results showed that different factors influence first and second-flush leaf infection rates. First flush infections were milder, primarily depending on provenance (an intrinsic factor), with early leaf development correlating with lower infection rates. Second flush leaf infections in summer were more severe, driven by extrinsic factors such as the microenvironment and water availability, with spore dispersal influenced by prevailing wind direction. We observed a significant effect of *E. alphitoides* on photosynthesis, along with reduction in infection rates under drought. This suggests that stomatal closure—reflected in reduced stomatal conductance—under drought stress may prevent pathogen penetration. Moreover, our results suggest that stomatal closure itself, rather than the size of the stomatal pore, may play a more critical role in limiting pathogen entry. Further assessment of biochemical defenses in drought-stressed seedlings could help clarify if additional mechanisms are involved.

Keywords Powdery mildew · Gas exchange · Stomata · Drought · Wind

Introduction

Pedunculate oak (*Quercus robur* L.) is a major tree species in European forests and one of the few that might benefit from climate change (Scharnweber et al. 2011; Dyderski et al. 2018; Wessely et al. 2024). Based on species distribution models, this species is considered a “winner” in Central European forests and is likely to gain new growth areas (Dyderski et al. 2018; Wessely et al. 2024). Although *Q. robur* is an important forest species in Europe and a model tree for genetic studies, surprisingly little is known about the disease resistance of this species (Bartholomé et al. 2020).

Quercus robur interacts with a diverse range of organisms, including numerous pests and pathogens (Jakuschkin et al. 2016). Among oak species, it is particularly susceptible to infection by multiple powdery mildew species (Mougou et al. 2008; Bartholomé et al. 2020), with *Erysiphe*

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alphitoides (Griffon & Maubl.) U. Braun & S. Takam. (syn. *Microsparella alphitoides*; *Erysiphaceae*, *Helotiales*, *Leotiomycetidae*, *Leotiomyces*, *Pezizomycotina*, *Ascomycota*) being the prevailing and globally distributed powdery mildew species (Limkaisang et al. 2006). Numerous studies on genetic analysis strongly suggest that the pathogen *Erysiphe alphitoides* originated in Asia and has since spread across Europe, affecting *Q. robur* populations (Takamatsu et al. 2007; Bartholomé et al. 2020).

Oak powdery mildew attacks young, developing leaves in spring and summer, making the young regeneration susceptible (Mougou et al. 2008). The infection process begins in early spring when sexual spores, known as chasmothecia, are released and infect oak leaves. Throughout the growing season, the pathogen produces asexual spores, or conidia, leading to multiple generations of the disease. The infection levels of oak powdery mildew are influenced by the leaf flush identity, with the highest infection observed on leaves from the second flush (Gaytán et al. 2022). The susceptibility of leaves to powdery mildew varies with their developmental stage. Developing leaves are more susceptible than mature ones, which explains why leaves from the first flush often escape infection due to lower spore loads in early spring (Gaytán et al. 2022). As the season progresses, spore loads increase, leading to higher infection levels on the second flush. Infected leaves produce abundant conidia, which facilitate the infection of subsequent leaf flushes and contribute to pathogen dispersal. Following conidial deposition on the leaf surface, the pathogen attaches, penetrates the host cuticle and cell wall, and initiates infection. *E. alphitoides* can penetrate directly or through stomata, forming specialized structures known as appressoria, which aid in invasion (Hückelhoven 2005). These hook-shaped appressoria develop on the epidermal surface, while fungal germ tubes use enzymatic and mechanical forces to breach the host cell wall (Panstruga and Schulze-Lefert 2003). Once inside, the pathogen establishes haustoria—specialized feeding structures—that extract nutrients from living cells to sustain fungal metabolism (Desprez-Loustau et al. 2011; Vaštag et al. 2022).

Although *E. alphitoides* rarely kills its hosts (Skwarek-Fadecka et al. 2022), it significantly impacts tree growth and physiology. Infected trees experience nutrient depletion (Hewitt and Ayres 1976), stunted growth, and altered gas exchange, though reports on the extent of these effects vary (Hajji et al. 2009). Hewitt and Ayres (1976), reported a 50% reduction in net photosynthesis, whereas Hajji et al. (2009) found that *E. alphitoides* had a relatively minor impact on gas exchange, causing only a 20–30% reduction in stomatal conductance and minimal effect on whole-plant transpiration. Instead, its most significant consequence was a severe

reduction in leaf lifespan, potentially leading to decreased carbon uptake over the growing season (Hajji et al. 2009).

In addition to uncertainties regarding the physiological effects of *E. alphitoides*, there is also considerable debate about the role of moisture in disease development. While powdery mildew is generally considered a xerophilic fungal parasite (Marçais and Desprez-Loustau 2014), oak powdery mildew epidemics are rarely linked to dry years, and some studies even suggest that its outbreak in the early twentieth century may have been associated with unusually wet conditions (Marçais and Desprez-Loustau 2014). These discrepancies highlight the need for further research into the interactions between host susceptibility and environmental factors on *E. alphitoides* infection under natural conditions.

Recent findings by Bartholomé et al. (Bartholomé et al. 2020) on genomic resistance in half-sibs of *Q. robur* indicate that resistance to *E. alphitoides* is likely heritable trait, offering potential for breeding disease-resistant *Q. robur* genotypes. However, critical knowledge gaps remain. The extent to which provenance-specific resistance, potentially driven by differences in leaf phenology and temporal mismatches with fungal spore dispersal, contributes to infection rates is not well characterized. Furthermore, the interactions between intrinsic -provenance specific factors and extrinsic -environmental conditions, require further investigation.

Here, we examined both intrinsic factors, such as provenance, and extrinsic factors, including soil moisture availability and microenvironmental conditions, to better understand their combined influence on *E. alphitoides* infection. Specifically, we assessed the natural infection rate in *Q. robur* seedlings under soil moisture controlled forest conditions, focusing on key physiological responses such as net photosynthesis, transpiration, and stomatal conductance. Given previous reports suggesting that first and second flush leaves differ in susceptibility to *E. alphitoides*, we incorporated this aspect into our study. Additionally, we investigated leaf stomatal morphology (e.g. size) as a potential pathway for fungal penetration, exploring its role in infection susceptibility.

More specifically, we targeted the following questions: (1) What is the relative importance of intrinsic and extrinsic factors and their interaction on the infection severity of first and second flush leaves? (2) To what extent does pathogen infection affect photosynthesis, and if so, is *E. alphitoides* infection associated with stomatal conductance (as a proxy for stomatal openness), stomatal and pore areas, and soil moisture?

By addressing these questions, this study aims to provide new insights into the complex interactions between oak provenances, environmental factors, and physiological traits that shape oak resistance to powdery mildew.

Methods

Plant material

Oak seeds were collected from seven provenances ranging from 44°N latitude to 54°N (from Croatia to northern Poland), representing different geographical and natural growing conditions (Fig. 1; Supplementary materials Fig. S1). The seeds were sown in spring 2021, and the experiment was conducted in 2022. Seedlings were grown in individual 5-L pots filled with a substrate consisting of 83–85% peat and 12–15% perlite, deacidified with dolomite to achieve a pH of 6.5. A slow-release fertilizer (OSMOCOTE 3 M 12–6–6) was added at a dosage of 1.5 g l^{-1} .

The experiment was established in the edge of forest gap ($20 \times 30 \text{ m}$). The potted seedlings were placed on a flat surface covered with a 3 cm high geogrid, allowing water drainage but preventing the soil from waterlogging. The experimental area was covered with transparent PCV sheets

to control irrigation and prevent access to rainwater. The meteorological conditions including irradiance under PCV sheet are provided in the repository (Niemczyk 2025).

A total of 530 oak seedlings were tested in the trial and were equally divided into plots. The trial area was divided into six blocks, each with three plots. The seedlings were randomly placed within the plot area. Each plot represented a specific irrigation regime: control, water deficit, and drought (no irrigation). In the control treatment, the volumetric soil water content (VWC) was maintained at an optimal level of approximately $0.25\text{--}0.30 \text{ m}^3 \text{ m}^{-3}$, serving as the reference for the water deficit treatment, which received 50% of the irrigation amount applied in the control. Each seedling (pot) was equipped with an individual drip irrigation system, with irrigation doses programmed separately for all three treatments using the Hunter NODE-BT application. Soil microclimate monitoring was carried out using a network of Decagon Em50 data loggers with 5TE sensors placed approximately 7 cm below the soil surface in

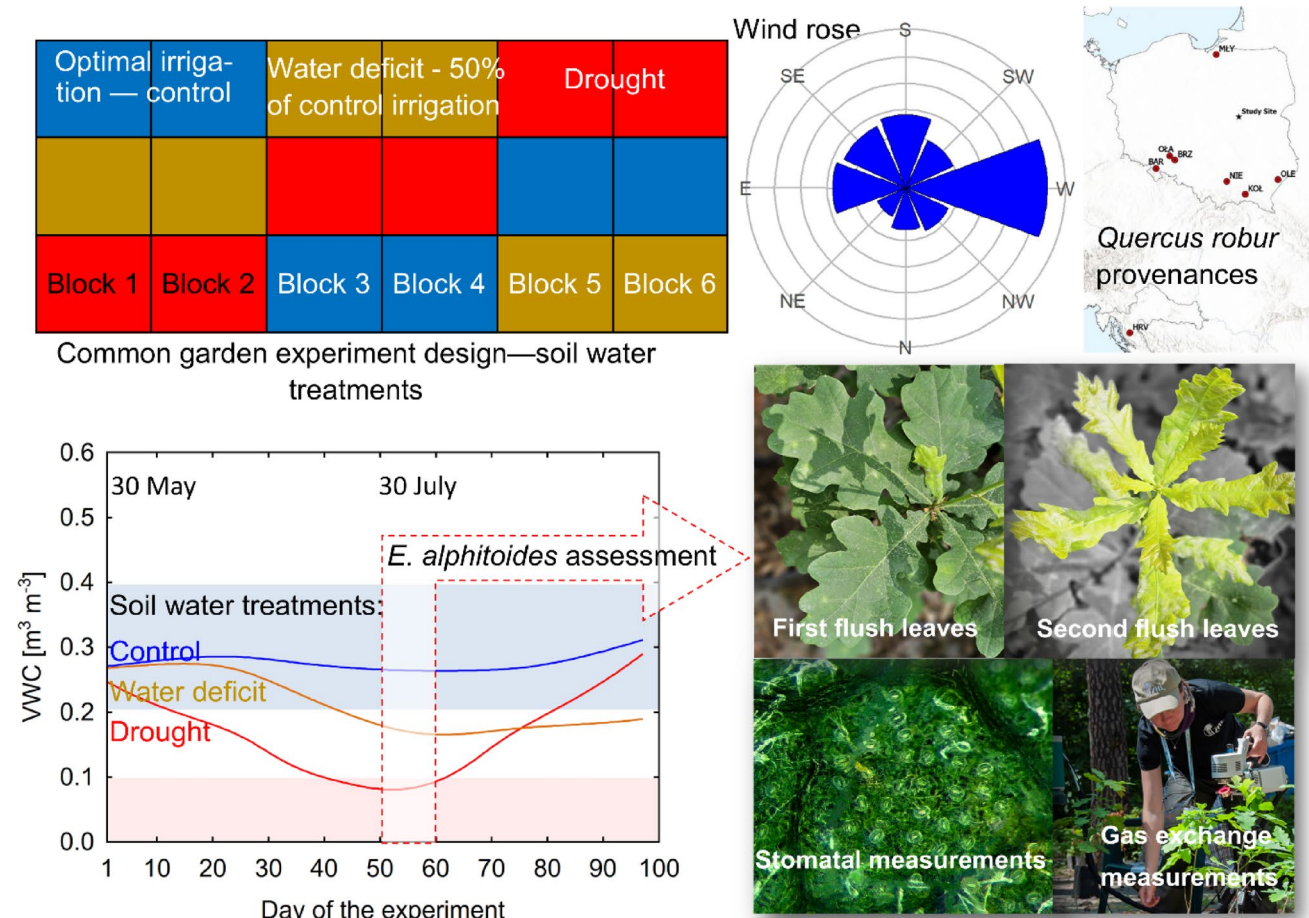


Fig. 1 Top: Experimental design showing soil water treatments and a wind rose indicating the dominant wind direction relative to the geographical location of the common garden. Seeds locations (map) of the tested *Q. robur* provenances. MŁY – Młynary, OLA – Oława, BRZ – Brzeg, BAR – Bardo, NIE – Niepołomice, OLE – Oleszyce, KOŁ –

Kołaczyce, HRV – Croatia Bottom left: Soil volumetric water content (VWC) under different irrigation regimes: optimal (control), water deficit (50% of optimal irrigation), and drought. Bottom right: Overview of measurements and observations conducted during the experiment

randomly selected pots in each block. The soil volumetric water content from soil monitoring was provided in the Fig. 1.

All pots were watered evenly before the start of the trial in the same manner as in control treatment (VWC was maintained at 25–30%). The experiment started on 30 May after the water treatments had been set up accordingly (optimal, deficit, and drought). The soil moisture conditions were strictly controlled throughout the experiment while the seedlings were naturally infected with *E. alphitoides* as many trees in the surrounding stand were diseased, and the pressure of the natural inoculum was high, as shown by the numerous natural infections in the vicinity of the oaks. Observations and measurements were made in the last week of July and the first week of August 2022, when VWC dropped below 10% in the drought treatment (Fig. 1).

Observation of leaf phenology and the infection rate by *E. alphitoides*

The phenology of leaf development was observed on May 12, 2022, using a scale from 1 to 5, where 1 indicates dormant winter buds and 5 represents fully expanded leaves (Scale values: 1 dormant winter bud; 2 buds expanding; 3 bud-burst (first green is visible); 4 leaves are flushing; 5 leaves are fully expanded; according to TREES4FUTURE protocol (de Cuyper et al. 2011)).

The infection rate of *E. alphitoides* on *Q. robur* seedlings was assessed in late July by separately evaluating spring leaves from the first budding phase (first flush, FF) and summer leaves from the proleptic second phase (second flush, SF). For each flush, the percentage of leaf area infected was visually estimated across all leaves within the flush, using a scale from 0% (no visible infection) to 100% (complete leaf surface coverage). Evaluation was conducted at the flush level to enable comparative analysis of infection patterns between older (FF) and younger (SF) foliage. To avoid biases, the same person (TW) made observations all 530 seedlings.

Gas exchange

Gas exchange measurements, including photosynthesis, transpiration and stomata conductance, were recorded in the last week of July when soil moisture dropped below 10% VWC in the drought treatment compared to 20–30% in the water deficit and control treatments (30–35%). To optimize the time and ensure the stability of the measurement conditions, while maintaining representativeness of both microenvironmental conditions and variability of provenances, we decided to carry out the measurements on four provenances in only three blocks and to omit every other

block (blocks I, III, and V were used for the measurements). These four provenances were selected to represent maximum geographical distances of seed sources and climatic distinctiveness of seed source environments. This selection was validated through a priori climatic clustering analysis (data not shown).

The gas exchange measurements were carried out on 64 seedlings per provenance (for KOL, MLY and BRZ) and 9 for HRV so that a total of 171 seedlings were measured. The measurements were carried out between 9:00 and 15:00 using a portable photosynthesis system (Li-Cor 6800; Li-Cor Inc.) equipped with an integrated fluorescence chamber head of 6 cm² aperture. The measurements of gas exchange were performed on the uppermost expanded leaf. We established a saturation light intensity of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Photosynthetically active radiation was provided using a light spectrum composed of 90% red and 10% blue light. In addition to light parameters, the following standardized conditions were maintained within the measurement chamber: leaf temperature (Txchg) at 25 °C, CO₂ concentration at 400 $\mu\text{mol mol}^{-1}$, relative humidity at 60%, and an airflow rate of 400 $\mu\text{mol s}^{-1}$. In addition, leaf-level VPD hereafter referred as VPD (Li-Cor 6800; Li-Cor Inc.) was simultaneously measured with gas exchange on each seedling. The soil VWC during measurements was (on average) 0.08, 0.16, and 0.26 m³m⁻³ for the drought, water deficit, and control treatments, respectively. The VPD was 1.42 kPa, and the air temperature was 25.04 °C. The detailed gas exchange protocol is described in Niemczyk et al. (2024).

Stomatal measurements

After visual observations of the appearance of pathogens, leaves from 35 seedlings were sampled to measure stomata characteristics on FF and SF leaves. To maintain the long-term research potential of this study, we limited the destructive sampling required to nine seedlings per provenance being analyzed across three soil moisture treatments, with one fewer seedling from the HRV provenance, ensuring a minimal yet robust sample size. The micro-relief method (replicate) was used to assess stomatal density (SD). Impressions were taken from the abaxial (lower) epidermis using colorless nail polish. All impressions were fixed on glass slides and examined and photographed under a light microscope (Zeiss microscope Primo Star with internal HD camera, Jena, Thuringia, Germany) at 40× magnification. At least three images of each leaf were taken randomly and extrapolated to the total number of stomata per 1 mm². The microscope images were analyzed using StomataCounter (Fetter et al. 2019). Each microscopic image was analyzed individually to avoid errors in stomata identification and counting. The stomatal area (including guard cells) and

stomatal pore area were measured using StoManager1 software developed by Wang et al. (2023). StoManager1 uses convolutional neural networks to identify and count stomata, separating the guard cell from the pore while measuring length, width, and area (Wang et al. 2023).

Statistical analysis

A General Linear Model (GLM) was used to analyze the differences in infection rates as a function of intrinsic and extrinsic factors. The models were constructed separately for leaf infections at the first flush in spring (FF) and the second flush in summer (SF), with provenance, treatment, blocks, and the interaction between factors as fixed effects. Blocks were treated as fixed effects to account for micro-environmental differences and their specific locations along the west–east gradient. Additionally, the non-parametric correlation between the phenological phase of leaf development and infection rate was analyzed using Kendall's tau test.

Infection rate was analyzed using a generalized additive model (GAM), with stomatal conductance and soil volumetric water content (VWC) as predictor variables. Block and provenance effects were included as quantitative variables. GAMs are particularly well-suited for modeling complex ecological processes, as they do not assume a priori any specific form of a relationship between the dependent variable and covariates, and apply non-parametric smoothers to each predictor independently, providing a more nuanced understanding of environmental influences (Guisan and Zimmermann 2000) on infection rates. For the normally distributed response data, we applied a logarithmic link function and evaluated model performance using the coefficient of determination (R^2).

Gas exchange characteristics were also subjected to GLMs. These models used quantitative data from vapour pressure deficit (VPD) and infection rates as covariates. Stomatal traits were also analyzed with general GLMs to compare morphometric traits in FF and SF leaves between different provenances, treatments, and blocks. We used the

sum of squares of type III (orthogonal) to determine the significance of these effects. The coefficient of determination (R^2) was used to measure the model's goodness of fit (Nakagawa and Schielzeth 2013), while the partial Eta squared (η^2) was used to describe the effect sizes (Richardson 2011). Post-hoc comparisons within factors were analyzed using the Tukey's honestly significant difference (HSD) test (Kramer 1956).

All statistical analyses were performed using the Statistica 13.0 statistical package ("TIBCO Software Inc. 2017).

Results

Infection rate as a function of extrinsic and intrinsic factors

The GLM for infection rates of *E. alphitoides* on *Q. robur* FF leaves found significant effects from provenance, block, and a block $\times$ soil moisture treatment interaction (Table 1). The GLM for SF leaves demonstrated significant effects for all factors (Table 1). The only interaction between the provenance and treatment showed no significant effect. It is also worth noting that in FF leaves, the highest effect size, expressed as partial eta-squared, was observed for the intrinsic factor of provenance (5.0%) and the extrinsic block effect (8.6%). In contrast, in SF leaves, extrinsic factors such as block (16.9%) and treatment (18.6%) had a greater influence on infections than the intrinsic factor of provenance (3.1%).

The Bardo (BAR; S-W mountain provenance) had the lowest infection rate in FF leaves at 7.1%, while Kołaczyce (KOŁ; S-E mountain provenance) had the highest at 16.3% (Fig. 2). The non-parametric correlation between the phenological phase of leaf development and infection rate showed a strong, negative correlation, suggesting that provenances that develop earlier in spring are less infected by *E. alphitoides* (Kendall's tau, $p=0.041$).

The infection rate on the SF leaves clearly exceeded that of the FF leaves, with the different provenances showing significant susceptibility to *E. alphitoides*. Oława (OŁA; 56.33%), Brzeg (BRZ 59.20%), and Niepołomice (NIE 60.51%), which grow naturally under the mildest climatic conditions among tested provenances in Poland, had the lowest infection rates, while Bardo (BAR; 71.30%) had the highest infection rate, which contrasts with the spring infection rate on this provenance, however the Tukey HSD test did not show significant differences between provenances in SF leaves (Tukey test; $p<0.05$) (Fig. 2).

The arrangement of blocks in relation to wind direction revealed a trend of increasing infection rates from west to east, which was minor for FF leaves but more pronounced

Table 1 Results of GLM of *E. alphitoides* infection rate for spring (first) flush (FF) and summer (second) flush (SF) leaves depending on oak provenance, block and soil moisture treatment [drought, water deficit, and control (optimal irrigation)]

Variable	FF			SF		
	Df	P-value	η_p^2	P-value	η_p^2	
Provenance	7	0.001	5.0	0.031	3.1	
Block	5	<0.001	8.6	<0.001	16.9	
Treatment	2	0.304	0.5	<0.001	18.6	
Provenance $\times$ Treatment	14	0.690	2.2	0.565	2.5	
Block $\times$ Treatment	10	<0.001	8.6	<0.001	6.5	

Bolded values denote statistically significant results at $\alpha \leq 0.05$

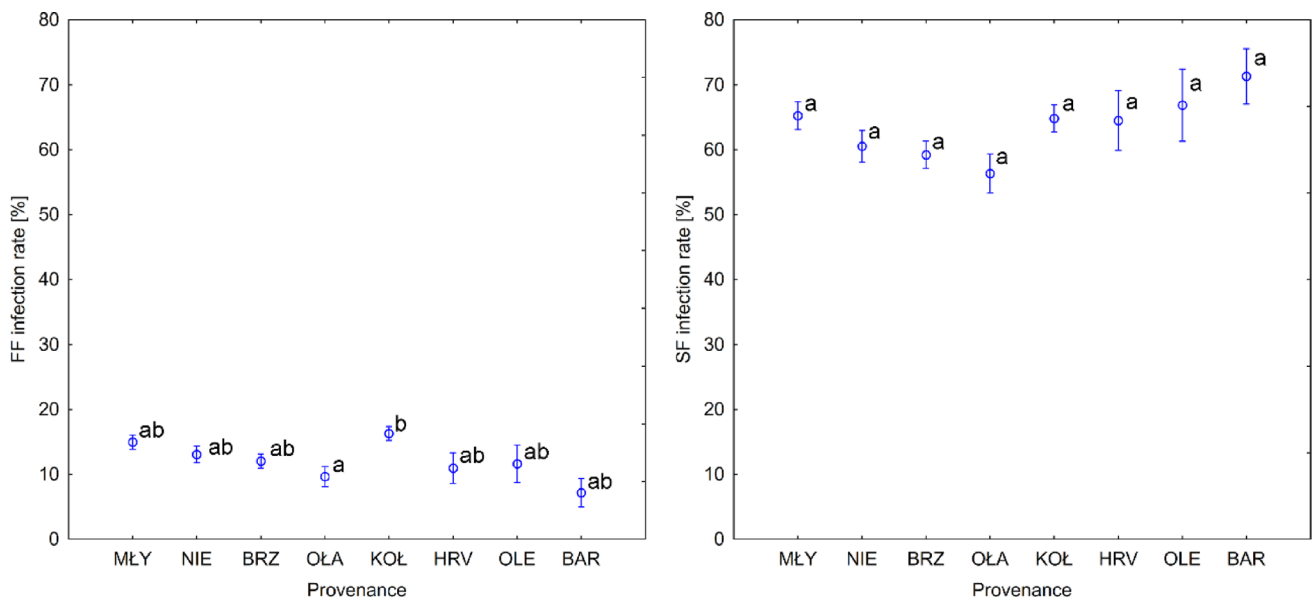


Fig. 2 Provenance effect upon the *E. alphitoides* Infection rate – mean values $\pm$ standard errors of *E. alphitoides* in blocks for first (left) and second (right) flush leaves across all blocks (study sites). Different letters denote statistically significant differences (Tukey test; $p < 0.05$)

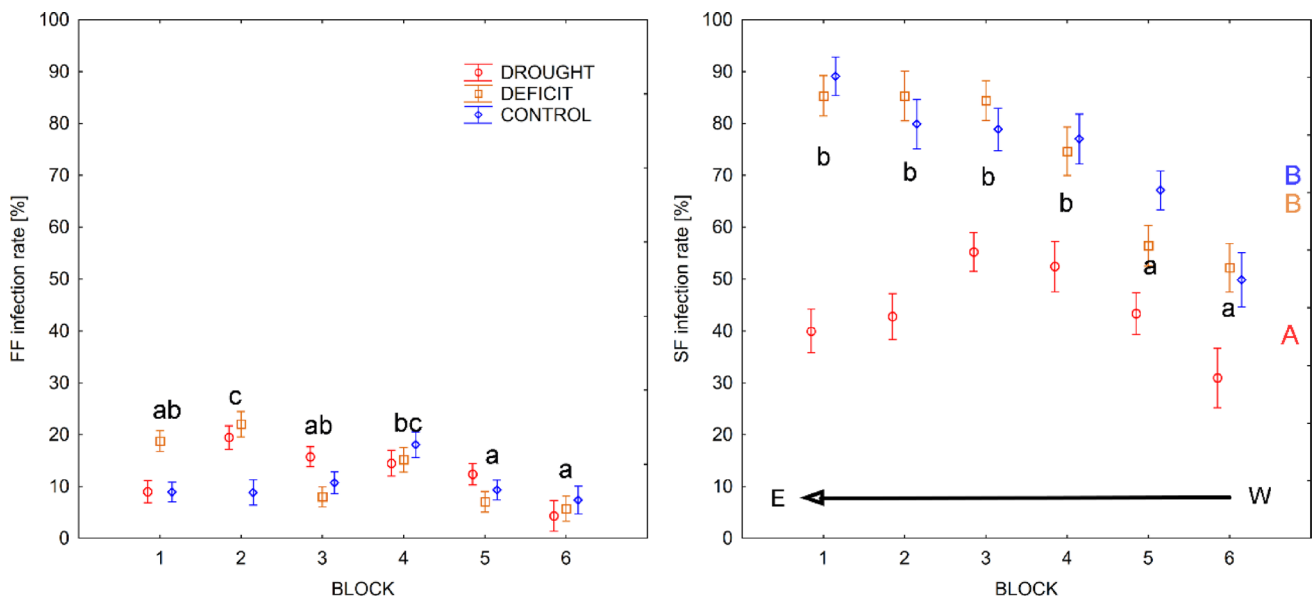


Fig. 3 Effects of block location and soil moisture treatment on infection rates — Mean infection rates ($\pm$ standard errors) of *E. alphitoides* in blocks for first flush (FF, left) and second flush (SF, right) leaves. The horizontal E–W line indicates the dominant wind direction at the

study site. Different capital letters indicate statistically significant differences between soil moisture treatments, while lowercase letters denote significant differences between blocks (Tukey test; $p < 0.05$)

for SF leaves (Fig. 3). Seedlings in block 6, located at the westernmost end, showed the lowest infection rate (44.52%), while seedlings in block 1, the easternmost, exhibited the highest (72.28%). This pattern was statistically supported by the Tukey HSD test ($p < 0.05$), which indicated significantly lower infection rates in the western blocks (5–6) compared to the more easterly blocks (1–4) (Fig. 3).

However, this spatial pattern was influenced by the soil water treatments. Soil moisture levels had a significant

effect on SF leaf infection rates. Specifically, the drought treatment resulted in a significantly lower infection rate in oak leaves, whereas in the control and water deficit treatments oak's leaves showed similar, higher infection rates (Tukey test; $p < 0.05$) (Fig. 3).

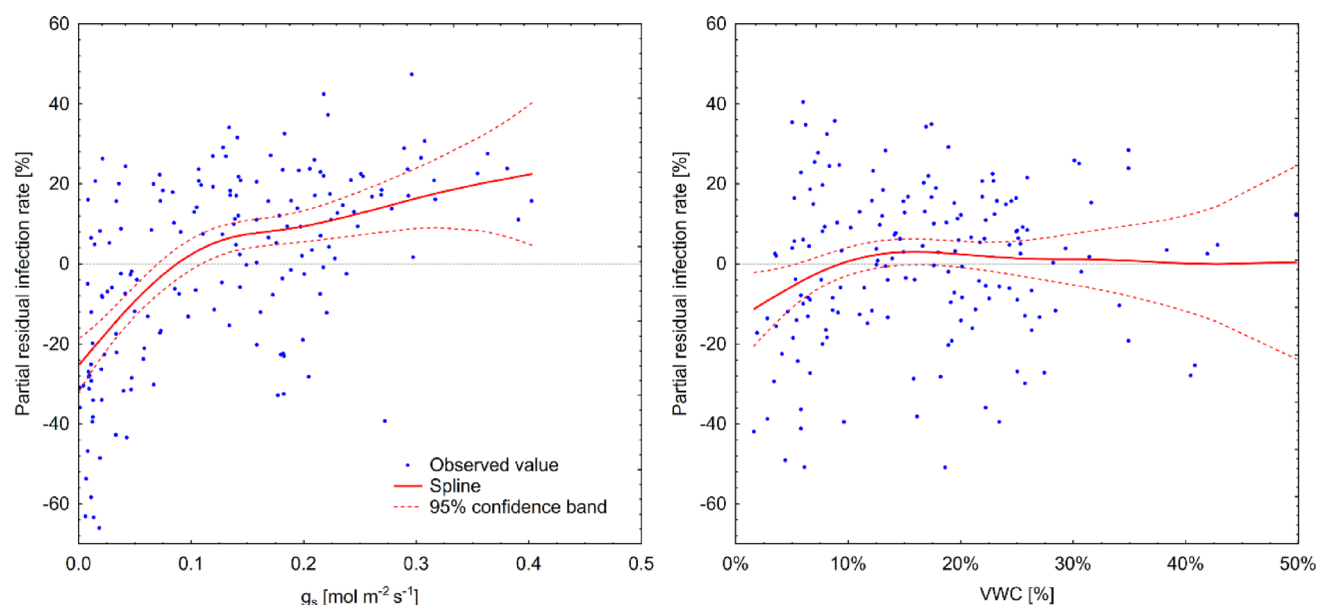


Fig. 4 Spline curves from the generalized additive model (GAM) showing the partial effects of stomatal conductance (g_s ; representing stomatal openness) and soil volumetric water content (VWC) on the infection rate of *E. althitoides* on the leaves where gas exchange was

measured. The solid lines represent the estimated smooth effect of each predictor on infection rate after adjusting for the other variable. The points represent the corresponding partial residuals (i.e., observed infection rates adjusted for other predictor)

Table 2 Results of GLM for net assimilation (A), transpiration (E), and stomatal conductance (g_s) in relation to intrinsic and extrinsic predictors

Variable	Df	A		E		g_s	
		p	η_p^2	p	η_p^2	p	η_p^2
Block	2	0.837	0.2	0.090	2.9	0.06	3.4
Provenance	3	0.486	1.5	0.600	1.1	0.597	1.1
Soil moisture treatment	2	<0.001	36.8	<0.001	36.5	<0.001	34.8
Provenance $\times$ soil moisture treatment	6	0.289	4.4	0.741	2.1	0.683	2.4
VPD leaf	1	0.006	4.6	0.310	0.6	0.002	5.7
<i>E. althitoides</i>	1	0.001	7.0	0.001	6.2	0.002	5.8

VPD and infection rate of *E. althitoides* were included as covariates in the analysis. Bolded values denote statistically significant results at $\alpha \leq 0.05$

Photosynthesis, transpiration, and stomatal conductance on infected leaves in different water regimes

GAM explained 53.2% of the variation in powdery mildew infection rates ($R^2=0.532$) and showed a significant influence of both soil moisture ($p=0.041$) and stomatal conductance ($p<0.001$) on the severity of infections (Fig. 4). Consequently, in the GLMs for gas exchange parameters, infection rate was included as a covariate. The soil moisture treatment was the main factor affecting the photosynthetic

rate in the GLMs ($\eta_p^2 \approx 36\%$). Both covariates, it means, infection rate and VPD also showed significant effect on seedling photosynthesis (Table 2).

Drought caused a significant decrease in seedling net assimilation compared to the control and water deficit treatments (Tukey test, $p<0.05$). A similar pattern of seedling

responses was also observed for stomatal conductance. The transpiration rate was also treatment-dependent. The highest transpiration rate was recorded in the control treatment. In contrast, the lowest transpiration was observed in drought conditions, indicating that the seedlings close their stomata (Fig. 5).

Stomatal characteristics

The GLM demonstrated statistically significant differences in stomata density for all factors (Table 3). The higher stomata density was stated for SF leaves than for FF leaves. Regarding intraspecific variation, the highest stomata density for both FF and SF leaves was stated for Kołaczyce (KOŁ) while the lowest for Croatian provenance (HRV) (Fig. 6). Also soil moisture treatment influenced stomata density indicating differences between drought treatment and the remaining treatments in FF leaves, while for SF

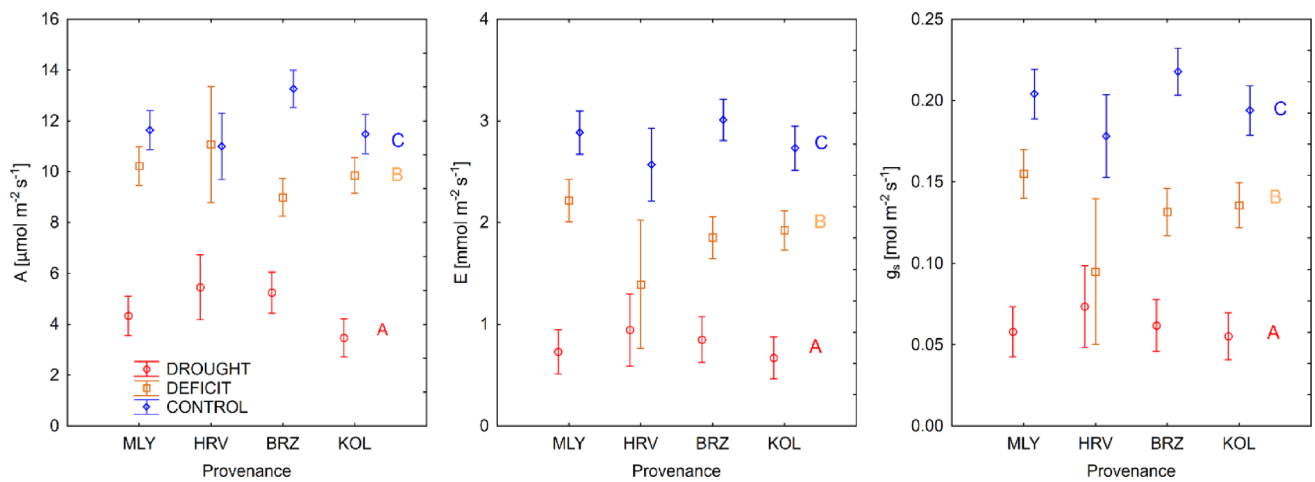


Fig. 5 The mean values $\pm$ standard errors of gas exchange parameters. From the left: net assimilation (A), transpiration (E), stomata conductance (g_s) in relation to provenance and treatment. Different capital letters denote significant difference between blocks in Tukey HSD test ($p < 0.05$)

Table 3 Results of the GLMs for stomatal traits: stomatal density and stomatal pore area

Variable	Df	Stomatal density				Stomatal pore area			
		p-value	η_p^2	p-value	η_p^2	p-value	η_p^2	p-value	η_p^2
Block	2	<0.001	55.7	<0.001	68.2	0.025	17.3	0.035	17.7
Provenance	3	<0.001	38.6	<0.001	58.7	0.214	6.5	0.002	21.4
Soil moisture treatment	2	<0.001	40.6	<0.001	27.8	0.004	15.7	0.070	8.5
Provenance $\times$ Soil moisture treatment	6	<0.001	62.2	<0.001	31.9	0.122	13.8	0.943	2.7
Individual(bxppt)	17	<0.001	67.6	<0.001	82.8	0.015	35.4	0.008	38.9

Bolded values denote statistically significant results at $\alpha \leq 0.05$

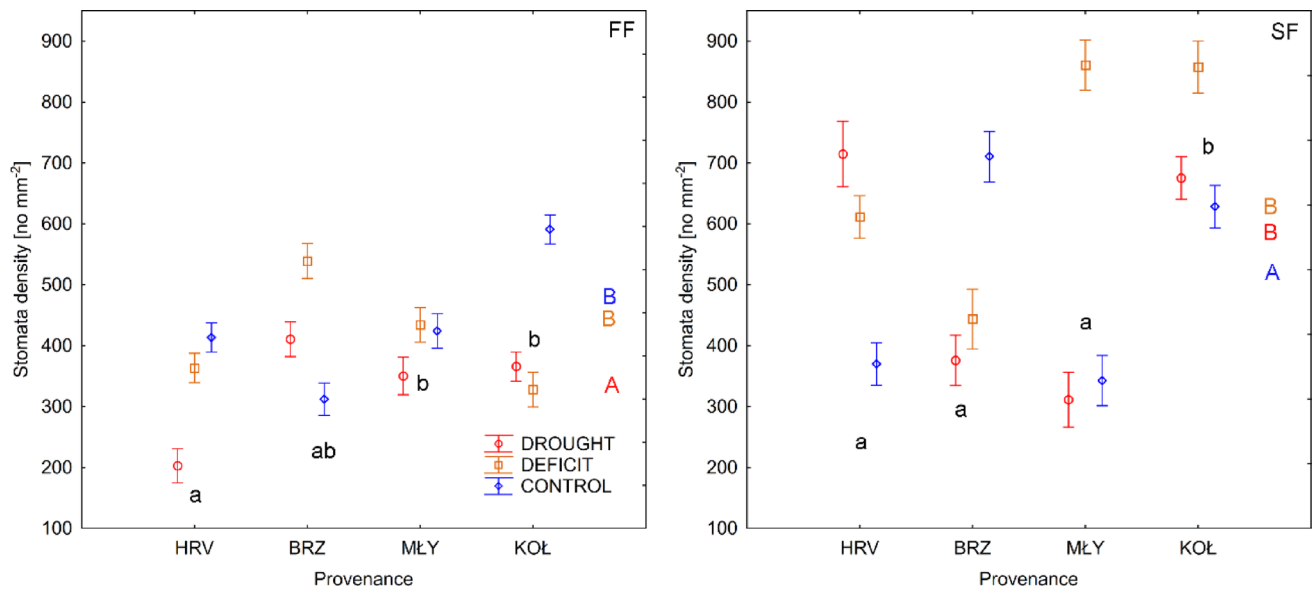


Fig. 6 Stomatal density, represented by a subset of the provenances and the soil moisture treatment (drought, deficit, control) for FF—first flush and SF—second flush. Different capital letters denote significant

difference between soil moisture treatments, while lower case letters denote differences between provenances in Tukey HSD test ($p < 0.05$)

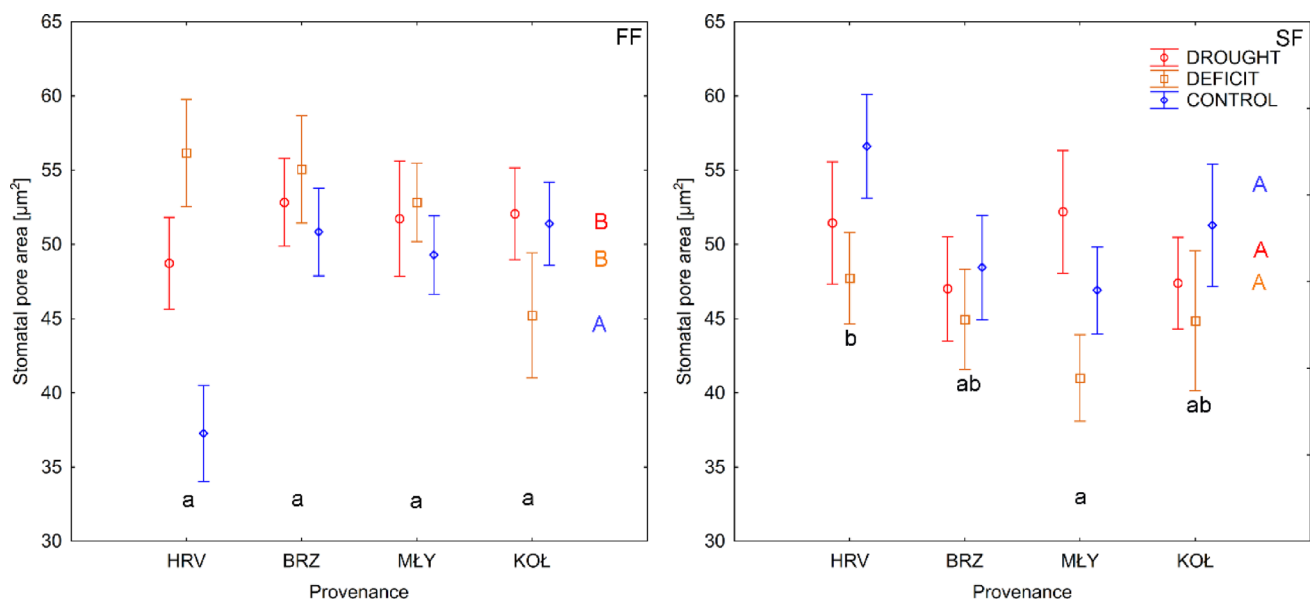


Fig. 7 Stomatal pore area as a function of provenance (FF – first flush; SF – second flush) and soil moisture treatment (drought, deficit, control). Different capital letters denote significant difference between

soil moisture treatments, while lower case letters denote differences between provenances in Tukey HSD test ($p < 0.05$)

leaves the stomata under drought and water deficit were more densely distributed in comparison to the stomata density on oak's leaves in control treatment. The statistically significant differences between soil moisture treatments, and provenance are presented in Fig. 6, while for blocks in Fig. S2 (supplementary materials).

The GLM for stomatal pore area showed statistical significance for treatment and provenance and block. However, it is important to note that we stated large variation within individual seedling (large $\eta_p^2=35\text{--}39\%$) in stomatal pore area. The other factors had smaller effect sizes (denoted as η_p^2) (Table 3). The southernmost provenance, HRV, had a significantly larger stomatal pore area on SF leaves compared to the northernmost provenance, Młyny (MŁY). In contrast, no clear pattern in stomatal pore area was observed in relation to soil moisture treatments (Fig. 7) or block location (Fig. S3 Supplementary materials).

Discussion

Our study provided insights into how intrinsic factors, such as the provenance of *Quercus robur*, and extrinsic factors, including soil moisture content and the location of seedlings relative to wind direction (block), influence the extent of *E. alphitoides* infection on one-year-old seedlings. By examining stomatal and pore areas and gas exchange, particularly stomatal conductance—which may serve as a proxy for stomatal openness—we shed light on the extent to

which pathogen infection affects photosynthesis and how *E. alphitoides* may enter the host plant.

Importance of intrinsic and extrinsic factors and their interaction on the infection severity of first and second flush leaves

Results of GLMs revealed that different factors contribute to the infection rates of first and second flush leaves. First flush leaf infections in spring were less severe and primarily dependent on provenance (an intrinsic factor). In contrast, second flush leaf infections in summer were more severe and strongly influenced by extrinsic factors such as the microenvironment (spatially influenced by location in the study area—block) and soil water availability (treatment), with intrinsic factors playing a minor role. This pattern may arise from the pathogen timing of ascospore production from chasmothecia, which serve as the primary inoculum for annual epidemics (Faticov et al. 2020; Gaytán et al. 2022). Ascospore production usually occurs 30–50 days after the budburst of the first oak flush, allowing the first flush to escape infection. Indeed, intraspecific variation in bud flush development played a role in infection rates. A statistically significant non-parametric correlation, indicated that provenances with faster bud development in spring had lower infection rates (FF leaves), which suggests that faster-developing leaves of provenances escaped peak ascospore abundance and avoided heavy infections. This is supported by previous studies that older leaves are more resistant to *E. alphitoides* infections (Marçais and Desprez-Loustau 2014).

However, this early development does not guarantee success for the subsequent buds regarding *E. alphitoides* infections. Marçais & Desprez-Loustau (Marçais and Desprez-Loustau 2014) observed that seedlings that produce multiple shoot flushes are more susceptible to infection if their foliage emerges under conditions of high inoculum availability, which can lead to severe powdery mildew infection. Indeed, we did not find any single provenance with a significantly lower infection rate (more resilient) to *E. alphitoides* on both first and second flush leaves. Increased spore availability in the mid-season made the second flush more susceptible, consistent with research by Marçais & Desprez-Loustau (Marçais and Desprez-Loustau 2014). The synchronization of spore release by *Erysiphe* fungi with the abundance of host leaf tissue following spring highlights the importance of both leaf age and season on infection rates (Marçais and Desprez-Loustau 2014; Bartholomé et al. 2020) (Desprez-Loustau et al. 2011; Hajji et al. 2009; Marçais & Desprez-Loustau 2014; Mougou-Hamdane et al., 2010). Provenance-dependent bud development plays a significant role in the infection of first flush leaves, as it is synchronized with the release of the pathogen's sexual spores. However, this factor has a minor influence on infection rates when abundant asexual spores are present in mid-season. These findings broaden the context of powdery mildew infections and suggest that, although the genetic background of provenances is important, its impact is somewhat limited by extrinsic factors. Nevertheless, a study by Bartholomé et al. (2020) indicates a strong genetic basis and heritability in powdery mildew resistance, identifying candidate genes coding for pseudo-response regulators (PRRs). Similarly, a recent study by Barrès et al. (2024) points to a few candidate genes for several fitness-related traits.

The environmental conditions considerably influenced the infection rate of second flush leaves. Notably, the location of the seedlings with respect to the wind direction played an important role. The lowest infection rates were observed in the westerly blocks (blocks 5–6), gradually increasing towards the easterly blocks (4, 3, 2, 1). This pattern is consistent with the prevailing wind direction in Poland. Similarly, a study by Augustin et al. (Augustin et al. 2005) on beech provenance trials in Germany found that disease incidence by *Nectria* canker was primarily influenced by proximity to diseased shelterwood and wind dispersal zone, while the influence of provenance was negligible. Our results suggest that wind (movement of the air) can influence the spore load and subsequent infection level.

Soil water availability also significantly affected second flush infection rates. Seedlings experiencing drought exhibited substantially lower infection rates (SF) than those under water-deficit and control treatments. This finding highlights

the role of environmental stress in modulating host–pathogen interactions.

Effects of *E. alphitoides* infection on photosynthesis: Associations with stomatal conductance, stomatal and pore areas, and soil moisture

Changes in gas exchange and stomatal characteristics could at least partially explain the observed relationship between soil water availability and second flush infection rates. Notably, we found a significant dependence of powdery mildew infection rate and soil moisture, and stomatal conductance. Furthermore, consistent with Hajji et al. (2009), our results confirmed that *E. alphitoides* negatively impacts host gas exchange, leading to reduced carbon assimilation and stomatal conductance. It is also well documented that drought-stressed plants close their stomata in response to water limitation (Farquhar and Sharkey 2003; Koller et al. 2013; Pirasteh-Anosheh et al. 2016; Martin-StPaul et al. 2017; Tominaga et al. 2018; Buckley 2019; Salmon et al. 2020; Wang et al. 2022). While drought had the dominant effect on photosynthesis, stomatal conductance, and transpiration, it remains unclear whether these physiological changes directly contribute to reduced infection rates or if other factors are involved. Two non-mutually exclusive mechanisms could potentially explain the observed decrease in infection rates under drought conditions: (1) Stomatal Closure as a Physical Barrier: Given that *E. alphitoides* may invade through the stomata, it is plausible that drought-induced stomatal closure restricts pathogen entry, thereby reducing infection rates. However, the extent to which this mechanism alone accounts for the observed pattern requires further investigation. (2) Carbohydrate Redistribution and Metabolic Shifts: Mild drought stress is known to alter carbohydrate metabolism, often leading to reductions in starch and sucrose concentrations while increasing glucose and fructose levels (Épron and Dreyer 1996). These shifts contribute to osmotic adjustment. However, under prolonged and severe drought, carbon starvation could occur (McDowell et al. 2008, 2022; Dai et al. 2018; He et al. 2020; Li et al. 2020; Chuste et al. 2020; Barker Plotkin et al. 2021), which might have consequences similar to *E. alphitoides* infection—both processes depleting carbohydrates in the growing points of seedlings. Additionally, in oak mildew, fructose and glucose are transferred from the host to fungal cells, where they are converted into polyols and trehalose (Hewitt and Ayres 1976). If drought-stressed seedlings have altered carbohydrate profiles, they might represent a less favorable resource for the pathogen, but this remains speculative.

As our study showed, stomatal density was higher in second flush (SF) leaves than in first flush (FF) leaves, which

may suggest a greater number of potential entry points for *E. alphitoides* to the host plant in SF leaves. However, the stomatal pore area was slightly smaller in SF leaves than in FF leaves which raises the question of whether stomatal size alone might play a limiting role in pathogen entry. Our findings suggest that stomatal closure, rather than stomatal pore area, may be a more critical factor in limiting pathogen entry. If drought-induced stomatal closure limits fungal penetration, this could partially explain the lower infection rates observed under drought conditions. However, the relative contributions of stomatal density, pore size, and dynamic stomatal responses to *E. alphitoides* infection remain unclear and warrant further investigation.

Future studies could explore whether stomatal behavior under varying environmental conditions influences infection success, particularly by examining real-time stomatal responses to fungal presence. Additionally, assessing whether other structural or biochemical changes occur in drought-stressed seedlings could help clarify whether the reduced infection rates under drought result from stomatal closure or other/ additional mechanisms are involved.

Conclusions

Our study highlights the complex interplay between intrinsic and extrinsic factors in shaping the susceptibility of *Quercus robur* to *E. alphitoides*. We found that different sets of factors influenced infection rates in FF and SF leaves. FF leaf infections were generally less severe and primarily governed by provenance (an intrinsic factor), which correlated with bud development timing. A significant negative correlation between early leaf development in spring and infection rates suggests that faster-developing provenances may escape peak ascospore abundance, thereby avoiding heavy infections. In contrast, SF leaf infections were more pronounced and largely driven by extrinsic factors. Spatial variation in infection severity aligned with prevailing wind direction (block effects) and water availability (treatment), while the role of intrinsic factors appeared limited.

Although our findings suggest a potential relationship between drought stress and reduced infection rates, the underlying mechanisms remain uncertain. We propose two possible explanations: (1) drought-induced stomatal closure may act as a physical barrier to pathogen entry, and (2) drought-driven shifts in carbohydrate allocation and metabolism may influence host–pathogen interactions. However, it is still unclear whether these physiological changes directly limit *E. alphitoides* infection or if other environmental and host-related factors contribute.

With the increasing frequency of drought events due to climate change, shifts in water availability are likely to

alter host–pathogen interactions and influence the future epidemiology of *E. alphitoides* in oak populations. Therefore, further research is needed to better understand the role of stomatal closure under soil drought stress in preventing *E. alphitoides* infections despite high inoculum pressure. Additionally, exploring the biochemical changes in drought-stressed plants, particularly metabolic shifts, could provide valuable insights into the metabolic mechanisms underlying altered susceptibility of oaks to *E. alphitoides* infections under drought conditions.

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Declarations

Conflict of interest The authors declare no competing interests.

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References

- Augustin NH, Kublin E, Metzler B et al (2005) Analyzing the spread of beech canker. *For Sci* 51:438–448. <https://doi.org/10.1093/forestscience/51.5.438>
- Barker Plotkin A, Blumstein M, Laflower D et al (2021) Defoliated trees die below a critical threshold of stored carbon. *Funct Ecol* 35:2156–2167. <https://doi.org/10.1111/1365-2435.13891>
- Barrès B, Dutech C, Saint-Jean G et al (2024) Demographic and genetic impacts of powdery mildew in a young oak (*Quercus robur* L.) cohort. *Ann for Sci*. <https://doi.org/10.1186/s13595-024-01259-2>
- Bartholomé J, Brachi B, Marçais B et al (2020) The genetics of exapted resistance to two exotic pathogens in pedunculate oak. *New Phytol* 226:1088–1103. <https://doi.org/10.1111/nph.16319>
- Buckley TN (2019) How do stomata respond to water status? *New Phytol* 224:21–36. <https://doi.org/10.1111/nph.15899>
- Chuste P-A, Maillard P, Bréda N et al (2020) Sacrificing growth and maintaining a dynamic carbohydrate storage are key processes for promoting beech survival under prolonged drought conditions. *Trees* 34:381–394. <https://doi.org/10.1007/s00468-019-01923-5>
- de Cuyper B, Bastien J, Paques L, et al (2011) D2.1-Common protocols and reference standards for selected traits and species TREES4FUTURE-Designing Trees for the Future. 102
- Dai Y, Wang L, Wan X (2018) Relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality caused by drought. *AoB Plants* 10:1–17. <https://doi.org/10.1093/aobpla/plx069>
- Desprez-Loustau M-L, Feau N, Mougou-Hamdane A, Dutech C (2011) Interspecific and intraspecific diversity in oak powdery mildews in Europe: coevolution history and adaptation to their hosts. *Mycoscience* 52:165–173
- Dyderski MK, Paź S, Frelich LE, Jagodziński AM (2018) How much does climate change threaten European forest tree species distributions? *Glob Chang Biol* 24:1150–1163. <https://doi.org/10.1111/gcb.13925>
- Épron D, Dreyer E (1996) Starch and soluble carbohydrates in leaves of water-stressed oak saplings. *Ann Sci for* 53:263–268. <https://doi.org/10.1051/forest:19960209>
- Farquhar GD, Sharkey TD (2003) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 33:317–345. <https://doi.org/10.1146/annurev.pp.33.060182.001533>
- Faticov M, Ekholm A, Roslin T, Tack AJM (2020) Climate and host genotype jointly shape tree phenology, disease levels and insect attacks. *Oikos* 129:391–401. <https://doi.org/10.1111/oik.06707>
- Fetter KC, Eberhardt S, Barclay RS et al (2019) StomataCounter: a neural network for automatic stomata identification and counting. *New Phytol* 223:1671–1681. <https://doi.org/10.1111/nph.15892>
- Gaytán Á, Moreira X, Castagnérol B et al (2022) The co-existence of multiple oak leaf flushes contributes to the large within-tree variation in chemistry, insect attack and pathogen infection. *New Phytol* 235:1615–1628. <https://doi.org/10.1111/nph.18209>
- Guisan A, Zimmermann NE (2000) Predictive habitat distribution models in ecology. *Ecol Modell* 135:147–186. [https://doi.org/10.1016/S0304-3800\(00\)00354-9](https://doi.org/10.1016/S0304-3800(00)00354-9)
- Hajji M, Dreyer E, Marçais B (2009) Impact of *Erysiphe alphitoides* on transpiration and photosynthesis in *Quercus robur* leaves. *Eur J Plant Pathol* 125:63–72. <https://doi.org/10.1007/s10658-009-9458-7>
- He W, Liu H, Qi Y et al (2020) Patterns in nonstructural carbohydrate contents at the tree organ level in response to drought duration. *Glob Chang Biol* 26:3627–3638. <https://doi.org/10.1111/gcb.15078>
- Hewitt Hg, Ayres Pg (1976) Effect of infection by *Microsphaera alphitoides* (powdery mildew) on carbohydrate levels and translocation in seedlings of *Quercus robur*. *New Phytol* 77:379–390. <https://doi.org/10.1111/j.1469-8137.1976.tb01527.x>
- Hückelhoven R (2005) Powdery mildew susceptibility and biotrophic infection strategies. *FEMS Microbiol Lett* 245:9–17. <https://doi.org/10.1016/j.femsle.2005.03.001>
- Jakuschkin B, Fievet V, Schwaller L et al (2016) Deciphering the pathobiome: intra-and interkingdom interactions involving the pathogen *Erysiphe alphitoides*. *Microb Ecol* 72:870–880
- Koller S, Holland V, Brüggemann W (2013) Effects of drought stress on the evergreen *Quercus ilex* L., the deciduous *Q. robur* L. and their hybrid *Q. × turneri* Willd. *Photosynthetica* 51:574–582. <https://doi.org/10.1007/s11099-013-0058-6>
- Kramer CY (1956) Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* 12:307–310
- Li Q, Zhao M, Wang N et al (2020) Water use strategies and drought intensity define the relative contributions of hydraulic failure and carbohydrate depletion during seedling mortality. *Plant Physiol Biochem* 153:106–118. <https://doi.org/10.1016/j.plaphy.2020.05.023>
- Limkaisang S, Cunnington JH, Wui LK et al (2006) Molecular phylogenetic analyses reveal a close relationship between powdery mildew fungi on some tropical trees and *Erysiphe alphitoides*, an oak powdery mildew. *Mycoscience* 47:327–335
- Marçais B, Desprez-Loustau ML (2014) European oak powdery mildew : impact on trees, effects of environmental factors, and potential effects of climate change. *Ann for Sci* 71:633–642. <https://doi.org/10.1007/s13595-012-0252-x>
- Martin-StPaul N, Delzon S, Cochard H (2017) Plant resistance to drought depends on timely stomatal closure. *Ecol Lett* 20:1437–1447. <https://doi.org/10.1111/ele.12851>
- McDowell N, Pockman WT, Allen CD et al (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178:719–739. <https://doi.org/10.1111/j.1469-8137.2008.02436.x>
- McDowell NG, Sapes G, Pivovarov A et al (2022) Mechanisms of woody-plant mortality under rising drought, CO₂ and vapour pressure deficit. *Nat Rev Earth Environ* 3:294–308. <https://doi.org/10.1038/s43017-022-00272-1>
- Mougou A, Dutech C, Desprez-Loustau M (2008) New insights into the identity and origin of the causal agent of oak powdery mildew in Europe. *For Pathol* 38:275–287
- Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods Ecol Evol* 4:133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Niemczyk M, Wrzesiński P, Szypl-Borowska I et al (2024) Coping with extremes: responses of *Quercus robur* L. and *Fagus sylvatica* L. to soil drought and elevated vapour pressure deficit. *Sci Total Environ*. <https://doi.org/10.1016/j.scitotenv.2024.174912>
- Niemczyk M (2025) Data meteo to common garden controlled water experiment. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.28737527>
- Panstruga R, Schulze-Lefert P (2003) Corruption of host seven-transmembrane proteins by pathogenic microbes: a common theme in animals and plants? *Microbes Infect* 5:429–437. [https://doi.org/10.1016/S1286-4579\(03\)00053-4](https://doi.org/10.1016/S1286-4579(03)00053-4)
- Pirasteh-Anosheh H, Saed-Moucheshi A, Pakniyat H, Pessarakli M (2016) Stomatal responses to drought stress. *Water Stress Crop Plants A Sustain Approach* 1–2:24–40. <https://doi.org/10.1002/978119054450.ch3>
- Richardson JTE (2011) Eta squared and partial eta squared as measures of effect size in educational research. *Educ Res Rev* 6:135–147. <https://doi.org/10.1016/j.edurev.2010.12.001>

- Salmon Y, Lintunen A, Dayet A et al (2020) Leaf carbon and water status control stomatal and nonstomatal limitations of photosynthesis in trees. *New Phytol* 226:690–703. <https://doi.org/10.1111/nph.16436>
- Scharnweber T, Manthey M, Criegee C et al (2011) Drought matters - Declining precipitation influences growth of *Fagus sylvatica* L. and *Quercus robur* L. in north-eastern Germany. *For Ecol Manage* 262:947–961. <https://doi.org/10.1016/j.foreco.2011.05.026>
- Skwarek-Fadecka M, Patykowski J, Nawrocka J et al (2022) Biochemical changes in *Quercus robur* L. leaves after *Erysiphe alphitoides* infection. *For Pathol* 52:e12756
- Takamatsu S, Braun U, Limkaisang S et al (2007) Phylogeny and taxonomy of the oak powdery mildew *Erysiphe alphitoides* sensu lato. *Mycol Res* 111:809–826. <https://doi.org/10.1016/j.mycres.2007.05.013>
- TIBCO Software Inc. (2017) Statistica 13.1.
- Tominaga J, Shimada H, Kawamitsu Y (2018) Direct measurement of intercellular CO₂ concentration in a gas-exchange system resolves overestimation using the standard method. *J Exp Bot* 69:1981–1991. <https://doi.org/10.1093/jxb/ery044>
- Vaštag E, Orlović S, Bojović M et al (2022) The influence of powdery mildew on chlorophyll a fluorescence and stomatal characteristics of pedunculate oak (*Quercus robur* L.). *Topola*. 209:31–46
- Wang Y, Wang Y, Tang Y, Zhu XG (2022) Stomata conductance as a goalkeeper for increased photosynthetic efficiency. *Curr Opin Plant Biol* 70:102310. <https://doi.org/10.1016/j.pbi.2022.102310>
- Wang J, Renninger HJ, Ma Q, Jin S (2023) StoManager1: An Enhanced, Automated, and High-throughput Tool to Measure Leaf Stomata and Guard Cell Metrics Using Empirical and Theoretical Algorithms. 1–15
- Wessely J, Essl F, Fiedler K et al (2024) A climate-induced tree species bottleneck for forest management in Europe. *Nat Ecol Evol* 8:1109–1117. <https://doi.org/10.1038/s41559-024-02406-8>

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