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Individual and combined effects of a simulated heatwave and the emerging *Phytophthora ×multiformis* on European alder species

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ABSTRACT

The decline of alder forests induced by increasing temperatures and the "*Phytophthora ×alni* species complex", a group of emerging non-native pathogens, represents a major threat to riparian ecosystems across the Northern Hemisphere. The intensification of heatwaves and the pathogen spread and disease expansion to Southern Europe underscores the importance of studying the responses of alder species to these stressors. This study assesses individual and combined effects of a simulated extreme heatwave and infection by the pathogen *P. ×multiformis* on *Alnus glutinosa* and *A. lusitanica* to identify more resilient genotypes and thus help to mitigate alder decline. 1760 two-year-old alder saplings from 11 populations and 6 genetic groups from Sweden to Morocco were subjected to individual and combined treatments of heatwave and subsequent pathogen infection. Mortality, stem and leaf necrosis, morphological and water-exchange parameters were periodically assessed. *Alnus lusitanica* showed higher tolerance to heat stress than *A. glutinosa*. Responses to the combined stressors differed significantly between populations and genetic groups of both alder species. Plant mortality was highest in the treatment with combined stressors, particularly for the southern populations of *A. lusitanica*. High genetic diversity and previous exposure to extreme temperatures and *Phytophthora* may provide alder populations an adaptive advantage when responding to individual or combined stressors. Our results suggest that *P. ×multiformis* infection after heatwave events may aggravate alder decline in riparian ecosystems, particularly in *A. lusitanica*. Further research on the disease effects in riparian forests of *A. lusitanica* is essential for the conservation and management of this endemic species.

1. Introduction

Ecosystems can be exposed to multiple stressors and the interaction between the combined effects may cause greater or lesser damage than the combination of the individual stressors, depending on the interaction being either synergistic or antagonistic, respectively (Côté et al., 2016). Climate change and pathogen invasions are among two of the most concerning global threats to riparian vegetation (Catford et al.,

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2013; Lapin et al., 2021), and their recurrent incidence can have cumulative negative effects (Stella and Bendix, 2018). Amongst other deleterious effects, vegetation decline in riparian corridors may lead to rising stream water temperature (Trimmel et al., 2018), impacting ecosystem functioning and reducing the capacity of aquatic and riparian ecosystems to adapt to climate change (Alonso et al., 2021; Capon et al., 2013; Ferreira et al., 2022). A deeper knowledge on the vegetation response to biotic and abiotic stressors and its interactions can benefit the identification of suitable management actions for the conservation of declining vegetation (Guignabert et al., 2024). Therefore, under increasingly challenging biological and environmental conditions, research on the consequences of individual and cumulative stresses (Rodríguez-González et al., 2022) may help shape new trends in riparian vegetation decline and demographics (Li et al., 2024; Janssen et al., 2020; Lemm et al., 2021; Scott et al., 2019).

Alders are paramount tree species in riparian ecosystems due to their nitrogen fixing ability, riverbank protection and food chain supply (Claessens, Oosterbaan, Savill, and Rondeux, 2010; Ferreira et al., 2022). *Alnus glutinosa* (L.) Gaertn. (common name: alder) and *A. lusitanica* Vít, Douda & Mandák (common name: Iberian alder) occur in European riparian swamps and riparian forests with permanent access to water. Before 2017, *A. glutinosa* was the most widespread alder species in Europe. In 2017, the *A. glutinosa* populations from the Iberian Peninsula and Morocco were designated as *A. lusitanica* Vít, Douda & Mandák based on genetic evidence (Vít et al., 2017). After 2017, only *A. glutinosa* populations occurring from the Pyrenees to Scandinavia and from the United Kingdom to Western Asia were considered as *A. glutinosa*. Recent studies also found possible intra specific grouping of populations based on its genetic diversity (Gomes Marques et al., 2024a, Martín et al., 2024). It has been suggested that the *A. lusitanica* populations possess traits associated with low water availability and may, therefore, have an adaptive advantage over *A. glutinosa* populations, if subjected to drought (Mandák et al., 2016; Gomes Marques et al., 2024a). There is also evidence of threat to the annual growth and survival of *A. lusitanica* populations in southern parts of the Iberian Peninsula due to ongoing climatic and hydrological changes in this region (Gomes Marques et al., 2018; Rodríguez-González et al., 2014). The increasing frequency and intensity of extreme temperatures in the Iberian Peninsula (Lorenzo et al., 2021) justifies research on the phenotypic responses of alders to such extremes.

Over the past 30 years, *A. glutinosa* and *A. lusitanica* populations in riparian forests and swamps across Europe have been experiencing a severe decline with high mortality rates due to root rot and stem bark cankers caused by invasive pathogens from the "*Phytophthora* ×*alni* species complex" (Gibbs, 1999; Husson et al., 2015; Bjelke et al., 2016). The "*P.* ×*alni* species complex" is specific to *Alnus* species and encompasses the hybrid *P.* ×*alni* and the hybrid's parental species *P. uniformis* from western North America and *P.* ×*multiformis*, also a hybrid assumed to originate from East Asia (Brasier et al., 2004; Husson et al., 2015; Jung et al., 2017). Tree dieback and mortality has been associated with these three pathogens in riparian alder stands throughout Europe, from Sweden to Portugal (Gibbs et al., 1999; Streito et al., 2002; Brasier et al., 2004; Jung and Blaschke, 2004; Černý and Strnadová, 2010; Solla et al., 2010; Pintos-Varela et al., 2012; Redondo et al., 2015; Jung et al., 2016, 2018; Kanoun-Boulé et al., 2016; Corcobado et al., 2020, 2023; Horta Jung et al., 2025). *Phytophthora* ×*multiformis* is widespread in riparian ecosystems and forest plantations (Jung and Blaschke, 2004; Aguayo et al., 2016; Pintos-Varela et al., 2017) and its aggressiveness to alders has been demonstrated to be similar or greater to that of *P.* ×*alni* (Zamora-Ballesteros et al., 2017; Trzewik et al., 2022). The southernmost stands of *P.* ×*alni* have been reported in the vicinities of Foz do Arouce and the Alva, Côa and Dão rivers in Portugal (Gomes Marques et al., 2024b; Horta Jung et al., 2025; Kanoun-Boulé et al., 2016) and Plasencia in Spain (Ferreira et al., 2022). These areas are characterized by high summer temperatures (record highs were registered in 2023), high seasonal and interannual variability in water availability, and heatwaves that can last several days (Copernicus, 2025).

Phytophthora survival and infection depends on both climatic and hydromorphological factors, decreasing with extreme maximum temperature values and increasing with presence of standing water (Gomes Marques et al., 2024b). Previous studies observed a predisposition of several tree species (*Fagus sylvatica*, *Quercus cerris*, *Q. pubescens, Q. ilex* and *A. rhombifolia*) to *Phytophthora* infection after extreme rainfall, flooding or drought (Corcobado et al., 2014) (Jung, 2009) Colangelo et al., 2018; Gomes Marques et al., 2022b), but there is a lack of research on the individual and combined effects of thermal stress and *Phytophthora* spp. infection on alders.

This study addresses and compares the individual and combined effects of heat stress and *P.* ×*multiformis* infection on a large set of *A. glutinosa* and *A. lusitanica* population provenances. Furthermore, the study aimed to identify populations or genetic groups that show some degree of tolerance to these stressors. More knowledge on alders' tolerance and susceptibility can support decision-making during management actions, notably in sites that are infested or prone to be infested by *Phytophthora* and subjected to heat stress (Gomes Marques et al., 2022b). To accomplish this, saplings grown from seeds collected from 11 populations across the latitudinal range of the two alder species were subjected to a simulated heatwave and infected with *P.* ×*multiformis*. Then, a large set of morphological and physiological traits were evaluated across the saplings. We hypothesized that (1) the combination of a heatwave and pathogen infection would increase plant mortality and negatively affect plant performance and (2) differences would exist between the alder species and/or intraspecific genetic groups in terms of their tolerance and susceptibility to the individual and combined stressors.

2. Methods

2.1. Plant material

Between February 2017 and March 2018, alder seeds were collected from five *Alnus glutinosa* and six *A. lusitanica* populations occurring in riparian forests or wetlands across the whole latitudinal range of European alder (Fig. 1, Table S1; Gomes Marques et al., 2022a). The seeds were collected from ten mother trees per population. In April 2018, the seeds were germinated and then grown for two years in greenhouse conditions in Oeiras, Portugal (38.6961ºN, 9.3202ºW). In the greenhouse, minimum and maximum temperatures were 11ºC and 38ºC, respectively, and relative humidity ranged from 20 % to 80 %. Genotypic analysis of the populations revealed six genetically distinct groups (Fig. 1; Gomes Marques et al., 2024a).

2.2. Experimental design and treatments

The two-year-old *A. glutinosa* and *A. lusitanica* saplings were subjected to heatwave temperatures and *P.* ×*multiformis* infection (Fig. 2). The saplings were arranged in 1-litre pots following a split-plot random design, replicated in four blocks, with thermic stress as the main factor (two categories: control and heatwave temperatures; whole plots) and pathogen infection as the split factor (two categories: non-infected and *P.* ×*multiformis*-infected). Each block had approximately 110 saplings and each treatment had four blocks. In each block, each of the 11 populations was represented by 10 open-pollinated families and each family by a single sapling whenever possible. Not all families were represented in all blocks due to the heterogeneity in germination rates among alder populations (Gomes Marques et al., 2022a). Individual saplings were randomly positioned within the blocks. In total, 1760 saplings were produced for the experiment, distributed as follows: 4 blocks × 2 temperatures × 2 infection treatments × 110 families × 1 individual, and a total of 1321 were healthy and used since beginning of the experiment.

Two adjoining greenhouse compartments were used to simulate an extreme heatwave and assess the effects on the alder saplings. The

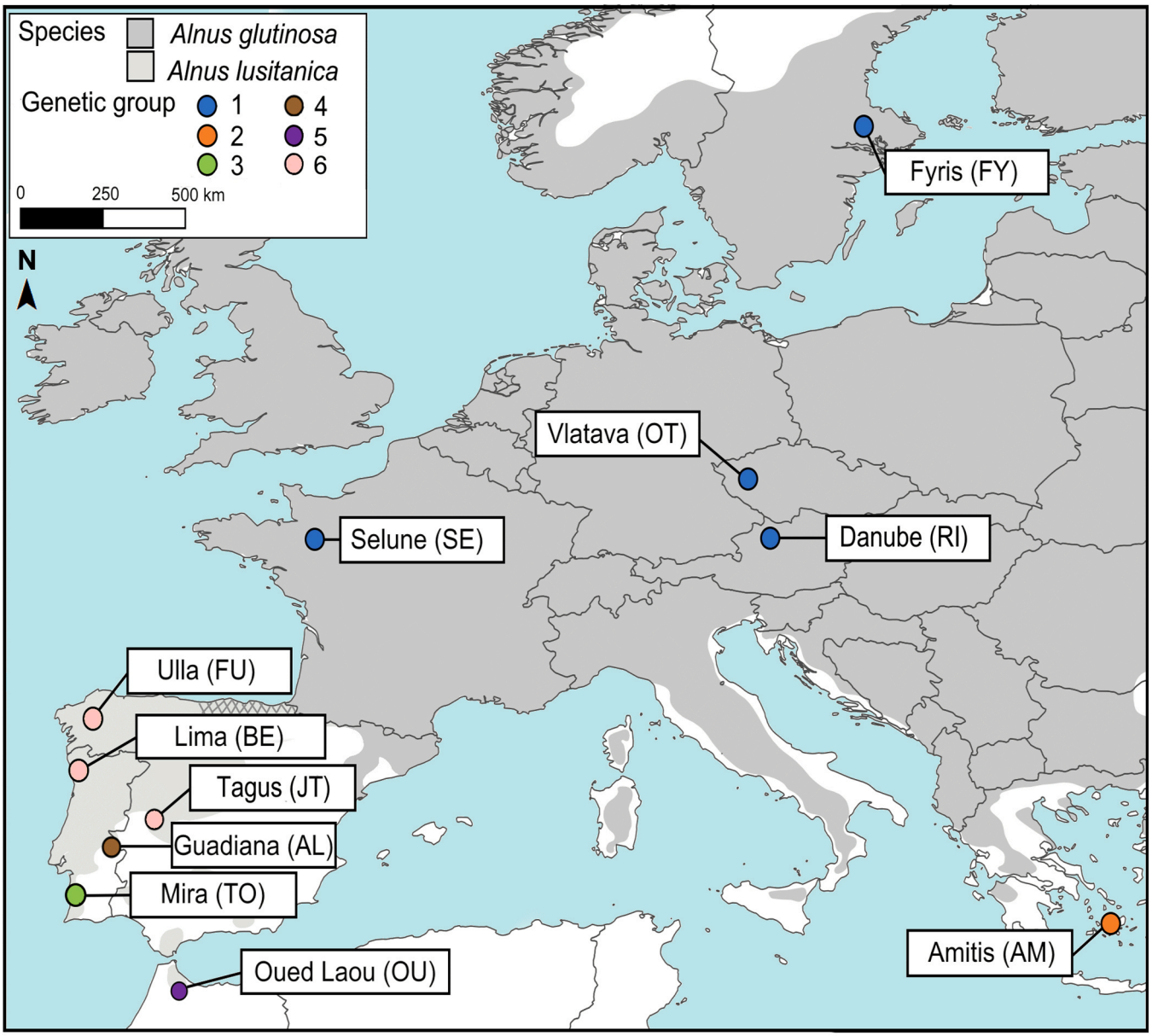


Fig. 1. Geographical locations and hydrographic basins identification of the 11 studied alder populations, each identified by a two-letter code in brackets. The geographical distribution of *Alnus glutinosa*, *A. lusitanica* and both species is represented by dark grey, light grey and pattern areas, respectively (adapted from EUFORGEN 2009 [www.euforgen.org], Vít et al., 2017 and Martín et al., 2024). The different location colours represent the genetic groups (Gomes Marques et al., 2024a). See Table S1 for further information about the studied populations. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

compartments were similar in size and sun exposure. On 14 June 2020, the saplings were divided into two groups, and one group was subjected to ambient temperatures and the other to heatwave temperatures. The heatwave treatment consisted of 21 days of maximum daytime (6 am to 9 pm) temperatures between 32ºC and 55ºC and minimum temperatures between 18 and 24 ºC (Supplementary Material Excel) thereby simulating predicted extreme heat events in the Mediterranean (Molina et al., 2020). Plants were watered regularly to field capacity. On 4 July 2020, the saplings under the control and extreme heatwave treatments were further divided into two groups (Fig. 2). One group was subjected to mock inoculation and the other to *P.* ×*multiformis* inoculation. Thus, by 25 July 2020, 112–153 saplings from each population, including saplings from all mother trees were subjected to the following four treatments: (i) control, (ii) infection, (iii) heatwave and (iv) heatwave and infection (Fig. 2).

2.3. Pathogen inoculation

The pathogen used was isolated in March 2020 from a bleeding stem canker of an *A. lusitanica* tree with defoliation and branch dieback growing at the Lima river, Portugal (41.726566ºN, 8.671680ºW). The obtained *Phytophthora* isolate Li109 (= TJ1460) was identified as *P.* ×*multiformis* based on (1) sequence analyses of three nuclear gene regions, i.e. the internal transcribed spacer region (ITS1–5.8S–ITS2) of the ribosomal RNA gene (ITS), β-tubulin (βtub) and translation elongation factor 1 alpha (tef-1α) and the mitochondrial genes cytochrome-c oxidase 1 (cox1) and subunit 1 of NADH dehydrogenase (nadh1) (Jung et al., 2017, 2024); (2) morphological characteristics, in particular high oogonial abortion rate (86 %), very strong ornamentation of the oogonial walls, a high proportion of > 20 % paragynous antheridia and a relatively low proportion of bicellular antheridia (24 %) (Brasier et al.,

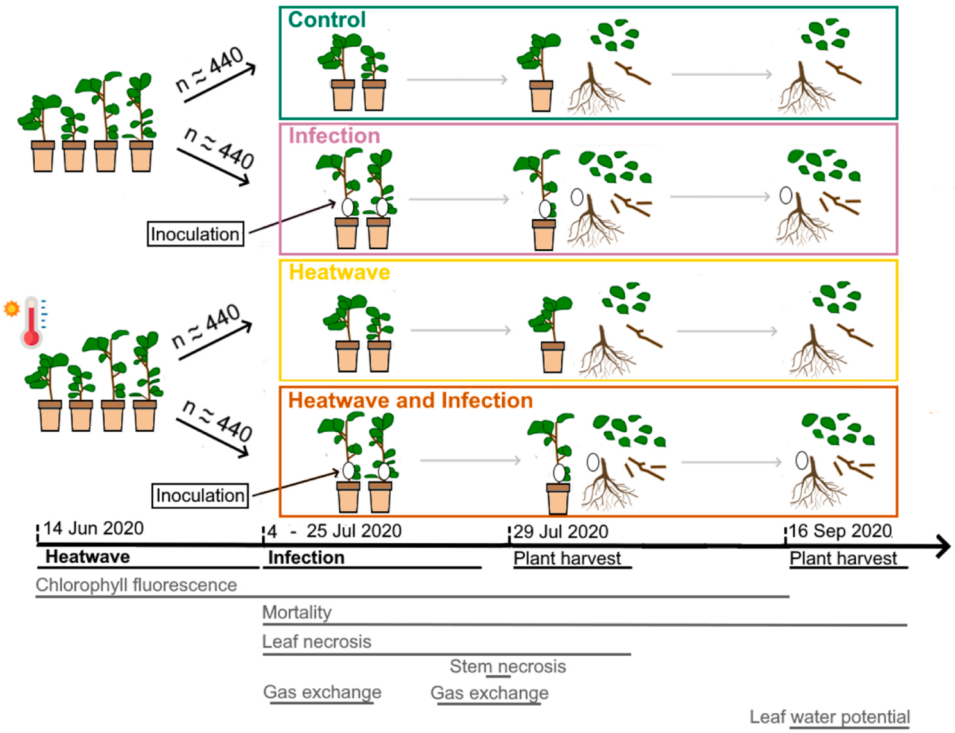


Fig. 2. Timeline of 'Control' (green), 'Infection' (pink), 'Heatwave' (yellow) and 'Heatwave and Infection' (orange) treatments and measurements applied to two-year-old *Alnus glutinosa* and *A. lusitanica* saplings from 11 populations. Infection was done by *Phytophthora ×multiformis* inoculation. For each treatment, 'n' is the theoretical number of saplings used.

2004; Jung et al., 2017); and (3) fast growth at 20°C on potato-dextrose agar (PDA; 5.6 mm/d) (Jung et al., 2017). The sequence data of isolate Li109 were deposited in GenBank (accession numbers: ITS = PQ344746; Btub = PQ356730; EF1a = PQ356731; cox1 = PQ356732; nadh1 = PQ356733).

Saplings were inoculated by placing a 7-mm-diameter mycelium disc onto a vertical stem incision with 1.0–1.5 cm length and situated 3–5 cm above the root collar. Discs were taken from the margin of a 7-day-old culture grown in V8-juice agar (V8A). Mock inoculations consisted of placing non-colonized V8A discs onto the stem wound. The inoculated area was covered with a sterile water-soaked cotton ball and Parafilm ® M sealing film. All saplings were then subjected to ambient temperatures (20–28 ºC) and watered regularly to field capacity for 21 days. On 25 July 2020, pieces of necrotic bark from the stem of inoculated saplings were plated onto selective PARPNH agar medium (Jung et al., 2017, 2024) to re-isolate *P. ×multiformis* and fulfil Koch's postulates.

2.4. Plant measurements

To assess the individual and combined effects of the simulated heatwave and *P. ×multiformis* infection on the performance of the alder saplings and the progression of the disease, measurements were conducted from April 2018 to September 2020 on plants subjected to all four treatments (Fig. 2). A comprehensive list of measured and calculated morphological and physiological traits is given in Supplementary Table S2. The physiological measurements were performed first, and morphological assessments were performed last.

After the heatwave treatment, sapling mortality was evaluated weekly. The percentage of necrotic leaves was visually estimated 12 days after heatwave treatment (i.e. 8 days before plant inoculation for the saplings subjected to infection), and at 46, 56 and 66 days after the heatwave treatment (i.e. 25, 35 and 45 days post inoculation -dpi- for the saplings subjected to infection, Figure S1). All plants were assigned a score from 0 (all leaves in the plant appear healthy) to 13 (dead plant; Table S3). At the end of the infection period (21 dpi), stem necrosis size (length and width) was measured in all plants after removing the sealing film to reveal the inoculated area. At 74 dpi, total lesion length and width was measured on each sapling and the lesion area was calculated.

The maximum photochemical efficiency of photosystem II (chlorophyll *a* fluorescence) was evaluated on leaves adapted to dark conditions for 30 min from a subsample of saplings that included both species and all the populations used in the experiment ($n = 171–428$, Fig. 2). The chlorophyll *a* fluorescence (Fv/Fm) was measured using a portable fluorometer (FluorPen FP100: ICT International, Armidale, NSW, Australia). See Gomes Marques et al., (2024a) for methods.

Leaf gas exchange was evaluated on a minimum of three saplings from each of the three populations that showed the most extreme chlorophyll fluorescence responses after the heatwave treatment ($n = 41$), namely the *A. lusitanica* population of Oued Laou, Morocco (OU), and the *A. glutinosa* populations of Fyris, Sweden (FY) and Amitis, Naxos Island, Greece (AM). Measurements of leaf gas exchange were made between 9 am and 12 am, 12 days after heatwave treatment (i.e. 8 days before plant inoculation for the saplings subjected to infection) and at 42 days after heatwave treatment (i.e., 21 dpi for the saplings subjected to infection, Fig. 2) using a portable open-system infrared gas analyser (Li-Cor 6400: LiCor, Lincoln, NE, USA) at a constant supply of photosynthetically active radiation of 1000 µmol photons m^{-2} s^{-1}. Measurements of net photosynthesis (Pn), stomatal conductance to water vapour (g_s) and transpiration (E) were also made three times on the same day on at least three randomly selected fully expanded leaves.

Predawn leaf water potential (Ψ_L) was measured in one leaf per plant detached from the same plants used for the leaf gas exchange measurements whenever the plant showed enough healthy leaves ($n = 87$). Measurements were performed before final plant harvest, at 95 days after heatwave treatment (i.e., 74 dpi for the saplings subjected to infection), using a Scholander-type (Scholander et al., 1965) pressure chamber (PMS 1000: PMS Instruments, Corvalis, OR, USA). See Gomes Marques et al., (2024a) for methods.

At 46 and 95 days after heatwave treatment (i.e., 25 and 74 dpi for the saplings subjected to infection, Figs. 2), 2–6 saplings per population

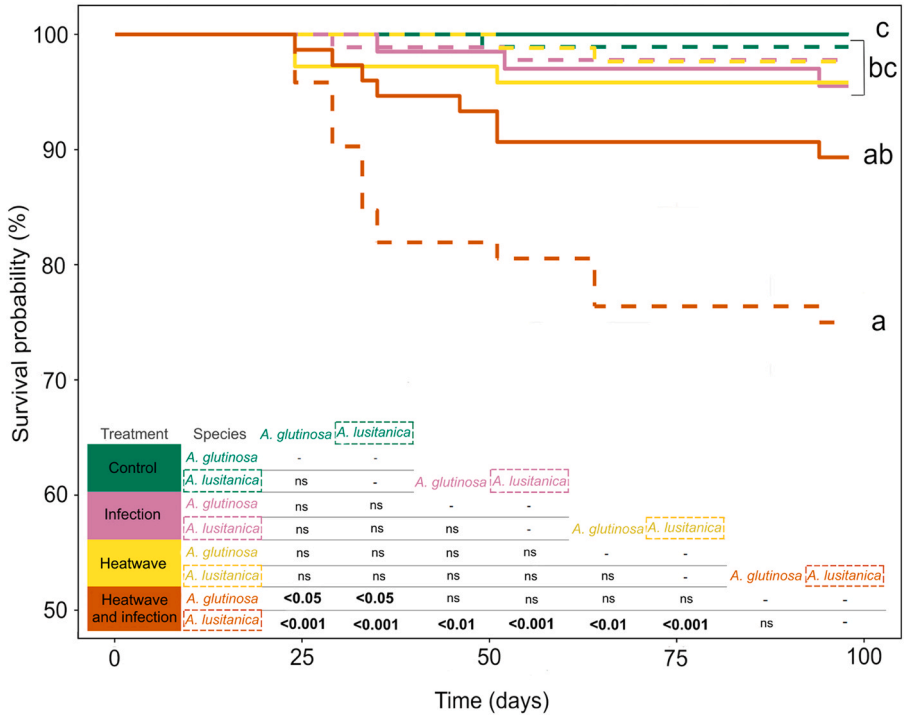


Fig. 3. Survival probability for *Alnus glutinosa* (solid lines) and *A. lusitanica* (dashed lines) saplings subjected to four different treatments (indicated by colour). Infection was done by *Phytophthora ×multiformis* wound inoculation of stems. Different letters indicate significant differences between treatments ($p < 0.05$). *P*-values of the comparisons between species and treatments are shown at the bottom left corner.

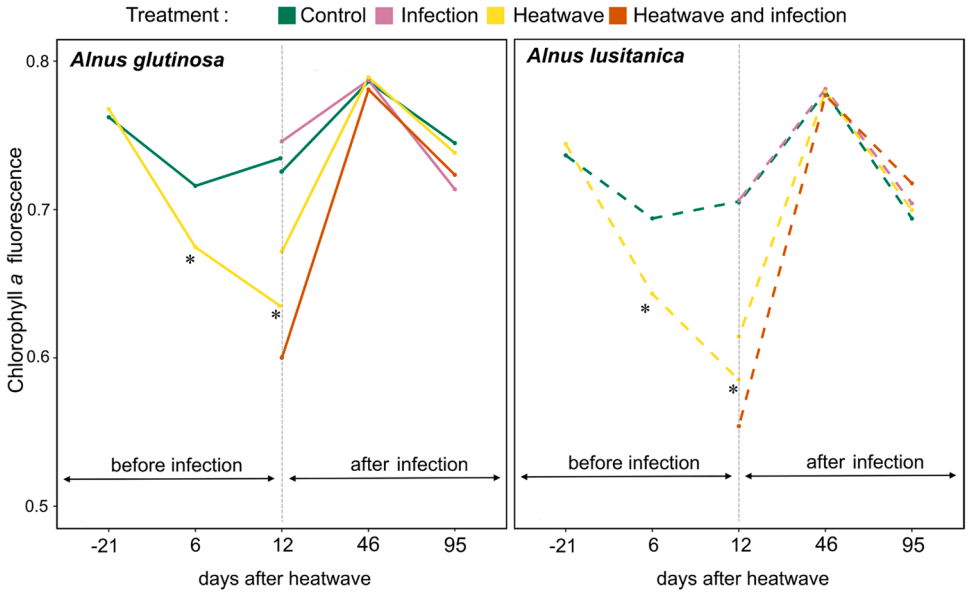


Fig. 4. Chlorophyll fluorescence for *Alnus glutinosa* (left, solid lines) and *A. lusitanica* (right, dashed lines) saplings subjected to four different treatments (indicated by colour). Infection was done by *Phytophthora ×multiformis* wound inoculation of stems. Asterisks indicate statistical significance at $p < 0.05$.

and per treatment ($n = 563$) were harvested for biomass assessment. The biomass of all saplings was partitioned into leaf, stem and root components and the fresh weight was assessed immediately. The dry-weight of each biomass component was assessed after oven-drying for 72 h at 65 °C. All fresh leaves and roots were scanned using the WinnSeedle and WinnRhizzo software packages (Regent Instruments) to obtain various plant parameters (Table S2).

2.5. Statistical analysis

The saplings' responses to the treatments were analysed at the population, genetic groups (Gomes Marques et al., 2024a) and species levels. All analyses were performed in R (v. 3.5.1; R Core Team, 2021).

Survival probabilities were calculated using the Kaplan–Meier estimate, and differences between treatments for each plant species were examined using the log-rank test in the *survival* package (Therneau, 2021; Therneau and Grambsch, 2000). Pairwise differences between

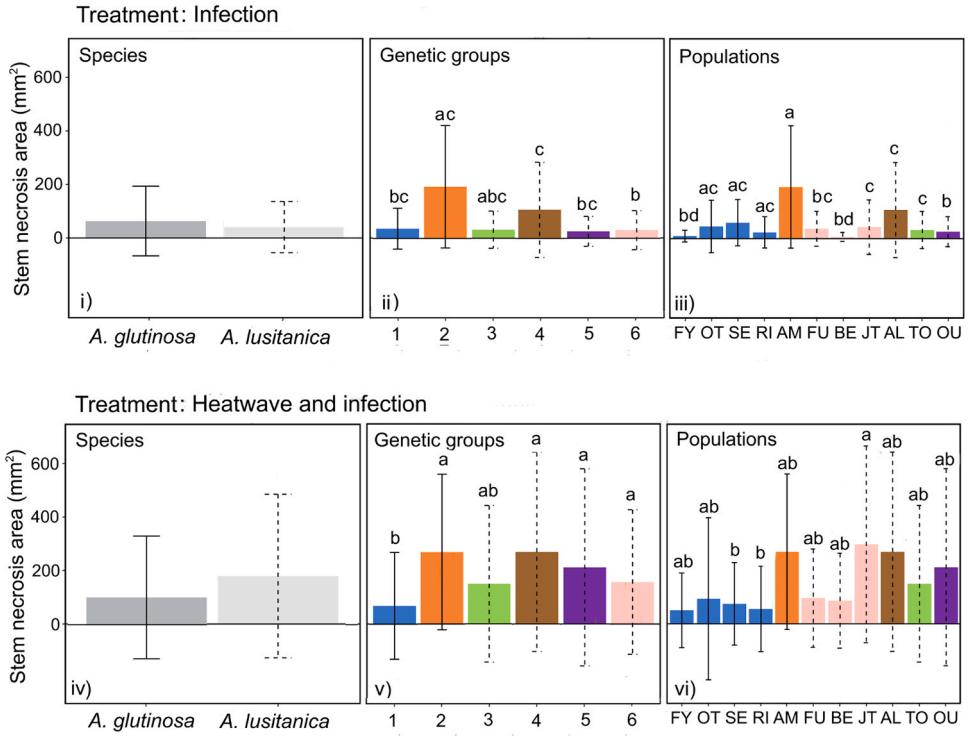


Fig. 5. Stem necrosis area (mm^2) on *Alnus glutinosa* (solid lines) and *A. lusitanica* (dashed lines) after infection by *Phytophthora ×multiformis* (i, ii and iii) and after the combination of heatwave and infection by *P. ×multiformis* (iv, v and vi). Results are presented by species (i and iv), genetic group (ii and v) and populations (iii and vi). Different colours indicate the six different genetic groups (legend in ii and v). Capital letters denote populations (see Fig. 1 and Supplementary Table S1). Different small letters indicate statistically significant differences between levels ($p < 0.1$).

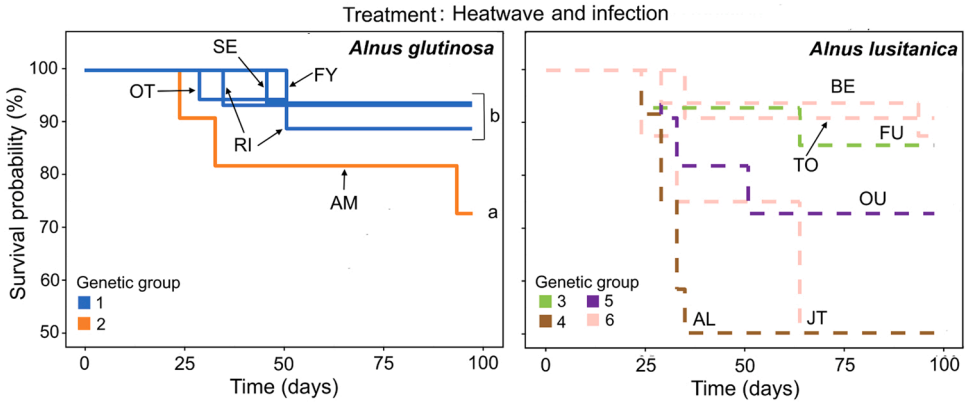


Fig. 6. Survival probabilities for *Alnus glutinosa* (left, solid lines) and *A. lusitanica* (right, dashed lines) saplings subjected to the combination of heatwave and infection by *Phytophthora ×multiformis*. Different colours indicate the six different genetic groups. Capital letters denote populations (see Supplementary Table S1). Different small letters indicate significant differences between groups ($p < 0.05$).

genetic groups and populations were determined using the *survminer* package (Kassambara, et al., 2021). The saplings' biomass allocation and stem necrosis size were assessed per species through mixed models using 'treatments' as fixed factor. Population was considered a random factor and was nested within genetic groups (Gomes Marques et al., 2024a). Linear mixed models were applied to the biomass allocation data using the *lmer* function in the *lme4* package. Generalized linear mixed models specifying a gamma distribution were applied to the stem necrosis area data > 0 using the *glmer* function in *lme4* package. All model residuals were tested for homoscedasticity and normality, and when assumptions were not validated, variables were transformed by the square root. Differences in physiology and leaf necrosis were assessed using the Kruskal–Wallis rank sum test, with Benjamini-Hochberg adjustment and pairwise Wilcoxon test.

3. Results

3.1. Responses to heatwave treatment

The heatwave treatment induced plant mortalities of 4.2 and 2.4 % in *A. glutinosa* and *A. lusitanica* saplings, respectively (n.s.; Fig. 3). The highest percentage of leaf necrosis was observed on the first day after heatwave treatment ($p < 0.001$; Figure S1). *Alnus glutinosa* saplings had higher leaf necrosis scores than *A. lusitanica* saplings until 56 days after heatwave treatment (Figure S1). The highest leaf necrosis scores occurred in the *A. glutinosa* population from Sweden (FY) at 46 days after heatwave treatment ($p < 0.05$; Figure S1).

Throughout the entire experiment, *A. lusitanica* control saplings showed lower chlorophyll fluorescence values than *A. glutinosa* control

saplings ($p < 0.05$; Fig. 4). Saplings from both species subjected to the heatwave treatment presented lower chlorophyll fluorescence and photosynthesis values and higher transpiration and stomatal conductance values ($p < 0.01$; Figures 4 and S2). At 46 days after the heatwave treatment, the heatwave treatment had a negative effect on the leaf area ratio and wood dry matter content (WDMC) of the *A. glutinosa* saplings ($p < 0.05$; Table 1). In contrast, *A. lusitanica* saplings showed 95 days after the heatwave treatment a positive effect on the leaf dry matter content (LDMC) ($p < 0.1$; Table 1).

3.2. Responses to Phytophthora ×multiformis infection

Plant mortality induced by *P. ×multiformis* infection was similar in *A. glutinosa* and *A. lusitanica* (4.5 and 2.2 %, respectively; n.s.; Fig. 3) and did not differ significantly from the mortality rate in the control saplings (0 and 1.1 %, respectively). Stem necrosis areas were also similar between the two species (Fig. 5i). Immediately after infection, the *A. lusitanica* saplings showed higher leaf necrosis percentages than *A. glutinosa* saplings ($p < 0.05$; Figure S1). At 56 days after the heatwave treatment (i.e., 35 dpi for the saplings subjected to infection), the infected saplings from both species, whether subjected to the heatwave treatment or not, showed higher leaf necrosis percentages ($p < 0.05$; Figure S1).

Saplings belonging to the *A. glutinosa* genetic group 2 (Greek population AM) showed the largest stem necrosis area ($p < 0.05$; Figures 5ii and 5iii). Saplings belonging to the *A. lusitanica* genetic group 6 (Portuguese population BE) and to the *A. glutinosa* genetic group 1 (Swedish population FY) showed the smallest stem necrosis areas ($p < 0.05$; Figures 5ii and 5iii).

The saplings' chlorophyll fluorescence values were not altered by *P. ×multiformis* infection (Fig. 4). However, the infected *A. lusitanica* saplings from the Moroccan population (OU) showed higher photosynthesis rates after infection than those subjected only to the heatwave treatment ($p < 0.05$; Figure S2). Changes in plant performance at 95 days after the heatwave treatment (i.e., 74 dpi for the saplings subjected to infection) due to *P. ×multiformis* infection were observed through a decrease in WDMC in both alder species, and through an increase in LDMC and a reduction in root to shoot ratio in *A. lusitanica* ($p < 0.1$; Table 1).

3.3. Responses to the combined stressors

The saplings subjected to the combination of heatwave and *P. ×multiformis* infection showed higher plant mortality (25 % for *A. lusitanica* and 11 % for *A. glutinosa*; Fig. 3) than those subjected to the individual stressors (2.4 and 2.2 % for *A. lusitanica*; 4.2 and 4.4 % for *A. glutinosa*; Fig. 3). Although survival curves between *A. lusitanica* and *A. glutinosa* were not statistically different, mortality was 127 % higher in *A. lusitanica* (25 vs 11 %, respectively, $p < 0.01$; Fig. 3). Differences between sapling mortality rates also occurred between genetic groups within *A. glutinosa* (Fig. 6). The *A. glutinosa* populations belonging to genetic group 1 showed lower mortality rates than the *A. glutinosa* population from the genetic group 2 (8 % and 27 %, respectively, $p < 0.05$; Fig. 6).

At 46 days after heatwave treatment (i.e., 25 dpi for the saplings subjected to infection), saplings from both species showed higher specific leaf areas (SLA; Table 1). The LDMC and LMR were lower in *A. lusitanica* (Table 1). At 95 days after heatwave treatment (i.e., 74 dpi for the saplings subjected to infection), the combination of heatwave and *P. ×multiformis* infection caused SLA to increase in *A. lusitanica* saplings ($p < 0.01$) and WDMC to decrease in saplings of both species ($p < 0.1$; Table 1).

The *A. lusitanica* populations showed larger stem necrosis areas than those of *A. glutinosa* (Fig. 5iv). The *A. glutinosa* populations belonging to genetic group 2 (Greek population AM) and 4 (southern Portuguese population AL) showed the largest stem necrosis areas (Figure 5 v). The *A. glutinosa* populations belonging to genetic group 1 (Austrian

Table 1
Morphological performance parameters at 46 and 95 days after heatwave treatment for *Alnus glutinosa* and *A. lusitanica* subjected to the four treatments. Different letters indicate significantly different means between treatments ($p < 0.1$). Values are means ± standard deviations.

	46 days after heatwave treatment (i.e. 25 dpi for the saplings subjected to infection)				**95 days after heatwave treatment (i.e. 74 dpi for the saplings subjected to infection)**			
Treatment	**Control**	**Heatwave**	**Infection**	**Heatwave and infection**	**Control**	**Heatwave**	**Infection**	**Heatwave and infection**
Specific leaf area								
Alnus glutinosa	22.78±3.86 *ab*	20.51±4.08 *a*	22.20±3.22 *ab*	24.91±3.03 *b*	19.04±3.43	21.76±7.18	21.47±4.25	21.32±3.85
Alnus lusitanica	19.63±2.04 *a*	21.45±3.04 *ab*	21.37±2.33 *ab*	22.91±3.49 *b*	21.69±2.39 *a*	23.95±13.00 *a*	21.13±4.37 *a*	24.48±3.61 *b*
Leaf area ratio								
Alnus glutinosa	2.70±0.96 *ab*	1.67±0.8 *a*	2.83±1.28 *b*	2.19±1.4 *ab*	0.92±0.49	1.06±0.45	1.05±0.59	1.01±0.64
Alnus lusitanica	2.11±0.51 *ab*	2.31±0.99 *b*	2.29±0.83 *ab*	1.97±1.10 *a*	1.38±0.44	1.34±0.51	1.40±0.63	1.60±0.53
Wood dry matter content								
Alnus glutinosa	0.49±0.03 *b*	0.47±0.03 *a*	0.47±0.08 *b*	0.44±0.07 *a*	0.50±0.04 *b*	0.58±0.35 *b*	0.38±0.04 *a*	0.39±0.06 *a*
Alnus lusitanica	0.43±0.12 *ab*	0.43±0.07 *a*	0.50±0.16 *b*	0.47±0.08 *a*	0.48±0.03 *c*	0.48±0.03 *c*	0.43±0.21 *b*	0.35±0.05 *a*
Leaf dry matter content								
Alnus glutinosa	0.39±0.05	0.36±0.04	0.37±0.04	0.34±0.07	0.47±0.13	0.40±0.08	0.45±0.13	0.43±0.10
Alnus lusitanica	0.38±0.02 *b*	0.37±0.04 *b*	0.37±0.02 *b*	0.35±0.05 *a*	0.37±0.04 *a*	0.38±0.08 *b*	0.39±0.05 *b*	0.35±0.04 *a*
Leaf mass ratio								
Alnus glutinosa	0.12±0.03 *ab*	0.08±0.04 *a*	0.12±0.05 *b*	0.09±0.05 *a*	0.05±0.03	0.05±0.02	0.05±0.03	0.05±0.02
Alnus lusitanica	0.11±0.02	0.11±0.04	0.11±0.04	0.08±0.04	0.06±0.02	0.06±0.02	0.07±0.03	0.07±0.02
Stem mass fraction								
Alnus glutinosa	0.53±0.16	0.50±0.12	0.50±0.10	0.57±0.15	0.44±0.12	0.41±0.10	0.53±0.15	0.35±0.20
Alnus lusitanica	0.52±0.12	0.49±0.12	0.51±0.10	0.53±0.15	0.49±0.11	0.40±0.15	0.52±0.17	0.51±0.21
Root to shoot ratio								
Alnus glutinosa	1.28±0.29	1.55±0.35	1.32±0.36	1.38±0.48	1.35±0.33 *ab*	1.60±0.81 *b*	1.27±0.30 *a*	1.35±0.36 *ab*
Alnus lusitanica	1.36±0.38	1.40±0.25	1.36±0.38	1.47±0.47	1.48±0.41 *ab*	1.64±0.70 *b*	1.25±0.43 *a*	1.92±1.11 *b*

population RI and French population SE) were the least affected by stem necrosis ($p < 0.05$; Fig. 5 v and vi).

4. Discussion

We report for the first time the morphological, physiological and performance effects on alders of (i) a simulated extreme heatwave, (ii) infection by *P. ×multiformis* and (iii) a combination of both stressors. This work also presents novel results on the genotypic and phenotypic variation between and within *A. glutinosa* and *A. lusitanica*, and differences between populations and genetic groups in terms of their susceptibility and tolerance to the individual and combined stressors.

4.1. Heatwave effects on alders

In Europe, heatwaves are progressively shifting to more early and late periods in summer (Pérez-García et al., 2025) as well as increasing in intensity and duration (Ruosteenoja and Jylha, 2023). In alder-dominated riparian ecosystems, water courses are typically perennial, and the occurrence of late spring heatwaves (i.e. when water uptake is still possible) poses a new threat to tree health, in addition to the more commonly studied compound drought-heat events. High temperature events can have various effects on plant species, depending on timing, duration and intensity (Niinemets, 2010). If the upper temperature threshold for plant performance and survival is reached, the heat stress alters the plants at the molecular and physiological levels: the plant's photosystem II can be damaged, photosynthesis rate and leaf area may decrease, and leaf senescence and proline content may increase (Dorado et al., 2022; Guion et al., 2022; Singh et al., 2020).

Our study demonstrated that, even though no water deprivation was imposed, heatwaves have a negative effect on *Alnus* species and particularly on *A. glutinosa*. This species is apparently not as well adapted to heat stress as *A. lusitanica*, which grows at the southern limits of the alder's geographical distribution, where extreme temperatures and low water availability occur regularly (Rodríguez-González et al., 2014). Accordingly, saplings from the *A. glutinosa* northernmost population of Fyris, Sweden, showed the highest average leaf necrosis area after heat stress.

Both *Alnus glutinosa* and *A. lusitanica* showed reduced chlorophyll fluorescence and photosynthetic rates in response to the heatwave treatment. Additionally, the aboveground growth of *A. glutinosa* was significantly limited, which is a typical response to heat in tree species. Leaf loss has been observed as a general plant response to heatwaves in southern Europe (Guion et al., 2022). Brown necrotic leaf margins, yellowing or browning of inner leaf areas and scorching are additional symptoms in trees subjected to heat stress (Dorado et al., 2023). Interestingly, the aboveground growth of *A. lusitanica* was promoted after the heatwave treatment, which indicated resilience to the thermal stress. The higher transpiration and stomatal conductance rates observed in both alder species after the heatwave treatment in our experiment, may contribute to leaf thermoregulation and help avoid thermic damage to the leaves (Blasini et al., 2022).

After the extreme heatwave, carbohydrate reserves and the lack of water limitation allowed saplings to quickly produce new leaves and sprouts afterwards, thereby recovering from leaf wilting. This study did not simulate a compound drought-heatwave event occurring during late summer or after prolonged and repeated extreme heat events, but rather an ecological event occurring in late spring, or early autumn. However, a recent study on the ecotypic variation of alders (Gomes Marques et al., 2024a) reported that *A. lusitanica* also displayed certain functional traits related to adaptation to low water availability, such as a high number of root tips. Perhaps under a compound drought-heatwave event, *A. lusitanica* seedlings would present increasing resilience, by relying on their higher root range for water uptake. The capacity of *A. lusitanica* to adapt to environmentally challenging conditions is still poorly studied and such information is essential to the management and conservation of the *Alnus* genus at the southern limits of its distribution, particularly under future climate. Further research should also focus on the responses of alders to low water availability and compound drought-heatwave events (Gomes Marques et al., 2022b).

4.2. *Phytophthora ×multiformis* infection effects on alders

The variation in susceptibility of alder to *P. ×multiformis* was found to be dependent on genetic groups and populations, and was mostly observable through differences in sapling stem necrosis and physiological performance. The southernmost *A. glutinosa* population (AM), located on the Greek island of Naxos, showed the highest susceptibility. This could be explained by (i) the lack of previous exposure of this population to the disease and (ii) the geographical isolation that contributed to a low genetic diversity in the population (Gomes Marques et al., 2024a). To our knowledge, none of the species from the "*P. ×alni* complex" have been detected in Greece yet. A study of the susceptibility of *A. glutinosa* to *P. uniformis* (Redondo et al., 2019) reported higher mortality of alders from sites without pathogen invasion, due to a low genetic variability and a lack of natural selection driven by pathogen pressures. Therefore, high genetic diversity, like that observed in the *A. lusitanica* populations FU and BE (Gomes Marques et al., 2024a) might provide trees with an adaptive advantage in their responses to individual or combined stressors, as observed in this study.

At the time of seed collection, all selected alders were apparently healthy, but most northern river basins with *A. glutinosa* populations and most of the mid-latitude river basins with *A. lusitanica* populations in the Iberian Peninsula had already been invaded by one or more species from the "*P. ×alni* complex" (Jung and Blaschke, 2004; Solla et al., 2010; Pintos-Varela et al., 2012, 2017; Bjelke et al., 2016; Jung et al., 2016; Kanoun-Boulé et al., 2016; Ferreira et al., 2022; Corcobado et al., 2023; Gomes Marques et al., 2024b). Natural selection could already have eliminated highly susceptible alder genotypes from these sites. Studies in Germany and Belgium demonstrated in river systems with long-term exposure to *P. ×alni* lower susceptibility of surviving healthy *A. glutinosa* trees as compared to declining trees, most likely resulting in gradually increasing proportions of tolerant genotypes in these alder populations (Jung and Blaschke, 2006; Chandlier et al., 2016). Similarly with the devastating ink disease caused by *P. cinnamomi* in *Castanea sativa* Mill. in Spain, northern chestnut populations, which were historically more exposed to the pathogen than southern populations, show lower susceptibility to the disease (Alcaide et al., 2020). Tolerance of the oak species *Quercus ilex* to *P. gonapodyides* has also been reported to be geographically dependent, with a trend of increasing susceptibility from north to south (Corcobado et al., 2017). In the particular case of riparian ecosystems, the dispersal of seeds or propagules through water can help transport certain genetic traits (Nilsson et al., 2010) over long distances from infected upstream populations with less susceptible trees downstream to recently infected sites with highly susceptible populations. Whether the susceptibility structure of *Alnus* populations is determined by pathogen-imposed selection or by other adaptive processes needs further studies. However, the present study confirms for the first time the spatial variation in the susceptibility of alders to *P. ×multiformis*.

Phytophthora ×multiformis infection induced changes in the performance of *A. lusitanica* saplings, such as increased leaf dry matter content and decreased root to shoot ratio. Infected saplings with a weaker root performance will not maintain their competitive ability over more tolerant genotypes of the same or other forest tree species due to their reduced ability to access water and nutrients. Alders are typical drought-avoiding species that generally thrive in wetlands and on riverbanks, hence, infected *A. lusitanica* saplings with reduced root growth may not survive summer drought.

4.3. Combined effects of heatwaves and *P. ×multiformis* infection on alders: future perspective

The host × pathogen interaction under environmental stress conditions is particularly complex because it is context- and species-specific. In our study, we observed that increased damage and mortality was induced in both *A. lusitanica* and *A. glutinosa* by the combination of a simulated heatwave and *P. ×multiformis* infection, thus confirming the first hypothesis (as stated in the introduction). The heatwave event reduced the photosynthetic activity of *A. lusitanica* and *A. glutinosa*, which can in turn reduce carbohydrate availability and, hence, affect the tree's reaction to *P. ×multiformis* infection. Moreover, the populations with the highest susceptibility to *P. ×multiformis* infection, i.e. the southern *A. lusitanica* and *A. glutinosa* populations, were also the most affected by the combined stressors.

While the two alder species showed broad similarities, their responses depended on genetic group and population, thus confirming the second hypothesis (as stated in the introduction). Indeed, more severe damage and longer lasting changes in the performance of saplings were observed in *A. lusitanica* than in *A. glutinosa*. Contrary to our hypothesis, populations originating from sites subjected to long and extreme heatwaves (Gomes Marques et al., 2022a) showed less tolerance to the combination of stressors. Perhaps those alder populations at the southern limits of the genus distribution are already experiencing sub-optimal conditions of resilience (Gomes Marques et al., 2018) and, as a result, their defence mechanisms are already weakened prior to pathogen exposure.

While research about the effects of environmental conditions on species from the "*P. ×alni* complex" has mostly focused on minimum temperatures and frost periods (Černý and Strnadová, 2012), the effects of high temperatures on the interaction between the pathogens and their alder hosts have been largely ignored. These pathogens prefer mild river-water and air temperatures (Brasier et al., 2004; Thoirain et al., 2007; Jung et al., 2017), therefore the hot summers occurring in the southern Iberian Peninsula might be expected to limit pathogen expansion (Bjelke et al., 2016; Gomes Marques et al., 2024a). However, the occurrence of *P. ×alni* in central regions of the Iberian Peninsula, where river-water temperatures can reach 27 ºC (Soto, 2018), may indicate that the pathogen is either adapting to high temperatures or spreads and infects alders only during the cooler seasons. Nonetheless, the predicted changes in temperatures and precipitation variability, particularly in southern Europe, raises concerns as to the potential infection capability of the "*P. ×alni* complex". The results obtained in this study suggest that, in case that *P. ×multiformis* and *P. ×alni* continue to spread southwards in the Iberian Peninsula, significant dieback of *A. lusitanica* in riparian ecosystems, particularly after heatwaves, may occur. This is especially concerning because *A. lusitanica* populations are at the limit of the genus distribution and valuable genetic diversity would be loss (Gomes Marques et al., 2024a, Martín et al., 2024).

5. Conclusions

Global change-related stressors such as heatwaves and the invasion by aggressive pathogens from the "*P. ×alni* complex" are threatening alder forests throughout Europe. As alders are paramount species in riparian ecosystems and have a fundamental role in increasing the spontaneous establishment of later successional species, alder decline disturbs the natural succession of plant communities in riparian corridors. The unregulated use of alder provenances in management and restoration actions could hamper the mitigation of decline spread. Our assessment of the combined effects of a heatwave and *P. ×multiformis* infection on the performance of European alder species shows how two alder species are differently affected by single and combined effects of two stressors. This study allowed the following conclusions: i) *A. glutinosa* is more affected by heatwaves than *A. lusitanica*; ii) there is geographical variation in the response of *A. lusitanica* and *A. glutinosa* to *P. ×multiformis* infection, with generally higher susceptibility of *A. lusitanica* compared to *A. glutinosa*, and iii) increased damage and mortality was induced in *A. lusitanica* and *A. glutinosa* by the combination of a simulated heatwave and *P. ×multiformis* infection. Most importantly, this study identified European and North African alder populations that present potential for adaptation to infection by *P. ×multiformis*, when combined with a heatwave. Consequently, management of riparian vegetation in infected sites that are under regular heat stress should consider the composition of tree species, in particular with regards to their ecological requirements, the individual or combined occurrence of pathogens and environmental stress, and the analysis of those factors in the context of disease spatial distribution across the hydrographic network (Gomes Marques et al., 2022; 2024b).

CRediT authorship contribution statement

Inês Gomes Marques: Conceptualization, Methodology, Investigation, Formal analysis, and Writing – original draft, Writing – review & editing. **Cristina Vieites-Blanco:** Investigation, Writing – review & editing. **Maria João Barrento:** Investigation, Writing – review & editing. **Arthur Cupertino:** Investigation, Writing – review & editing. **José N. Semedo:** Investigation, Resources, Writing – review & editing. **Ana Paula Rodrigues:** Conceptualization, Investigation, Resources, Writing – review & editing. **Paula Scotti-Campos:** Resources, Writing – review & editing. **Thomas Jung:** Investigation, Writing – review & editing. **Alejandro Solla:** Conceptualization, Methodology, Writing – review & editing. **Teresa Soares David:** Conceptualization, Methodology, Resources, Writing – review & editing. **Patricia M. Rodríguez-González:** Conceptualization, Methodology, Writing – review & editing and Funding acquisition. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2025.123202.

Data availability

Data will be made available on request.

References

Aguayo, J., Halkett, F., Husson, C., Nagy, Z.Á., Szigethy, A., Bakonyi, J., Frey, P., Marçais, B., 2016. Genetic diversity and origins of the homoploid-type hybrid *Phytophthora* ×*alni*. Appl. Environ. Microbiol. 82 (24), 7142–7153. https://doi.org/10.1128/AEM.02221-16.

Alcaide, F., Solla, A., Cherubini, M., Mattioni, C., Cuenca, B., Camisón, Á., Martín, M.Á., 2020. Adaptive evolution of chestnut forests to the impact of ink disease in Spain. J. Syst. Evol. 58 (4), 504–506. https://doi.org/10.1111/jse.12551.

Alonso, A., Pérez, J., Monroy, S., López-Rojo, N., Basaguren, A., Bosch, J., Boyero, L., 2021. Loss of key riparian plant species impacts stream ecosystem functioning. Ecosystems 24, 1436–1449. https://doi.org/10.1007/s10021-020-00592-7.

Bjelke, U., Boberg, J., Oliva, J., Tatterrsdill, K., Mckie, B.G., 2016. Dieback of riparian alder caused by the *Phytophthora alni* complex: projected consequences for stream ecosystems. Freshw. Biol. 61 (5), 565–579. https://doi.org/10.1111/fwb.12729.

Blasini, D.E., Koepke, D.F., Bush, S.E., Allan, G.J., Gehring, C.A., Whitham, T.G., Day, T. A., Hultine, K.R., 2022. Tradeoffs between leaf cooling and hydraulic safety in a dominant arid land riparian tree species. Plant Cell Environ. 45 (6), 1664–1681. https://doi.org/10.1111/pce.14292.

Brasier, C.M., Kirk, S.A., Delcan, J., Cooke, D.E.L., Jung, T., Man In't Veld, W.A., 2004. *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycol. Res. 108 (10), 1172–1184. https://doi.org/10.1017/S0953756204001005.

Capon, S.J., Chambers, L.E., Mac Nally, R., Naiman, R.J., Davies, P., Marshall, N., Pittock, J., Reid, M., Capon, T., Douglas, M., Catford, J., Baldwin, D.S., Stewarsdon, M., Roberts, J., Parsons, M., Williams, S.E., 2013. Riparian ecosystems in the 21st century: hotspots for climate change adaptation? Ecosystems 16, 359–381. https://doi.org/10.1007/s10021-013-9656-1.

Catford, J.A., Naiman, R.J., Chambers, L.E., Roberts, J., Douglas, M., Davies, P., 2013. Predicting novel riparian ecosystems in a changing climate. Ecosystems 16, 382–400. https://doi.org/10.1007/s10021-012-9566-7.

Černý, K., Strnadová, V., 2010. *Phytophthora* alder decline: disease symptoms, causal agent and its distribution in the Czech Republic. Plant Prot. Sci. 46 (1), 12–18. https://doi.org/10.17221/43/2009-PPS.

Černý, K., Strnadová, V., 2012. Winter survival of *Phytophthora alni* subsp. *alni* in aerial tissues of black alder. J. For. Sci. 58 (7), 328–336. https://doi.org/10.17221/11/2012-JFS.

Claessens, H., Oosterbaan, A., Savill, P., Rondeux, J., 2010. A review of the characteristics of black alder (*Alnus glutinosa* (L.) Gaertn.) and their implications for silvicultural practices. For. Int. J. For. Res. 83 (2), 163–175. https://doi.org/10.1093/forestry/cpp038.

Colangelo, M., Camarero, J.J., Borghetti, M., Gentilesca, T., Oliva, J., Redondo, M.A., Ripullone, F., 2018. Drought and *Phytophthora* are associated with the decline of oak species in Southern Italy. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.01595.

Copernicus (2025). Major heatwave in southwestern Europe during the third-warmest August on record. Retrieved from ⟨https://climate.copernicus.eu/major-heatwave-southwestern-europe-during-third-warmest-august-record⟩.

Corcobado, T., Cubera, E., Juárez, E., Moreno, G., Solla, A., 2014. Drought events determine performance of *Quercus ilex* seedlings and increase their susceptibility to *Phytophthora cinnamomi*. Agric. For. Meteorol. 192-193, 1–8. https://doi.org/10.1016/j.agrformet.2014.02.007.

Corcobado, T., Miranda-Torres, J.J., Martín-García, J., Jung, T., Solla, A., 2017. Early survival of *Quercus ilex* subspecies from different populations after infections and co-infections by multiple *Phytophthora* species. Plant Pathol. 66 (5), 792–804. https://doi.org/10.1111/ppa.12627.

Corcobado, T., Cech, T.L., Brandstetter, M., Daxer, A., Hüttler, C., Kudláček, T., Horta Jung, M., Jung, T., 2020. Decline of European beech in Austria: involvement of *Phytophthora* spp. and contributing biotic and abiotic factors. Forests 11, 895. https://doi.org/10.3390/f11080895.

Corcobado, T., Cech, T.L., Daxer, A., Ďatková, H., Janoušek, J., Patra, S., Jahn, D., Hüttler, C., Milenković, I., Tomšovský, M., Horta Jung, M., Jung, T., 2023. *Phytophthora*, *Nothophytophthora* and *Halophytophthora* diversity in rivers, streams and riparian alder ecosystems of central Europe. Mycol. Prog. 22 (7), 50. https://doi.org/10.1007/s11557−023−01898−1.

Core Team, R., 2021. R: a language and environment for statistical computing. R. Found. Stat. Comput.

Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in conservation. Proc. R. Soc. B Biol. Sci. 283, 20152592. https://doi.org/10.1098/rspb.2015.2592.

Dorado, F.J., Solla, A., Alcaide, F., Martín, M.Á., 2022. Assessing heat stress tolerance in *Castanea sativa*. Forestry 95 (5), 667–677. https://doi.org/10.1093/forestry/cpac021.

Dorado, F.J., Pinto, G., Monteiro, P., Chaves, N., Alías, J.C., Rodrigo, S., Camisón, Á., Solla, A., 2023b. Heat stress and recovery effects on the physiology and biochemistry of *Castanea sativa* mill. Front. For. Glob. Change 5, 1072661. https://doi.org/10.3389/ffgc.2022.1072661.

Ferreira, V., Pazianoto, L.H.R., Solla, A., 2022. Invasive forest pathogens affect the characteristics, microbial colonisation, and decomposition of leaf litter in streams. Freshw. Biol. 67 (2), 416–429. https://doi.org/10.1111/fwb.13851.

Gibbs, J.N., Lipscombe, M.A., Peace, A.J., 1999. The impact of *Phytophthora* disease on riparian populations of common alder (*Alnus glutinosa*) in Southern Britain. Eur. J. For. Pathol. 29, 39–50. https://doi.org/10.1046/j.1439-0329.1999.00129.x.

Gomes Marques, I., Campelo, F., Rivaes, R., Albuquerque, A., Ferreira, M.T., Rodríguez-González, P.M., 2018. Tree rings reveal long-term changes in growth resilience in Southern European riparian forests. Dendrochronologia 52, 167–176. https://doi.org/10.1016/j.dendro.2018.10.009.

Gomes Marques, I., Faria, C., Conceição, S.I.R., Jansson, R., Corcobado, T., Milanovic, S., Laurent, Y., Bernez, I., Dufour, S., Mandák, B., Ennouni, H., Sahli, A., Ater, M., Dorado, F.J., Caperta, A.D., Soares, D.S., Solla, A., Rodríguez-González, P.M., 2022a. Germination and seed traits in common alder (*Alnus* spp.): the potential contribution of rear-edge populations to ecological restoration success. Restor. Ecol. 30, e13517. https://doi.org/10.1111/rec.13517.

Gomes Marques, I., Solla, A., David, T.S., Rodríguez-González, P.M., Garbelotto, M., 2022b. Response of two riparian woody plants to *Phytophthora* species and drought. For. Ecol. Manag. 518, 120281. https://doi.org/10.1016/j.foreco.2022.120281.

Gomes Marques, I., Vieites-Blanco, C., Barrento, M.J., Semedo, J.N., Rodrigues, A.P., Scotti-Campos, P., Martín, M.A., Solla, A., David, T.S., Rodríguez-González, P.M., 2024a. Phenotypic variation and genetic diversity in European *Alnus* species. For. cpae039. https://doi.org/10.1093/forestry/cpae039.

Gomes Marques, I., Vieites-Blanco, C., Rodríguez-González, P.M., Segurado, P., Marques, M., Barrento, M.J., Fernandes, M.R., Cupertino, A., Almeida, H., Biurrun, I., Corcobado, T., Costa e Silva, F., Díez, J.J., Dufour, S., Faria, C., Ferreira, M.T., Ferreira, V., Jansson, R., Machado, H., Marçais, B., Moreira, A.C., Oliva, J., Pielech, R., Rodrigues, A.P., David, T.S., Solla, A., Jung, T., 2024b. The ADnet Bayesian belief network for alder decline: integrating empirical data and expert knowledge. Sci. Total Environ. 947, 173619. https://doi.org/10.1016/j.scitotenv.2024.173619.

Guignabert, A., Jonard, M., Messier, C., André, F., Coligny, F., Doyon, F., Ponette, Q., 2024. Adaptive forest management improves stand-level resilience of temperate forests under multiple stressors. Sci. Total Environ. 948, 174168. https://doi.org/10.1016/j.scitotenv.2024.174168.

Guion, A., Turquety, S., Polcher, J., Pennel, R., Bastin, S., Arsouze, T., 2022. Droughts and heatwaves in the Western Mediterranean: impact on vegetation and wildfires using the coupled WRF-ORCHIDEE regional model (RegIPSL). Clim. Dyn. 58, 2881–2903. https://doi.org/10.1007/s00382-021-05938-y.

Horta Jung, M., Maia, C., Mora-Sala, B., Abad-Campos, P., Schena, L., Mosca, S., Carella, G., Moricca, S., Nechwatal, J., Dionísio, L., Cravador, A., Jung, T., 2025. High diversity of *Phytophthora* species in natural ecosystems and nurseries of Portugal: detrimental side effect of plant introductions from the age of discovery to modern globalization. Plant Pathol. 74, 330–362. https://doi.org/10.1111/ppa.14022.

Husson, C., Aguayo, J., Revellin, C., Frey, P., Ioos, R., Marçais, B., 2015. Evidence for homoploid speciation in *Phytophthora alni* supports taxonomic reclassification in this species complex. Fungal Genet. Biol. 77, 12–21. https://doi.org/10.1016/j.fgb.2015.02.013.

Janssen, P., Stella, J.C., Piégay, H., Räpple, B., Pont, B., Faton, J.M., Cornelissen, J.H.C., Evette, A., 2020. Divergence of riparian forest composition and functional traits from natural succession along a degraded river with multiple stressor legacies. Sci. Total Environ. 721, 137730. https://doi.org/10.1016/j.scitotenv.2020.137730.

Jung, T., 2009. Beech decline in central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. For. Pathol. 39 (2), 73–94. https://doi.org/10.1111/j.1439-0329.2008.00566.x.

Jung, T., Blaschke, M., 2004. *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread and possible management strategies. Plant Pathol. 53, 197–208. https://doi.org/10.1111/j.0032-0862.2004.00957.x.

Jung, T., Blaschke, M., 2006. Management strategies for the *Phytophthora* root and collar rot epidemic of alders in Bavaria. In: Brasier, In.C.M., Jung, T., Oßwald, W. (Eds.), Progress in Research on Phytophthora Diseases of Forest Trees. Forest Research, Farnham, Surrey, UK, pp. 61–66.

Jung, T., Orlikowski, L., Henricot, B., Abad-Campos, P., Aday, A.G., Aguín Casal, O., Bakonyi, J., Cacciola, S.O., Cech, T., Chavarriaga, D., Corcobado, T., Cravador, A., Decourcelle, T., Denton, G., Diamandis, S., Dogmus-Lehtijärvi, H.T., Franceschini, A., Ginetti, B., Glavendekić, M., Green, S., Hantula, J., Hartmann, G., Herrero, M., Ivic, D., Horta Jung, M., Lilja, A., Keca, N., Kramarets, V., Lyubenova, A., Machado, H., Magnano di San Lio, G., Mansilla Vázquez, P.J., Marçais, B., Matsiakh, I., Milenkovic, I., Moricca, S., Nagy, Z.Á., Nechwatal, J., Olsson, C., Oszako, T., Pane, A., Paplomatas, E.J., Pintos Varela, C., Prospero, S., Rial Martínez, C., Rigling, D., Robin, C., Rytkönen, A., Sánchez, M.E., Sanz Ros, A.V., Scanu, B., Schlenzig, A., Schumacher, J., Slavov, S., Solla, A., Sousa, E., Stenlid, J., Talgø, V., Tomic, Z., Tsopelas, P., Vannini, A., Vettraino, A.M., Wenneker, M., Woodward, S., Peréz-Sierra, A., 2016. Widespread *Phytophthora* infestations in european nurseries put forest, semi-natural and horticultural ecosystems at high risk

of Phytophthora diseases. For. Pathol. 46, 134–163. https://doi.org/10.1111/efp.12239.
Jung, T., Horta Jung, M., Scanu, B., Seress, D., Kovács, D.M., Maia, C., Pérez-Sierra, A., Chang, T.-T., Chandelier, A., Heungens, A., van Poucke, K., Abad-Campos, P., Léon, M., Cacciola, S.O., Bakonyi, J., 2017. Six new *Phytophthora* species from ITS clade 7a including two sexually functional heterothallic hybrid species detected in natural ecosystems in Taiwan. Persoonia 38, 100–135. https://doi.org/10.3767/003158517X693615.
Jung, T., Pérez-Sierra, A., Durán, A., Jung, M.H., Balci, Y., Scanu, B., 2018. Canker and decline diseases caused by soil- and airborne *Phytophthora* species in forests and woodlands. Persoonia 40, 182–220. https://doi.org/10.3767/persoonia.2018.40.08.
Jung, T., Milenković, I., Balci, Y., Janoušek, J., Kudláček, T., Nagy, Z.Á., Baharuddin, B., Bakonyi, J., Broders, K.D., Cacciola, S.O., Chang, T.-T., Chi, N.M., Corcobado, T., Cravador, A., Đorđević, B., Durán, A., Ferreira, M., Fu, C.-H., Garcia, L., Hieno, A., Ho, H.-H., Hong, C., Junaid, M., Kageyama, K., Kuswinanti, T., Maia, C., Májek, T., Masuya, H., Magnano di San Lio, G., Mendieta-Araica, B., Nasri, N., Oliveira, L.S.S., Pane, A., Pérez-Sierra, A., Rosmana, A., Sanfuentes von Stowasser, E., Scanu, B., Singh, R., Stanivuković, Z., Tarigan, M., Thu, P.Q., Tomić, Z., Tomšovský, M., Uematsu, S., Webber, J.F., Zeng, H.-C., Zheng, F.-C., Brasier, C.M., Horta Jung, M., 2024. Worldwide forest surveys reveal forty-three new species in *Phytophthora* major clade 2 with fundamental implications for the evolution and biogeography of the genus and global plant biosecurity. Stud. Mycol. 107, 251–388. https://doi.org/10.3114/sim.2024.107.04.
Kanoun-Boulé, M., Vasconcelos, T., Gaspar, J., Vieira, S., Dias-Ferreira, C., Husson, C., 2016. *Phytophthora alni* and *Phytophthora lacustris* associated with common alder decline in central Portugal. For. Pathol. 46 (2), 174–176. https://doi.org/10.1111/efp.12273.
Kassambara, A., Kosinki, M., Biecek, P., & Fabian, S. (2021). Drawing Survival Curves using "ggplot2". Retrieved from ⟨https://cran.r-project.org/package=survminer⟩.
Lapin, K., Bacher, S., Cech, T., Damjanić, R., Essl, F., Georges, F.I., Hoch, G., Kavcic, A., Koltay, A., Kostic, S., Oettel, J., Orlovic, S., Poljakovic-Pajnik, L., Sallmannshofer, M., Steinkellner, M., Stojnic, S., Westergen, M., Zlatkovic, M., Zolles, A., de Groot, M., 2021. Comparing environmental impacts of alien plants, insects and pathogens in protected riparian forests. NeoBiota 69, 1–28. https://doi.org/10.3897/neobiota.69.71651.
Lemm, J.U., Venohr, M., Globevnik, L., Stefanidis, K., Panagopoulos, Y., van Gils, J., Posthuma, L., Kristensen, P., Feld, C.K., Mahnkopf, J., Hering, D., Birk, S., 2021. Multiple stressors determine river ecological status at the european scale: towards an integrated understanding of river status deterioration. Glob. Change Biol. 27 (9), 1962–1975. https://doi.org/10.1111/GCB.15504.
Li, H., Johnson, C.J., Rex, J.F., Todd, M., 2024. Long-term riparian forest loss around streams, lakes, and wetlands in ecologically diverse managed and unmanaged landscapes. For. Ecol. Manag. 562, 121931. https://doi.org/10.1016/j.foreco.2024.121931.
Lorenzo, N., Díaz-Poso, A., Royé, D., 2021. Heatwave intensity on the Iberian Peninsula: future climate projections. Atmos. Res. 258, 105655. https://doi.org/10.1016/j.atmosres.2021.105655.
Mandák, B., Vít, P., Krak, K., Trávníček, P., Havrdová, A., Hadincová, V., Zákravsky, P., Jarolímová, V., Bacles, C.F.E., Douda, J., 2016. Flow cytometry, microsatellites and niche models reveal the origins and geographical structure of *Alnus glutinosa* populations in Europe. Ann. Bot. 117 (1), 107–120. https://doi.org/10.1093/aob/mcv158.
Martín, M.A., Moreno, R., Die, J.V., Cabrera, A., Castro, P., Pérez, M.D., Palomino, C., Cuenca, B., Pérez, F., Solla, A., 2024. Distribution, diversity and genetic structure of alders (*Alnus lusitanica* and *A. glutinosa*) in Spain. For. Ecol. Manag. 562, 121922. https://doi.org/10.1016/j.foreco.2024.121922.
Molina, M.O., Sánchez, E., Gutiérrez, C., 2020. Future heat waves over the Mediterranean from an Euro-CORDEX regional climate model ensemble. Sci. Rep. 10, 8801. https://doi.org/10.1038/s41598-020-65663-0.
Niinemets, Ü., 2010. Mild versus severe stress and BVOCs: thresholds, priming and consequences. Trends Plant Sci. 15 (3), 145–153. https://doi.org/10.1016/j.tplants.2009.11.008.
Nilsson, C., Brown, R.L., Jansson, R., Merritt, D.M., 2010. The role of hydrochory in structuring riparian and wetland vegetation. Biol. Rev. 85 (4), 837–858. https://doi.org/10.1111/j.1469-185X.2010.00129.x.
Pérez-García, L., García-Hernández, C., Ruiz-Fernández, J., 2025. Trends, atmospheric patterns, and spatial variability of heatwaves in an oceanic climate area of NW iberia. Land 14 (2), 310. https://doi.org/10.3390/land14020310.
Pintos-Varela, C., Rial Martínez, C., Aguín Casal, O., Mansilla Vázquez, J.P., Ares Yebra, A., 2012. First report of *Phytophthora alni* subsp. *uniformis* on black alder in Spain. Plant Dis. 96 (4). https://doi.org/10.1094/pdis-10-11-0891-pdn.
Pintos-Varela, C., Rial-Martínez, C., Aguín-Casal, O., Mansilla-Vázquez, J.P., 2017. First report of *Phytophthora* ×*multiformis* on *Alnus glutinosa* in Spain. Plant Dis. 101 (1). https://doi.org/10.1094/PDIS-08-16-1092-PDN.
Redondo, M.A., Boberg, J., Olsson, C.H.B., 2015. Winter conditions correlate with *Phytophthora alni* subspecies distribution in Southern Sweden. Phytopathology 105 (4), 1191–1197. https://doi.org/10.1094/PHYTO-01-15-0020-R.
Redondo, M.A., Stenlid, J., Oliva, J., 2019. Genetic variation explains changes in susceptibility in a naïve host against an invasive forest pathogen: the case of alder and the *Phytophthora alni* complex. Phytopathology 110 (2), 517–525. https://doi.org/10.1094/PHYTO-07-19-0272-R.
Rodríguez-González, P.M., Campelo, F., Albuquerque, A., Rivaes, R., Ferreira, T., Pereira, J.S., 2014. Sensitivity of black alder (*Alnus glutinosa* [L.] Gaertn.) growth to hydrological changes in wetland forests at the rear edge of the species distribution. Plant Ecol. 215, 233–245. https://doi.org/10.1007/s11258-013-0292-9.
Rodríguez-González, P.M., Abraham, E., Aguiar, F., Andreoli, A., Baležentienė, L., Berisha, N., Bernez, I., Bruen, M., Bruno, D., Camporeale, C., Čarni, A., Chilikova-Lubomirova, M., Corenblit, D., Ćušterevska, R., Doody, T., England, J., Evette, A., Francis, R., Garófano-Gómez, V., del Tánago, M.G., Gultekin, Y.S., Guyard, F., Hellsten, S., Hinkov, G., Jakubínský, J., Janssen, P., Jansson, R., Kail, J., Keles, E., Kelly-Quinn, M., Kidová, A., Kiss, T., Kulvik, M., La Porta, N., Laslier, M., Latella, M., Lorenz, S., Mandžukovski, D., Manolaki, P., Martinez-Fernández, V., Merritt, D., Michez, A., Milovanović, J., Okruszko, T., Papastergiadou, E., Penning, E., Pielech, R., Politti, E., Portela, A., Riis, T., Škvorc, Z., Slezák, M., Stammel, B., Stella, J., Stesevic, D., Stupar, V., Tammeorg, O., Tammeorg, P., Fosholt, T.M., Urbanič, G., Villar, M., Vogiatzakis, I., Vrchovsky, P., Yousefpour, R., Zinke, P., Zlatanov, T., Dufour, S., 2022. Bringing the margin to the focus: 10 challenges for riparian vegetation science and management. Wiley Interdiscip. Rev. Water 9 (5), e1604. https://doi.org/10.1002/WAT2.1604.
Ruosteenoja, K., Jylha, K., 2023. Average and extreme heatwaves in Europe at 0.5-2.0°C global warming levels in CMIP6 model simulations, 4281 Clim. Dyn. 61, 4359. https://doi.org/10.1007/s00382-023-06798-4.
Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.A., 1965. Sap pressure in vascular plants. Science 148 (3668), 339–346. https://doi.org/10.1126/science.148.3668.339.
Scott, P., Bader, M.K.F., Burgess, T., Hardy, G., Williams, N., 2019. Global biogeography and invasion risk of the plant pathogen genus *Phytophthora*. Environ. Sci. Policy 101, 175–182. https://doi.org/10.1016/j.envsci.2019.08.020.
Singh, A.K., Dhanapal, S., Yadav, B.S., 2020. The dynamic responses of plant physiology and metabolism during environmental stress progression. Mol. Biol. Rep. 47, 1459–1470. https://doi.org/10.1007/s11033-019-05198-4.
Solla, A., Pérez-Sierra, A., Corcobado, T., Haque, M.M., Diez, J.J., Jung, T., 2010. *Phytophthora alni* on *Alnus glutinosa* reported for the first time in Spain. Plant Pathol. 59 (4), 798. https://doi.org/10.1111/j.1365-3059.2009.02254.x.
Soto, B., 2018. Climate-induced changes in river water temperature in north iberian peninsula. Theor. Appl. Climatol. 133, 101–112. https://doi.org/10.1007/s00704-017-2183-9.
Stella, J.C., Bendix, J., 2018. Multiple stressors in riparian ecosystems. In Multiple Stressors in River Ecosystems: Status, Impacts and Prospects for the Future. Elsevier, pp. 81–110. https://doi.org/10.1016/B978-0-12-811713-2.00005-4.
Streito, J.-C., Legrand, P., Tabary, F., Jarnouen de Villartay, G., 2002. *Phytophthora* disease of alder (*Alnus glutinosa*) in France: investigations between 1995 and 1999. For. Pathol. 32, 179–191. https://doi.org/10.1046/j.1439-0329.2002.00282.x.
Therneau, T. (2021). A Package for Survival Analysis in R; Version 3.2-13. Retrieved from ⟨http://cran.r-project.org/package=survival⟩.
Therneau, T.M., Grambsch, P.M., 2000. Modeling survival data: extending the cox model. Springer, New York.
Thoirain, B., Husson, C., Marçais, B., 2007. Risk factors for the *Phytophthora*-induced decline of alder in northeastern France. Phytopathology 97, 99–105. https://doi.org/10.1094/PHYTO-97-0099.
Trimmel, H., Weihs, P., Leidinger, D., Formayer, H., Kalny, G., Melcher, A., 2018. Can riparian vegetation shade mitigate the expected rise in stream temperatures due to climate change during heat waves in a human-impacted pre-alpine river? Hydrol. Earth Syst. Sci. 22 (1), 437–461. https://doi.org/10.5194/hess-22-437-2018.
Trzewik, A., Maciorowski, R., Orlikowska, T., 2022. Pathogenicity of *Phytophthora alni* isolates obtained from symptomatic trees, soil and water against alder. Forests 13 (1), 20. https://doi.org/10.3390/f13010020.
Vít, P., Douda, J., Krak, K., Havrdová, A., Mandák, B., 2017. Two new polyploid species closely related to *Alnus glutinosa* in Europe and north Africa – an analysis based on morphometry, karyology, flow cytometry and microsatellites. Taxon 66 (3), 567–583. https://doi.org/10.12705/663.4.
Zamora-Ballesteros, C., Haque, M.M.U., Diez, J.J., Martín-Garcia, J., 2017. Pathogenicity of *Phytophthora alni* complex and *P. plurivora* in *Alnus glutinosa* seedlings. For. Pathol. 47 (2), e12299. https://doi.org/10.1111/efp.12299.