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Demographic and genetic impacts of powdery mildew in a young oak (*Quercus robur* L.) cohort

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Abstract

Key message By monitoring a field experiment over nine years, we investigated the impacts of the two main pathogen species *Erysiphe quercicola* S. Takam. and U. Braun and *Erysiphe alphitoides* (Griffon and Maubl.) U. Braun and S. Takam causing powdery mildew on a young cohort of pedunculate oak (*Quercus robur* L.), both from a demographic and genetic point of view using SNP markers. We show that survival rate is affected by mean disease severity. But while the growth-related tolerance to infection of the oak individual seems to be more determinant than resistance against infection, no equalizing effect of the disease could be detected.

Context Studies on the effects of pathogens on the survival and population dynamics of forest trees are scarce. Yet a better understanding of these interactions could prove strategic in the challenging context of climate change.

Aims Our general objective was to characterize the demographic and genetic impact of the two main pathogen species *Erysiphe quercicola* S. Takam. and U. Braun and *Erysiphe alphitoides* (Griffon and Maubl.) U. Braun and S. Takam causing powdery mildew in the early stages of a *Quercus robur* L. population.

Methods An ad hoc field design with two disease exposures, natural and protected, was surveyed over nine years. This enabled a detailed phenotypic monitoring of 1733 emerging individuals from 15 progenies, and the genotyping of 68% of them.

Results The pathogen induced high levels of seedling mortality several years after sowing, associated with reduced growth and capacity to overwinter. Fast-growing families showed the highest survival rate under both natural and protected disease exposure. Contrary to a possible trade-off hypothesis between growth and defense, family height potential was not negatively related to disease resistance across the studied oak mother trees. While supporting a deleterious effect of very low individual heterozygosity on the probability of survival, average genomic diversity was not significantly affected by mortality associated with powdery mildew. Our study also points to a few candidate genes for several fitness-related traits.

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Conclusion Overall, our results suggest that in oak natural populations, infection levels (related to resistance *sensu stricto*) may be less determinant than growth-related tolerance to infection for the fate of seedlings. However, an equalizing effect of powdery mildew on relative oak genotype performances cannot be excluded at later stages.

Keywords Pedunculate oak, Oak regeneration, Disease-diversity relationship, Trade-off

1 Introduction

Seedling establishment and early growth stages are crucial phases in the tree life cycle. Most forest tree species show a typical concave mortality curve, characterized by a very high juvenile mortality (e.g., Harcombe 1987; Peñuelas et al. 2007; Hampe and Petit 2005; Kelly 2002). Under natural conditions, both abiotic and biotic factors affect tree seedling survival, in addition to stochastic processes (e.g., Shibata et al. 2010; Petritan et al. 2014; Martini et al. 2019). Among biotic factors, many pathogens may affect seedling, and more generally juvenile survival. Seedlings and saplings are especially susceptible to pathogens due to their mostly non-woody tissues, both in roots and stems (Dominguez-Begines et al. 2020; Jankowiak et al. 2022). For example, Augspurger (1984) reported that damping-off pathogens (soilborne fungi and oomycetes) accounted for the largest proportion of seedling deaths within the first year in several species of tropical forest trees.

By their negative effect on the individual fitness of their host (by definition), pathogens can strongly affect plant population demographic patterns. On the other hand, at community level, their positive role in maintaining between and within species diversity has received increasing support (Dobson and Crawley 1994; Mordecai 2011; Bever et al. 2015). The impact of pests and pathogens on seedlings has been extensively studied as a possible mechanism promoting tree species coexistence (maintenance of spatial diversity) in species-rich tropical forests. According to the Janzen-Connell model, species-specific herbivores and pathogens provide a frequency-dependent spacing (thus diversifying) mechanism by causing increased mortality of seedlings growing at a short distance from their mother tree (Janzen 1970; Connell 1971; Summers et al. 2003). Many studies have provided support to this model both in tropical and temperate environments (Packer and Clay 2000; Bell et al. 2006; Yamazaki et al. 2009; Terborgh 2020), although the magnitude and generality of Janzen-Connell effects are still a matter of debate (Song et al. 2021). Such frequency-dependent and density-dependent processes are especially important for specialized pathogens, as in Janzen-Connell effects, or in co-evolutionary dynamics at population level (Mundt et al. 2008; Parker and Gilbert 2018; Burdon and Laine 2019). Pathogens may also affect competitive interactions between genotypes, between or within species, in a non-frequency-dependent or density-dependent manner, by causing a differential cost on the fitness of the competing plants (Mundt et al. 2008; Creissen et al. 2016). For example, foliar diseases have a debilitating effect on highly infected seedlings, which may result in a competitive disadvantage in the presence of less affected neighbors (Wiener 1990; Gilbert 2002; Power and Mitchell 2004). When the competitively dominant genotypes in the absence of disease experience a greater cost to disease than less competitive genotypes in the presence of pathogens, pathogens reduce fitness differences and therefore promote plant diversity (Mordecai 2011). This occurs when the fast-growing/strongest competitors are the most vulnerable to pathogens (Summers et al. 2003; Bever et al. 2015; Cope et al. 2021). The prevailing hypothesis in the literature to explain this negative correlation is the growth-defense trade-off concept, based on the premise that defense is costly and thus requires allocation of resources at the expense of growth (Monson et al. 2022). Growth-defense trade-offs have been reported at inter- and intra-specific levels in many groups of plants under various environments, including for tree species (Lind et al. 2013; Heckman et al. 2019; Kruger et al. 2020; Cope et al. 2021).

Studies on the impact of pathogens on plant populations have been extensively performed in an agricultural context, in relation to yield losses (e.g., Savary et al. 2019). Studies in natural systems are fewer and mainly focused on some model systems (Burdon and Thrall 2014), e.g., flax rust (Thrall et al. 2012), Arabidopsis pathogens (Creissen et al. 2016), anther smut of Silene (Bernasconi et al. 2009), Plantago powdery mildew (Laine 2004; Safdari et al. 2021). In this study, we aimed to characterize the impacts of powdery mildew on fitness-related traits and genetic diversity during the early life-stages of an oak cohort. Powdery mildew is one of the most important diseases on temperate oaks in Europe, in particular pedunculate oak, *Quercus robur* L. (Mougou et al. 2008; Lonsdale 2016). In Europe, powdery mildew was shown to be associated with a complex of cryptic (morphologically similar) species, of which *Erysiphe alphitoides* (Griffon and Maubl.) U. Braun and S. Takam is nowadays the most prevalent throughout Europe, often in a mixture with *Erysiphe quercicola* S. Takam. and U. Braun in southern Europe and with *Erysiphe hypophylla* (Nevod.) U. Braun and Cunningt. in northern Europe (Mougou et al. 2008; Desprez-Loustau et al. 2018; Gross et al. 2021). Demeter et al. (2021) suggested that powdery mildew could be one of the major factors involved in regeneration failures in pedunculate oak throughout Europe. Seedlings and young trees, with a relatively high amount of young, succulent, fast-growing tissues, are especially susceptible to disease (Pap et al. 2012; Marçais and Desprez-Loustau 2014). A significant negative effect of powdery mildew on the

height and radial growth of oak saplings was demonstrated in comparison with controls protected by fungicide applications (Pap et al. 2012; Desprez-Loustau et al. 2014). Powdery mildew, as an obligate parasite, derives nutrients produced by plant photosynthesis to its own benefit thanks to specialized feeding structures (called haustoria) that penetrate into living cells of the leaf parenchyma (Hewitt and Ayres 1976). As a consequence, several types of damage have been described: reduced net assimilation rate, reduced height and radial growth, and greater susceptibility to frost (Hajji et al. 2009; Marçais and Desprez-Loustau 2014; Pap et al. 2014; Bert et al. 2016). However, how the impacts of powdery mildew scale up at the oak population level have rarely been explored.

The spatial, demographic, and genetic structure of oak populations (especially *Q. robur* and *Quercus petraea* (Matt.) Liebl.) has nevertheless received much attention owing to the ecologic, cultural and economic importance of these species in Europe (e.g., Kremer and Petit 1993; Streiff et al. 1998; Gömöry et al. 2001; Vakkari et al. 2006; Kesić et al. 2021). Overall, oak populations exhibit a high level of genetic diversity, with no significant or little differences among cohorts of different ages in the same stand (Vranckx et al. 2014a from adults to established seedlings; Gerzabek et al. 2020 from emergence to 3-year-old seedlings). In a natural context, the various biotic and abiotic factors affecting oak seedling recruitment can vary in space and time (e.g., Crawley and long 1995; Alberto et al. 2011; Gerzabek et al. 2020). The diversity and fluctuation of selective pressures acting on different genetic components have been proposed as possible explanations for the maintenance of genetic diversity in plant populations (Ennos 1983; Delph and Kelly 2014).

Genetic changes in plant populations under pathogen pressure have been reported in a few pathosystems (Thrall et al. 2012). In this case, with alleles being selected due to their positive association with a greater resistance and/or tolerance to the disease, it may be possible to identify some of these variants using an association genetics approach. Genome-wide association studies (GWAS) are a powerful tool to link phenotypic variation with genetic polymorphisms, allowing the identification of the underlying biological mechanisms (Korte and Farlow 2013; Tibbs Cortes et al. 2021). High-quality genomic resources are now available for *Q. robur* (Lepoittevin et al. 2015; Plomion et al. 2018; Lang et al. 2021). Both genetic variation among families (Desprez-Loustau et al. 2014) and putative candidate genomic regions for oak susceptibility to powdery mildew (Bartholomé et al. 2020) were previously demonstrated in independent studies. Together with a very high genomic diversity within oak populations and a rapid decay of linkage disequilibrium among variants across the oak genome (Lang et al. 2021), these species characteristics provide an advantageous setting for performing GWAS.

Our general objective was to characterize the demographic and genetic impact of powdery mildew in the early stages of an oak population. We used an original experimental field design with two levels of powdery mildew exposure and a half-sib family genetic structure, that we analyzed with a large range of methods, in order to address the following questions:

1. How does powdery mildew affect juvenile survival? Phenotypic monitoring was carried out during the first nine years after sowing. We analyzed the effect of powdery mildew on the probability of seedling survival with various logistic regression models and used structural equation modeling (SEM) to describe the multiple relationships between the measured phenotypic variables and survival.
2. Does the impact of powdery mildew, in terms of survival, vary among oak families, i.e., does powdery mildew differentially affect the reproductive success of different oak mother trees? In particular, do the families performing best (i.e., with greatest survival and growth) in conditions of low powdery mildew pressure also perform best in conditions of high powdery mildew pressure? We hypothesized that seedling and juvenile survival is strongly affected by early growth, this trait itself being sensitive to both maternal effects such as those due to acorn weight and average family or individual genotypic effects, but that growth could be negatively correlated with resistance to the pathogen (i.e., associated with a growth-resistance trade-off).
3. As a consequence, does powdery mildew reduce fitness differences of mother trees, measured by the mean survival of their progenies, i.e., has powdery mildew an equalizing effect? If so, is the surviving population more or less diverse in terms of family composition under high powdery mildew pressure than under low disease pressure?
4. Does powdery mildew impact the genetic diversity of the oak population, not only in terms of family composition? In order to address this question, a large number of emerging seedlings were genotyped at several hundred SNPs, and heterozygosity statistics were calculated. The genetic diversity was then compared in the surviving and initial populations under the two contrasted disease pressures. Furthermore, we tested whether possible genetic changes could be associated with a difference in individual heterozygosity between dead and living seedlings, in agreement with the HFC (Heterozygosity-Fitness-Correlations) hypothesis (Vranckx et al. 2014b).
5. Finally, given our experimental setting with a known family structure, are there significant genetic associations between some loci and seedling survival or other related traits (growth, infection)?

2 Material and methods

2.1 Experimental design

The experimental design and trait definitions were thoroughly described in the phenotypic monitoring study during the first three years (Desprez-Loustau et al. 2014). Briefly, the progeny of 15 isolated (i.e., with no overlapping canopies) oak trees (*Q. robur*) was collected in 2008 in Cestas, France. *Q robur* is a diploid, monoecious, wind-pollinated tree species, with a highly outcrossing breeding system; it is a light-demanding species (especially at juvenile stages) with a moderate tolerance to drought, compared with other oaks such as *Q. petraea* (Eaton et al. 2016). We thus considered a population made of 15 open-pollinated half-sib families. The weight of each acorn was recorded for its importance on the initial seedling developmental stage (Sánchez-Montes de Oca et al. 2018). Acorns were then sown in April 2009 on a 10×10 cm grid in a field design with 9 unit plots, each containing 296 acorns, and distributed in 3 blocks (Appendix Figs. 11 and 12). The field design was located in the INRAE experimental domain in Pierroton, Cestas, France. The unit plots were randomly attributed to one of two powdery mildew exposures: either Natural or Protected, i.e., with a protection provided by myclobutanil (Dow AgroSciences, Sophia Antipolis, France), a fungicide authorized for usage in nurseries. There were six unit plots with natural exposure (corresponding to the pooled "Medium" and "High" disease treatments described in Desprez-Loustau et al. 2014, which did not differ much in seedling infection and mortality) and three with fungicide (myclobutanil, a systemic fungicide which inhibits the ergosterol biosynthesis of the fungus) application. Although fungicide application limits the level of infection, it does not completely prevent the disease on treated trees. Acorns from different families were randomly distributed among plots, with 173 acorns per family on average (minimum = 118; maximum = 285; Appendix Fig. 12).

At the end of each growing season from 2009 to 2012, survival was noted and height was measured for each individual. In following years, survival and height were assessed in early spring, a few weeks after budburst. Tree height was defined as the height of the highest living bud (i.e., with leaves). Apical bud mortality occurred in some years, i.e., the upper stem and branches did not show bud burst, resulting in a negative net annual height growth (Desprez-Loustau et al. 2014).

Powdery mildew infection was monitored and scored on several occasions each year, particularly in the first years. To characterize annual disease severity, we used data corresponding to the highest annual infection score (generally from the last assessment at the end of the season, in September or October, but sometimes earlier, depending on the year's powdery mildew epidemic dynamic). For example, in 2011, early and severe epidemics occurred, resulting in premature defoliation. Disease monitoring was therefore stopped in July. Disease severity assessments are therefore not directly comparable between years. No assessment was done in 2014 and 2015, during which infection was low. Powdery mildew infection was estimated visually by trained observers as a percentage of the total infected leaf area for each individual. From the molecular analysis of various samplings in the experimental field, in different years (206 analyses in total), it was confirmed that the most prevalent species was *E. alphitoides* (more than 90% samples). *E. quercicola* was the other species detected, albeit at a much lower prevalence.

A late frost occurred in spring 2013, resulting in leaf damage in some seedlings. The occurrence of such damage was recorded as present/absent for each seedling. The details of the variables used in the statistical analyses are described in the Appendix, Table 1.

2.2 Statistical analyses

2.2.1 Logistic and structural equation modeling for analysis at the individual level

In order to explore the impacts of powdery mildew on survival, we used two statistical approaches.

First, logistic models were used to test the effects of different variables (see Appendix, Table 2) on tree survival ("Survival (2017)", dead or living). In the first and simplest model, two explanatory variables of survival were included: "Acorn weight" and "Powdery mildew exposure" (Natural exposure or Protected by fungicide, Model 1). In order to further detail the powdery mildew effects, we replaced the exposure variable by a quantitative variable corresponding to the mean disease severity over the first 5 years ("Mean infection (2009–2013)", Model 2). Another model was also run with seedling height at the end of the first growing season in place of acorn weight ("Height in 2009", Model 3). The binary (i.e., yes/no) variable "Frost damage (2013)" was then added to Model 1 (Model 4). Finally, a full logistic model of survival included previously studied factors (powdery mildew exposure, acorn weight and frost damage), with a family effect and an interaction effect between powdery mildew exposure and family (Model 5).

Second, we used structural equation modeling (SEM) in order to estimate multiple and interrelated dependencies among measured phenotypic variables and survival. Based on a pre-defined causal model, the SEM method gives quantitative estimates of direct and indirect effects of several inter-correlated variables on a variable of interest, according to different "paths." Standardized coefficients (i.e., relationships expressed in terms of standard deviations) are produced by the analysis, enabling the comparison of the relative strengths of the effects of different explanatory variables, along the different paths. In addition, the total effect of each explanatory variable on the variable of interest is broken down into its direct and indirect

effects, according to the specified paths. In our case, the main objective was to understand how powdery mildew affects the final survival of seedlings (in 2017). The phenotypic variables used in SEM were the mean seedling infection over the first five growing seasons (2009–2013), the height at the end of 2012, and the presence of frost damage in early 2013. These variables were selected because mortality only started in 2014 thus phenotypic data needed for the model were available for almost all seedlings. We assumed that differences in infection and height in the first five years, as well as frost damage, were important determinants of subsequent survival. Moreover, susceptibility and growth expressed during the first 5 years are likely correlated with susceptibility and growth in subsequent years. Taking into account previous knowledge on powdery mildew, we constructed and tested a model with three "paths" (corresponding to potentially different mechanisms) relating disease severity ("Mean infection (2009–2013)") to survival ("Survival 2017", Fig. 1).

One path was an indirect effect of powdery mildew infection on survival through an effect on height. This was based on the assumption that infection has a direct (negative) effect on growth (as reported in Bert et al. 2016), thus on height (arrow "1") and that seedling height is expected to have a direct effect on survival (arrow "5"). The second path was an indirect effect of powdery mildew on survival through a direct effect on frost damage ("Frost damage 2013", arrow "2"), and a direct effect of frost damage on survival ("Survival (2017)", arrow "4"). This path is consistent with previous observations on the same field experiment (Desprez-Loustau et al. 2014) or made by other authors (reviewed in Marçais and Desprez-Loustau 2014) that suggested that powdery mildew infection could affect the cold hardening process of shoots at the end of the season, resulting in greater shoot mortality during winter. A third path was a direct effect of powdery mildew infection on survival (arrow "3") which may include toxic effects of the pathogen on its host or other effects not taken into account by the other paths. Finally, we included two other effects not related to powdery mildew infection: a direct effect of height on frost damage and one of acorn weight on height (arrows "6" and "7", respectively).

2.2.2 Analyses at family level

Since the same 15 families were tested under both powdery mildew exposures (Natural *versus* Protected due to fungicide use), their relative performance in both environments could be compared based on family mean phenotypic value. In particular, the proportion of individuals having survived in each family is an estimate of one component of the reproductive success of their mother tree under each environment. Moreover, the family mean of each trait could be considered as an estimate of the genetic value of the corresponding mother tree. The relationship between family growth potential (i.e., defined as the mean progeny height in a reduced disease environment provided by the protected exposure) and family disease resistance (inversely related to mean progeny infection scores under natural conditions) can then be analyzed.

In order to assess temporal changes in the relative family composition of the surviving populations under both disease exposures, we calculated a Shannon index in each plot and year as $H' = -\sum p_i * \log_2(p_i)$, with p_i the proportion of each family in the population. H' can vary between a maximum value of $\log_2(N)$, with N the number of groups, if all groups have the same frequency (here $N = 15$ and $H'max = 3.91$), and a minimum value of 0 if the population is composed of a single entity.

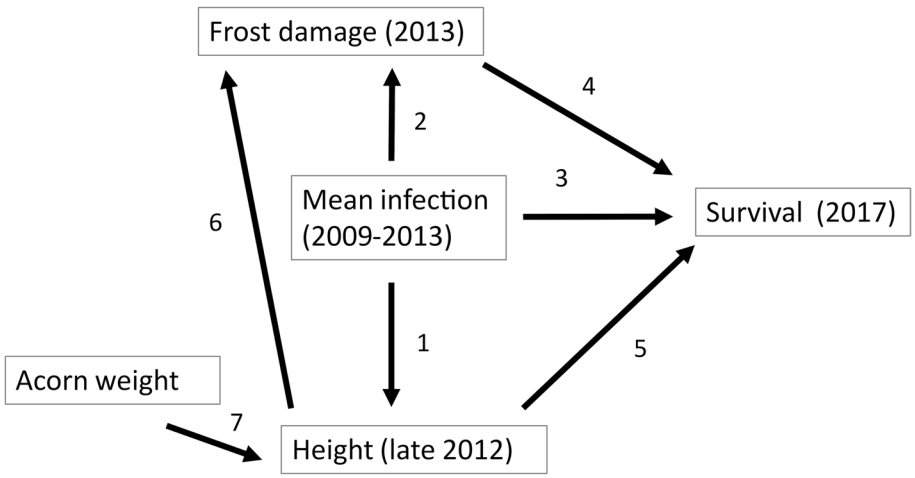

Fig. 1 Path diagram of the structural equation model (SEM) of survival. The measured phenotypic variables are drawn in a rectangular shape. A model with three paths (two indirect and one direct) relating "Mean infection (2009–2013)" to "Survival 2017" was tested. The direct effects of one variable on another are indicated by numbered black arrows (see the Sect. 2 for details)

All analyses were performed with the SAS software Version 9.4 (Copyright © 2013, SAS Institute Inc., Cary, NC, USA), in particular the Logistic, GLM and Calis (for SEM) procedures (scripts and data can be found in Barrès and Desprez-Loustau 2024).

2.3 Genetic analyses

2.3.1 Sample collection and DNA extraction

Three to six leaves were sampled on each emerged seedling at the 15-leaf stage so as not to compromise the survival of individuals. Nine 9-mm-diameter leaf discs were cut off from the dried-leaves for each individual and stored at – 80 °C in 96-well plates until DNA extraction. DNA was extracted using the Invisorb DNA plant HTS 96 kit (Invitek, Germany). We followed the manufacturer's instructions except that samples were disrupted with two 4-mm tungsten carbide beads during 2×1 min, at 30 Hz, and that the lysis step lasted 1 h (instead of 30 min) at 65 °C. A Mixer Mill MM300 (Retsch, Germany) was used to disrupt the leaf samples. DNA was eluted in a final volume of 60 µl of elution buffer. SNP genotyping required high DNA quality and quantity. Genomic DNA sample quantity was assessed using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen™) according to the manufacturer's instructions. Absence of DNA degradation was controlled on 1% agarose gel by the DNA bank platform of the Genotyping National Center, CNG (CEA-IG, Evry, France). A second genomic DNA extraction was performed for samples where concentration was lower than 45 ng.µl^{-1}, or if the total amount of DNA was lower than 1 µg. Assignment of individuals to their half-sib families was checked using nine microsatellites (Guichoux et al. 2011).

2.3.2 SNP selection and array design

The SNPs were chosen among a subset of 8078 polymorphic SNPs from the allelic resequencing of more than 800 initial targeted genic regions within the genome of 13 *Q. robur*, using a high quality SNP database from Sanger sequence data, (Lang et al. 2021; https://github.com/garniergere/Reference.Db.SNPs.Quercus). Two Perl scripts, *SNP_statistic* from the SeqQual pipeline (https://github.com/garniergere/SeqQual) and *snp2_illumina* (Lepoittevin et al. 2010) were used to compute statistics for each SNP and to design a template file compatible with the Illumina Assay Design Tool (ADT) software respectively. Within the 13 *Q. robur* sequence data, three criteria were used to further filter SNP genotypes: a minimum depth of 8 reads, a minor allele frequency higher than 7% (allowing exclusion of variants found only once for the minimum of 8 diploid individuals sequenced) and an Illumina ADT score greater than 0.4, which yielded 2447 SNPs. Moreover, in the case of two SNPs within 60 bp of each other, only one was kept, following Illumina's recommendations, the chosen SNPs fulfilling the same previous quality criteria (Appendix, Fig. 13), which yielded 1670 SNPs. Finally, two stringent filters were added: (i) SNPs with an ADT score lower than 0.6 and with only 2 sequences for one of the alleles and (ii) SNPs with a minor allele frequency lower than 10%, with no heterozygous individuals identified and with only 2 sequences for one of the alleles. These two categories of SNPs were excluded. Finally, 1536 SNPs were included in the genotyping assay.

2.3.3 SNP Genotyping

The SNP genotyping experiment was performed on the subset of seedlings with the highest quality and quantity of extracted DNA, 1185 individuals being finally retained (i.e., 71% of those that underwent DNA extraction) with 759 and 426 individuals for the Natural and Protected exposure, respectively. For each 96-well plate, we checked the quality and reproducibility of the genotyping essay with one negative control (water) and four positive controls (DNA samples of two well-known genotypes, 3P and A4, duplicated twice). We also included across plates 59 DNA samples of parents and potential parents to further test for possible Mendelian inconsistencies between parents and offsprings. A total of 30–50 ng of genomic DNA per individual was used for SNP genotyping by the INRA-EPGV group using the Illumina BeadArray platform of the Genotyping National Center, CNG (CEA-IG, Evry, France) and following the GoldenGate Assay manufacturer's protocol (Illumina Inc., San Diego, CA, USA). Three assays, over a 3-day period each, were performed to genotype 1284 samples for the 1536 SNPs. The protocol was similar to the one described by Hyten et al. (2008), except for the number of oligonucleotides involved in a single DNA reaction, which comprised 4608 custom oligonucleotides in the Oligo Pool Assay (OPA). Raw hybridization intensity data processing, clustering, and genotype calling were performed using the genotyping module of the BeadStudio/GenomeStudio package (Illumina, San Diego, CA, USA) with a GeneCall score cutoff of 0.25 to obtain valid genotypes for each individual at each SNP.

2.3.4 SNP quality criteria for genotyping reliability

After a first genotype calling of the raw data, we assessed SNP genotype quality across individuals using the methodology proposed by Illumina (Tindall et al. 2010). Briefly, 50% GC score and 10% GC score were plotted as a function of the sample call rate. Poorly performing samples were obvious outliers across many genotypes when compared to the majority. In our experiment, these outliers corresponded to samples with 50% GC score and call rate lower than (mean(50% GeneCall score) − 0.0.1) and (mean(call rate) − 0.015) respectively or to samples with 10% GeneCall score and call rate lower than (mean(10% GeneCall score) − 0.0.15) and (mean(call rate) − 0.015), respectively

(Appendix. Fig. 14). After discarding those poor-quality samples, a new genotype calling was performed on remaining individuals using the same GeneCall score cutoff. SNP quality was further determined automatically using a call frequency greater than 0.99, a 10% GeneCall score greater than 0.6, a heterozygote frequency greater than 1%, and a very low level of inconsistencies for Parent–Child or Parent-Parent–Child testing. To avoid discarding valuable SNPs or keeping poor quality SNPs, a visual inspection of all SNPs clusters was further performed after the automatic pipeline. SNP markers that displayed either compression or unexpected clustering patterns were discarded (Appendix, Figs. 15 and 16). A total of 819 SNPs were finally kept for further analyses (the detailed list is provided in the Data S2, Barrès and Desprez-Loustau 2024).

2.3.5 Multi-locus individual genetic diversity

Genetic diversity indices were computed for different groups of individuals: the 1185 individuals that were representative of the initial populations, and individuals that survived or not under both powdery mildew exposures (Natural and Protected). Observed and expected heterozygosities (H_o and H_e, sensu Nei 1973), and F_{ST} indicating differentiation between the initial and the surviving populations for both exposures were estimated (Weir and Cockerham 1984), using "adegenet" (Jombart 2008) and "Genepop" (Rousset 2008) R packages. Differentiation between populations and deviation of populations from Hardy–Weinberg equilibrium were tested using "Genepop" R package (Rousset 2008). Five heterozygosity statistics were estimated for each individual based on the 819 successfully genotyped SNPs, using the GENHET function in R (Coulon 2010): the proportion of heterozygous loci (PHt), two standardized heterozygosities based on the mean expected heterozygosity and on the mean observed heterozygosity (Hs_exp and Hs_obs, respectively), the internal relatedness (IR) and the homozygosity by locus (HL). The preliminary analyses showed that these five statistics were highly correlated (absolute Spearman's rank correlation coefficient between 0.96 and 1.00; Appendix, Fig. 17). Therefore, only PHt was kept for further analyses. Mean estimates of PHt were compared among the group of individuals that did survive *versus* the ones that did not for each exposure (Natural and Protected) using Mann–Whitney tests. Equality of variances was also assessed using Fligner-Killeen tests among the same group of individuals (survivors *versus* dead individuals, both for Natural and Protected exposures). The mean values of PHt in Natural *versus* Protected, initial and surviving, and subpopulations were compared by running a GLM model. The effect of PHt on survival was also tested by logistic regression along with four other main explanatory variables (Appendix, Table 2, Model 6).

2.3.6 GWAS analysis

The physical position of SNP markers was obtained by aligning the flanking sequence of the SNP markers (with a maximum of 100 base pairs on each side of the SNP; Data S1, Barrès and Desprez-Loustau 2024) using Blast (Johnson et al. 2008) on the *Q. robur* genome assembly available on The Darwin Tree of Life project (accession PRJEB51283). This confirmed that the 819 SNPs were scattered across the whole genome, on all chromosomes, in 426 different genic regions (see Data S2, Barrès and Desprez-Loustau 2024), with an average distance between regions of 2.11 Mb (ranging from 0.0029 to 15.3 Mb). The associations between SNP markers and the four phenotypic traits of interest ("Mean infection (2009–2013)", "Height in 2012", "Acorn weight" and "Survival (2017)") were tested using the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK, Huang et al. 2019) in GAPIT3 R package (Wang and Zhang 2021). Default parameters were used for all analyses. This method allows to account for the different relatedness levels among individuals, building from the multilocus mixed model of Segura et al. (2012) by iteratively incorporating associated markers as covariates, but with a special optimization criterion (Tibbs Cortes et al. 2021). Because of the large number of tests, a false discovery rate (FDR) analysis was used to control for false positive associations (Benjamini and Hochberg 1995), using a threshold of 0.01 for the FDR-corrected p-value. Deviation of the observed p-values from the expected values was assessed with a QQ-plot (Appendix, Figs. 18 and 19 for Natural and Protected exposures, respectively). Both powdery mildew exposures were analyzed separately. All R scripts used and related datasets are available in a Zenodo repository (Barrès and Desprez-Loustau 2024).

3 Results

3.1 Seedling and juvenile survival

Oak survival was very high during the first five years, close to or greater than 90% in both powdery mildew exposures, i.e., the protected or fungicide-treated one, and the natural or non-protected one that was submitted to natural powdery mildew infection (Fig. 2A). Survival decreased in the following 4 years. The decrease was much steeper for non-protected trees. Mortality was observed mainly at the beginning of spring when individuals failed to flush, and not during the growing seasons. The annual mortality rate was highest in 2014 in all non-protected plots but one, compared to the fungicide-treated plots. Survival at the end of the monitoring period was 49.7% on average in plots with natural powdery mildew infection, compared to 79.5% in fungicide-protected plots. As expected for an efficient fungicide treatment, disease severity was much lower in fungicide-treated plots than in non-protected plots throughout the experiment (Fig. 2B). Seedlings showed higher height

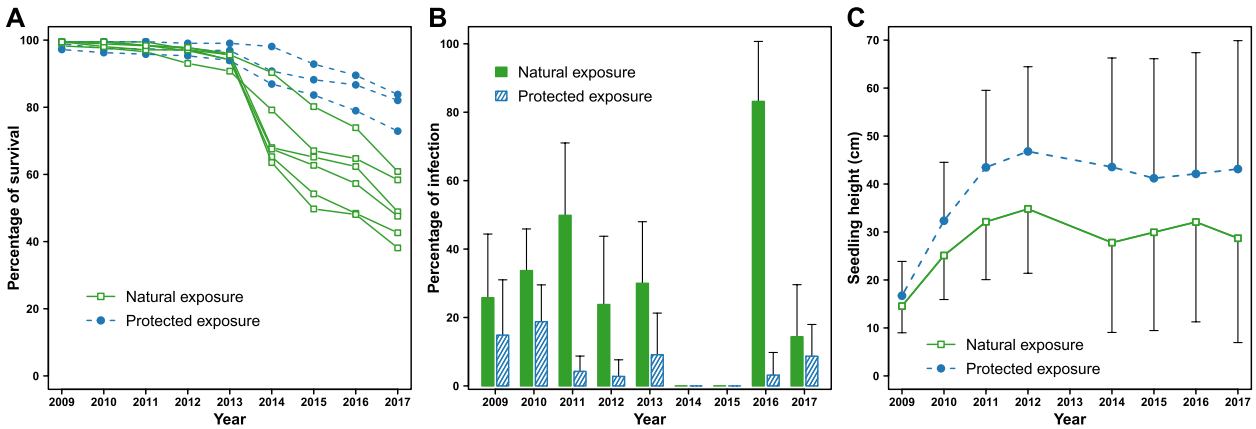

Fig. 2 (**A**) Time course of individuals' survival rate in the three protected replicates and the six natural infection plots; (**B**) mean annual infection across plots under both powdery mildew exposures; (**C**) seedling height across studied years in protected versus natural exposure plots

in the protected plots than in the naturally infected plots, as soon as the second year (Fig. 2C).

The powdery mildew exposure and the acorn weight were both significant predictors of survival in the last year of observation, i.e., 2017 (Wald Khi2 = 4.00, $P = 0.045$ and Wald Khi2 = 27.1, $P < 0.0001$, respectively; Appendix, Table 3 and Fig. 20). The natural mildew exposure was associated with a four-fold increase in the odds of mortality compared to the fungicide-protected one, which corresponds to an odds ratio of 4.0 with a 95% confidence interval (C.I.) of 3.19 to 5.06. For the acorn weight, a 23% increase of the odds of survival per additional gram was observed on average (odds ratio of 1.23 with a 95% C.I. of 1.14 to 1.32). The interaction effect between acorn weight and powdery mildew exposure was not significant in the model (Appendix, Table 3), as well as the block effect (not shown in the final analysis).

Model 2 provided a quantitative assessment of the effect of the mean percentage of infection across the first five years, on survival (Appendix, Tables 2 and 4), with an estimated odds ratio of 0.954 (95% C.I of 0.946 to 0.962). This means that each additional percentage of leaf infection is expected to reduce the odds of survival by 4.6% (Fig. 3).

Using seedling height at the end of the first growing season (Model 3, Appendix Table 2) instead of acorn weight as a predictor variable (Model 1, Appendix Table 2) had little influence on the results, with a very slight improvement of the concordance of the association between predicted probabilities and observed responses (68.7% instead of 68.1%). Thus, seedling height at the end of the first growing season was a good predictor of survival at the end of the monitoring period (i.e., 8 years later), with a strong negative impact of the "Powdery mildew exposure" at a given height (Appendix, Fig. 21). The "Height in 2009:Powdery mildew exposure" interaction was not significant in this model. When "Frost damage (2013)" was added to the logistic model (Model 4), this variable had a significant negative effect on survival (Wald Khi2 = 5.99, $P = 0.014$, odds ratio = 0.804; C.I. of 0.649 to 0.996) in addition to the effects of "Acorn weight" and the "Mean infection (2009 and 2013)" (Wald Khi2 = 17.8, $P < 0.0001$ and Wald Khi2 = 112.8, $P < 0.0001$, respectively; Appendix, Fig. 22).

The Structural Equation Model showed almost equal but opposite effects of "Height (late 2012)" and "Mean infection (2009–2013)" onto final survival, with total standardized coefficients (not displayed on Fig. 4) of 0.30 (positive) and −0,28 (negative), respectively. The total negative effect of powdery mildew infection corresponds to a direct negative effect of −0.19 (path 3) and indirect negative effects of −0.09. The most important indirect effect is −0.087 (= −0.28*0.31, according to paths 1 and 5) through "Height (late 2012)", the indirect effect through "Frost damage (2013)" being much less (0.08* −0.09 = −0.007, according to paths 2 and 4) (Fig. 4). The direct contribution of "Frost damage (2013)" on final survival (path 4) was a mild negative effect (−0.09) (Fig. 4).

3.2 Differential impact of powdery mildew among open-pollinated families

Average proportions of individuals having survived varied among families, ranging from 35 to 93% in the fungicide-protected plots and from 23 to 72% in the plots submitted to natural powdery mildew exposure (Fig. 5).

The full logistic model of survival, including previously studied factors (powdery mildew exposure, acorn weight, and frost damage), a family effect, and an interaction between powdery mildew exposure and family (Model 5) was highly significant and showed an improved concordance percent between predictions and observations of 76.7%. All previously studied factors remained significant but the family effect further explained the probability of survival (Wald Khi2 = 126.68, $P < 0.0001$; Appendix, Table 5).

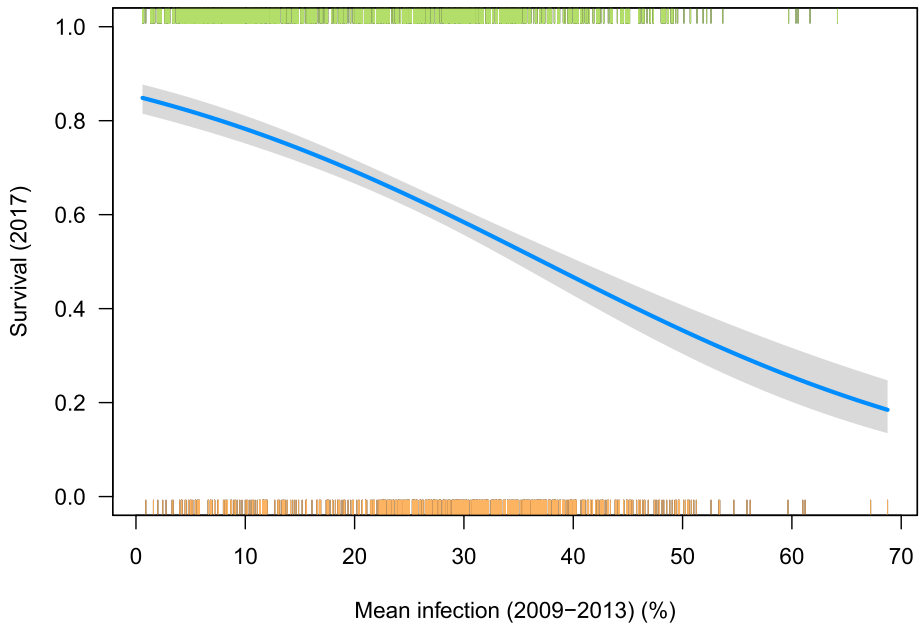

Fig. 3 Logistic model predicting juvenile survival in year 2017, based on mean infection between 2009 and 2013 (at mean acorn weight = 5.028 g). The fraction of surviving individuals is indicated on the *y*-axis. Distributions of the individual values of the variable "Mean infection (2009–2013)" were displayed as orange marks at the bottom of the figure and light-green marks at the top of the figure for dead and live trees, respectively. The gray envelop around the blue line represents the 95% confidence interval

However, the interaction between family and powdery mildew exposure was not significant. This means that overall, in this experiment, exposure to powdery mildew had a similar negative effect on the survival of all families without any strong changes in their ranking for survival (Spearman correlation = 0.86; $P = 0.0001$, and see Fig. 5).

Mean family survival (percent surviving progeny in 2017) was significantly correlated with family height (i.e., mean value over the progeny) from 2014 onwards in both disease exposures (e.g., $r = 0.71$ and 0.69 with height in 2017 in fungicide-protected and powdery mildew natural exposures, respectively). In plots under the natural exposure, the relationship between family survival and family "height potential," i.e., mean height of the same family measured in protected plots in 2017, was even stronger than with realized height ($r = 0.82$, $P = 0.0002$). No significant correlation was observed at the family level between height potential (in any year) and powdery mildew susceptibility (= mean infection observed in powdery mildew exposed plots in 2009–2013), although both variables showed a significant family effect. The height of surviving juveniles at the end of the monitoring period (in 2017) varied significantly among families: from 19.2 to 53.4 cm in fungicide-treated plots ($F = 5.01$—df = 14

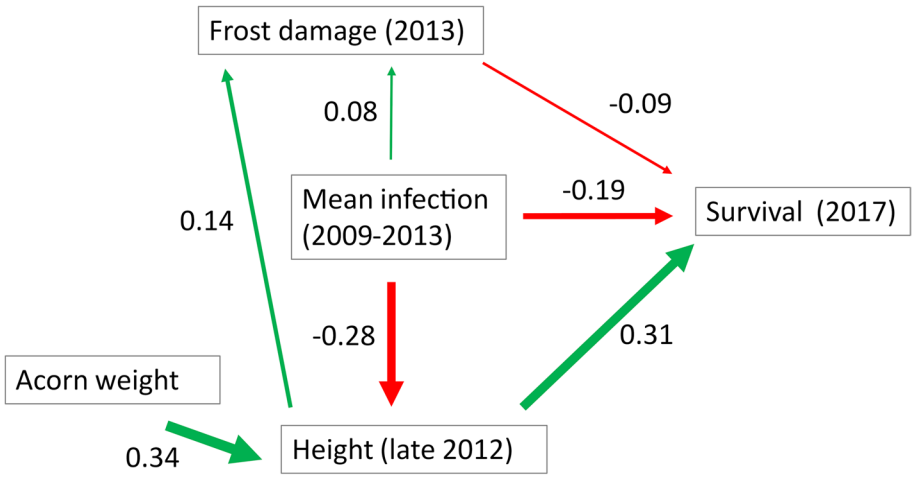

Fig. 4 Results of the structural equation model—line width is proportional to the effect value

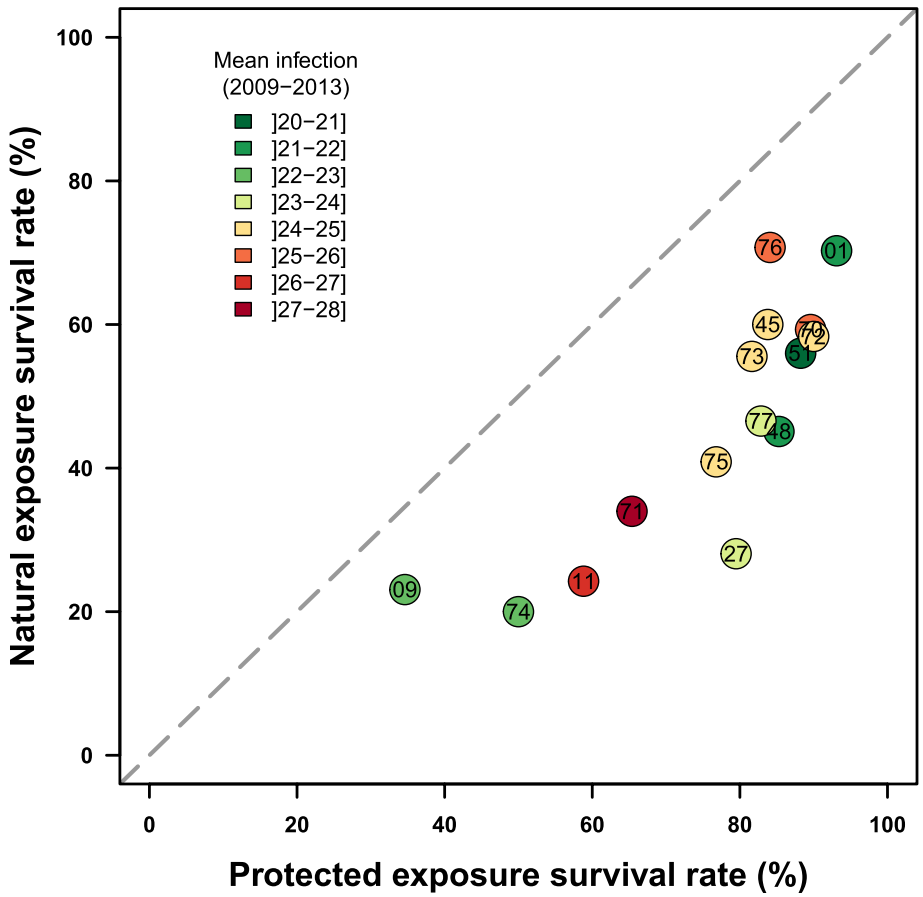

Fig. 5 Progeny survival percentages across families in Protected plots versus Natural powdery mildew-infected plots. Each dot corresponds to a family identified by its number. The mean powdery mildew infection (2009–2013) for each family in the Natural infection exposure is color-coded from dark-green = low mean powdery mildew infection (minimum = 20.9) to dark-red = high mean powdery mildew infection (maximum = 27.8)

$P < 0.0001$), and from 17.8 to 35.7 in powdery mildew plots ($F = 2.38$—df = 14 $P < 0.0032$) (Fig. 6).

The between-family coefficient of variation for final height (standard deviation/mean) was lower in the natural disease exposure than in the protected by fungicide exposure (21.5 and 26.4, with standard deviations = 5.84 and 10.54, respectively) (Fig. 6). Within-family variation (SD) was also reduced in the natural disease exposure compared to the protected exposure ($t = -2.4$; $P = 0.0306$). Mean powdery mildew infection over the first 5 years under the naturally exposed plots varied significantly among families from 28.5 to 35.7% ($F = 5.28$—df = 14 $P < 0.0001$).

The Shannon index, measuring diversity within plots in terms of family composition, remained very high in fungicide-treated plots throughout the experiment, but decreased after 2013 in all plots naturally exposed to powdery mildew, as a result of increasing differences across years in the relative numbers (percent of surviving individuals) of the different families (Fig. 7).

3.3 Multi-locus heterozygosity

Out of the 1,185 individuals included in the SNP genotyping experiment, 1143 were successfully genotyped with less than 0.08% of missing data. Observed and Expected heterozygosity did not vary between initial and surviving populations in both disease exposures, with values of Ho and He of 0.32–0.33 in all cases (Appendix, Fig. 23). Genetic differentiation between initial and surviving populations were very low and not significant (Appendix, Fig. 23). The distribution of the proportion of heterozygous loci (PHt, see methods) values estimated on all individuals was negatively skewed: while most individuals had a PHt value in the range of 0.235–0.335, very few individuals (2.54%) showed lower values (< 0.235, minimum 0.18) (Appendix, Fig. 24). The mean PHt across individuals was very similar in both disease exposures when comparing initial *versus* surviving populations: 0.283 ± 0.022 *versus* 0.284 ± 0.020 for the natural exposure; 0.283 ± 0.025 *versus* 0.285 ± 0.019 for the protected exposure (Appendix, Fig. 25 and Table 6). However, in both exposures and across all families, the individuals

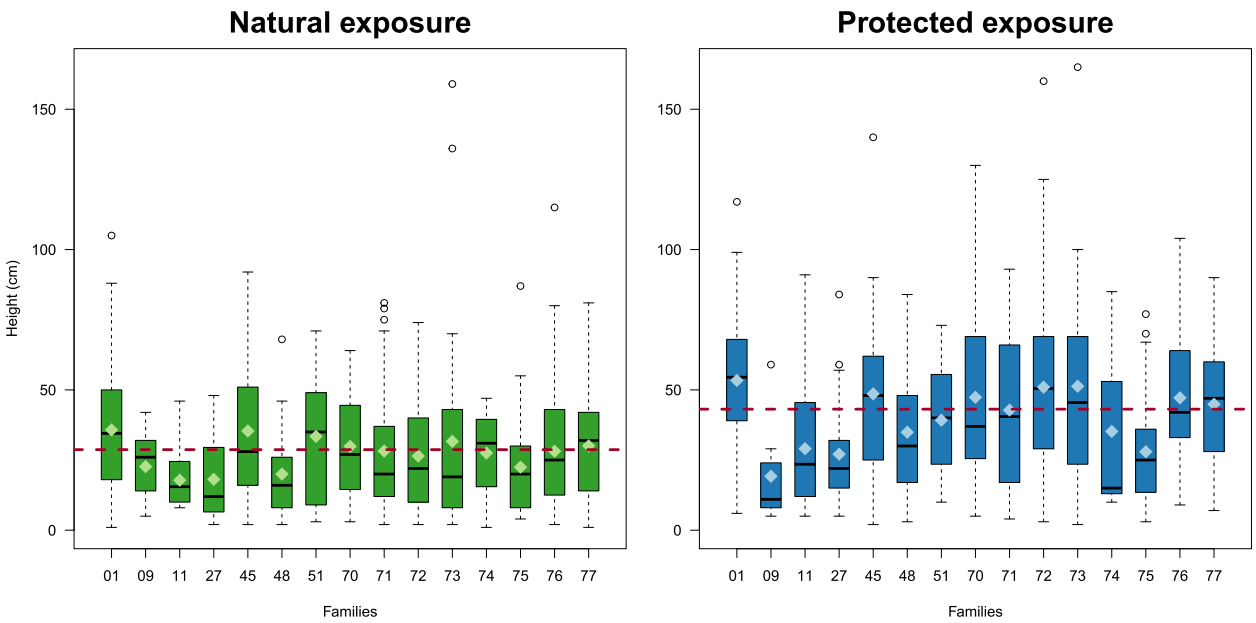

Fig. 6 Boxplot of recorded heights in 2017 in both Natural (left) and Protected (right) exposures across families. The black lines represent the median height for each family and the light-colored diamonds represent their mean height. The red dotted lines represent the overall averages of juvenile height in each exposure

with very low PHt were over-represented in dead seedlings (Fig. 8, Appendix, Fig. 26). This resulted in a decrease in the variance of the PHt between surviving and dead individuals (Khi2 = 0.567, $P = 0.45$ and Khi2 = 11.4, $P = 0.0007$ for Natural and Protected exposure, respectively).

The logistic model of survival including PHt in addition to the exposure (Natural *versus* Protected), family, acorn weight, and frost effects (Model 6, Appendix Table 2) demonstrated a significant positive effect of individual heterozygosity on survival, but the "Powdery mildew exposure:PHt" interaction was not significant. This suggests that the effect of low heterozygosity was not more deleterious in naturally exposed than in protected seedlings but simply added to the negative effect of infection.

3.4 Tests of genetic associations

Overall, 16 significant genetic associations were found between 14 loci (SNPs) and the four phenotypic traits investigated, mostly on chromosomes 2, 6, and 8 (Fig. 9). In the Natural exposure, one SNP was statistically associated with "Mean infection (2009–2013)" on chromosome 6. This SNP belonged to the same gene as another close one (distant from 336 bp) that was significantly associated to "Acorn weight" (Fig. 9). One SNP located on chromosome 8 was associated with "Survival (2017)". Two SNPs were significantly associated with "Height in 2012", one of which was also associated with "Acorn weight" (Fig. 9). In the protected exposure, a different SNP located on chromosome 2 was associated with "Survival (2017)". This SNP was also associated with "Acorn weight".

The SNP *CL7647CT8535_01-89* linked to "Mean infection (2009–2013)" is located in a gene predicted to be an ethylene response factor C3. While no significant association of this SNP was found with seedling survival in the natural exposure, the genotypic classes with lower mortality are consistent with those showing less infection and thus increased resistance (Fig. 10, top row). "Acorn weight" and "Height in 2012" did not show any association with this marker. The SNP *CL8450CT11856_03_04-703* linked to survival in the natural exposure is located in a gene coding for a putative histone H4. No differences were observed for the three other traits among genotypic classes at this locus (Fig. 10, middle row). The SNP *CL8754CT10139_03-29* significantly associated with the survival in the protected exposure is situated in a gene coding for a putative pentatricopeptide repeat-containing protein. This marker was also significantly associated with "Acorn weight" but not with "Height in 2012" or "Mean infection (2009–2013)" traits (Fig. 10, bottom row).

4 Discussion

4.1 Strong negative powdery mildew impact on juvenile survival

The negative impact of powdery mildew on oak regeneration was pointed out by many authors (Pap et al. 2012; Marçais and Desprez-Loustau 2014; Demeter et al. 2021). However, we could not find any quantitative data on pathogen-induced mortality of seedlings in forest. Our experimental approach, with a comprehensive individual monitoring of seedlings across nine years under two

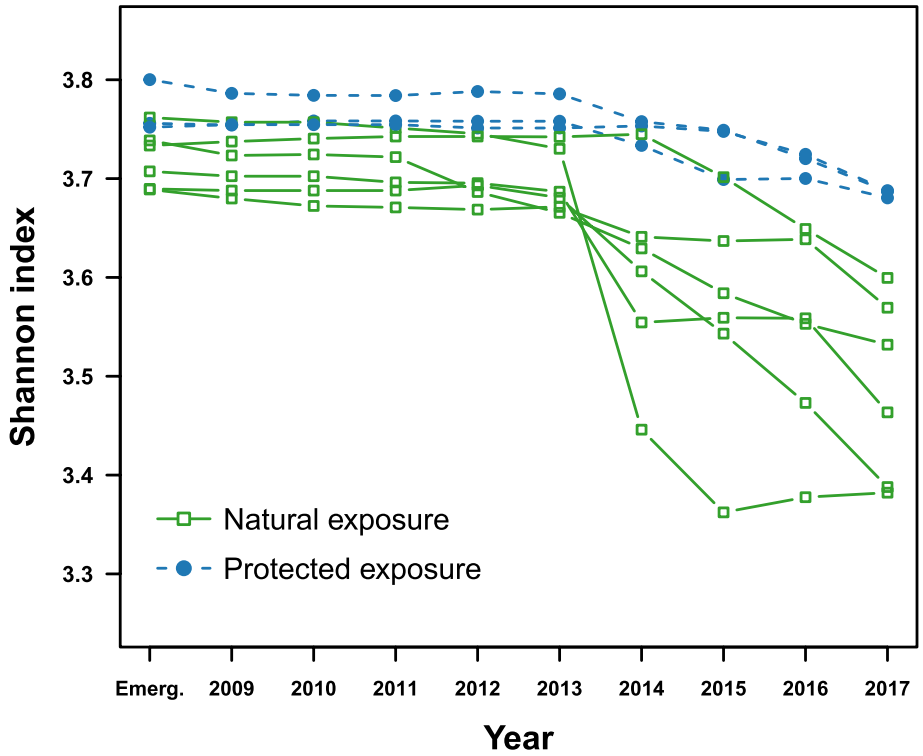

Fig. 7 Temporal changes in Shannon index, used as a family-diversity index calculated from the proportional family abundances in plots under natural or protected by fungicide powdery mildew exposure. The index was calculated for each of the nine experimental plots: six under natural powdery mildew exposure (green) and three under protected by a fungicide powdery mildew exposure (blue) every year

contrasted disease exposures, provides supporting evidence of a causal association between powdery mildew infection and mortality of seedlings, under field conditions. Mortality was indeed much higher in the plots exposed to natural infection than in plots treated with fungicide. Moreover, the probability of mortality could be quantitatively related to disease severity in the previous years.

The high mortality rates in early stages of naturally regenerated forest stands are generally attributed to an intense competition among tree seedlings (Collet and Le Moguedec 2007). However, mortality patterns in our experiment suggest that powdery mildew effects overcame competition effects. Indeed, mortality rates remained very low in the fungicide-treated plots during the monitoring period even though plants were taller and maintained at a greater density than in plots without fungicide (where seedlings progressively died), thus at a potentially stronger competition level. Maybe the competition-related mortality (self-thinning stage) (Peet and Christensen 1987; Collet and Le Moguedec 2007) will simply be delayed in our conditions, characterized by full light availability and an initial seedling density (1 acorn per 10*10 cm) which may be lower than in some spots of natural regeneration (Diaci et al. 2008; Annighöfer et al. 2015; Kuehne et al. 2020).

Infection-induced mortality has been reported for other powdery mildew diseases, such as *Podosphaera plantaginis* (Castagne) U. Braun & S. Takam on *Plantago lanceolate* L. (Laine 2004), or *Erysiphe cruciferarum* Opiz ex L. Junell on *Alliaria petiolate* (M.Bieb.) Cavara &Grande (Enright and Cipollini 2007), and rust diseases (other plant biotrophic pathogens), such as myrtle rust (Carnegie et al. 2016), *Melampsora medusae* f. sp. *deltoidae* Shain on Poplar (Newcombe et al. 1994) or *Puccinia lagenophorae* Cooke on groundsel (Paul and Ayres 1986). Mortality started only five years after sowing in our experiment, which suggests cumulative and delayed effects of infection, as expected for this kind of pathogen. As biotrophic parasites, powdery mildews strongly affect the carbon economy of their host plant, by direct consumption of carbon fixed by photosynthesis (through their haustoria) but also by forcing allocation of plant carbon to defense (Hückelhoven 2005; Oliva et al. 2014). In addition, powdery mildew infection has a direct negative effect on net carbon assimilation by photosynthesis, as was demonstrated for *E. alphitoides* (Hewitt and Ayres 1975, 1976; Hajji et al. 2009; Pap et al. 2014). The depletion of carbon by the pathogen likely explains growth reduction. Cumulative and delayed effects of powdery mildew were previously described on radial growth in young oak trees (Bert et al. 2016). Then it is reasonable to assume, although the full demonstration remains to be made (Martinez-Vilalta 2014), that severe infections, recurring in successive years, can lead to exhaustion of reserves and

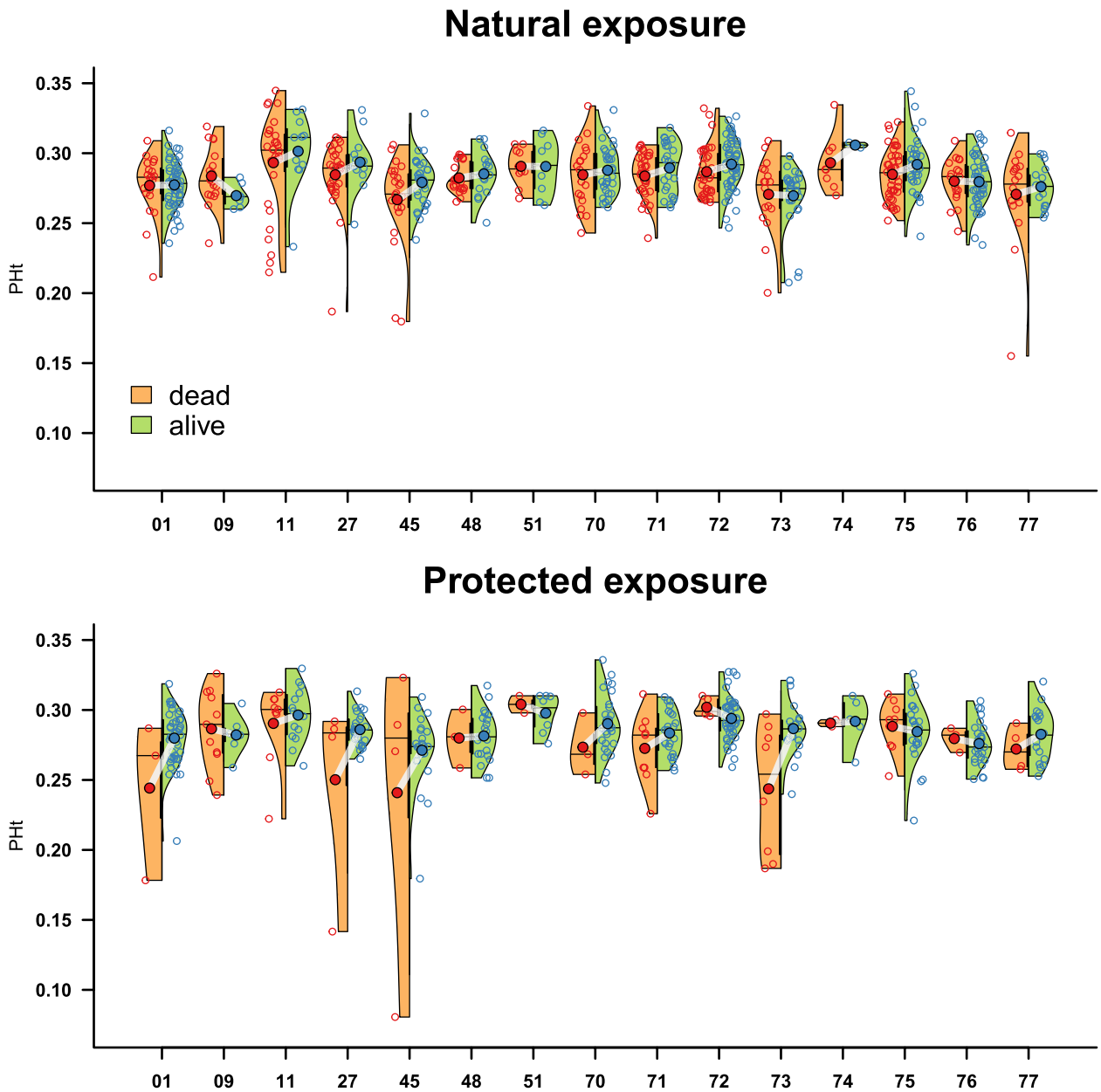

Fig. 8 Violin plots of the PHt values for Natural (above) and Protected (below) exposures across families. Red and blue empty (or full) circles represent the PHt values, across dead and alive individuals (or families), respectively. Black horizontal lines delimit the median values for dead and alive individuals across families

(See figure on next page.)

Fig. 9 Manhattan plot for the genome-wide association study results across the four phenotypic traits investigated: "Survival (2017)", "Mean infection (2009–2013)", "Height in 2012" and "Acorn weight" and across both exposures (Natural in blue, Protected in green). Each dot represents a SNP. The negative logarithm of the association *p*-value corrected for multiple tests is displayed on the vertical axis. SNPs with a significant association with a trait are indicated with yellow, orange, and red dots. The SNP markers are ordered along the genome and grouped by chromosome. 1 to 12: chromosome number; *Na*, unknown location; *Mt*, mitochondrial; *Cp*, chloroplast

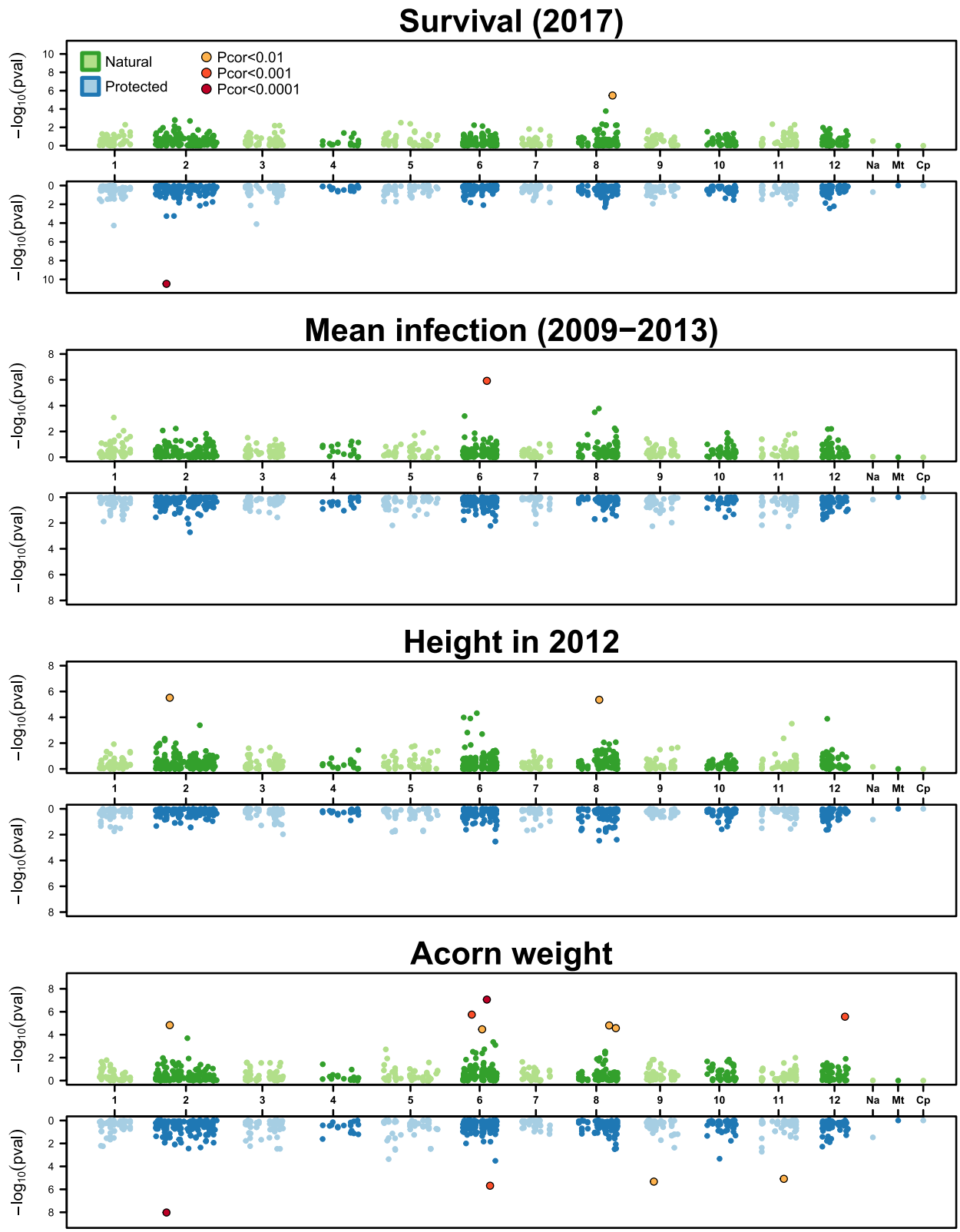

Fig. 9 (See legend on previous page.)

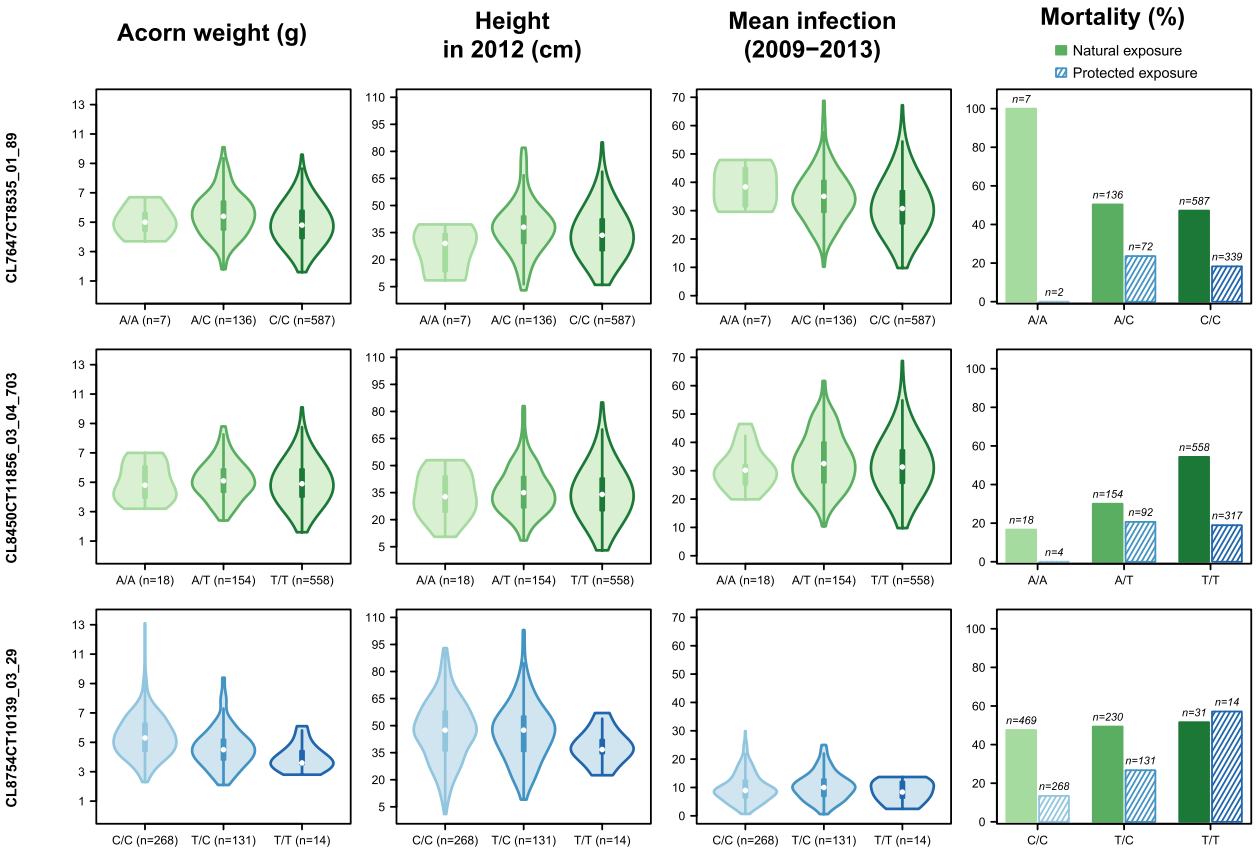

Fig. 10 Genotypic classes distribution and mean trait values across traits for SNPs *CL7647CT8535_01-89*, *CL8450CT11856_03_04-703* and *CL8754CT10139_03-29* that were significantly associated with "Mean infection (2009–2013)" under natural exposure, "Survival (2017)" under natural exposure and "Survival (2017)" under protected exposure (last column), respectively. Genotypic classes are named using the IUPAC nucleotide symbol convention: A = adenine, C = cytosine, and T = thymine

ultimately death (Oliva et al. 2014). The structural equation model that we tested is consistent with a strong direct effect of powdery mildew infection on seedling survival, twice as important as the effect mediated by decreased height. One possible mechanism could be reduced root growth in infected plants resulting from the alteration of the carbon metabolism. In the case of oak, the development of a large root system facilitates survival when aerial parts are affected or killed (Larsen and Johnson 1998). Finally, the SEM also suggested an indirect effect of powdery mildew through frost sensitivity, in agreement with previous observations of severe shoot mortality following winter in infected seedlings (Desprez-Loustau et al. 2014). The late spring frost of 2013 could have given the "coup de grâce" to already weakened seedlings. Paul and Ayres (1986) also reported that heavy infection could compromise the ability of plants to tolerate winter stress in groundsel infected by rust. Jarosz and Burdon (1992), with flax rust, noted that the main effect of disease was to reduce survivorship during the winter following infection which could lead to pathogen-generated cycles in the host population size (Susi et al. 2017).

4.2 Differential impact of powdery mildew across families

The observations of progenies from identified mother trees allowed to assess fitness components that were either linked to growth, disease resistance, or progeny survival of the mother trees that were originally sampled. Powdery mildew infection was quite high across years in our experiment and families showed different levels of disease severity (% leaf area infected). The progenies of all 15 mother trees were negatively affected in their survival under higher disease pressure. However, the ranking of the mean family values for survival was very similar under both powdery mildew exposures.

In particular, progenies from most competitive mother trees (i.e., with the greatest progeny survival under low disease pressure, with fungicide) were also among those with the greatest survival under high powdery mildew pressure. The hypothesis of changes in mother tree survival ranking related to powdery mildew exposure was therefore not supported. This hypothesis was based on the assumption of a negative relationship between resistance to powdery mildew and growth (considered as an

important component of fitness at the seedling stage). In our experiment, seedling survival was indeed strongly related to growth, as estimated by seedling height. However, the results suggested there was no apparent trade-off between growth and disease resistance: the families with the greatest mean height in the fungicide-treated plots (i.e., representing the growth trait) did not have the highest infection rates when exposed to disease (representing the defense trait). In both disease environments, the families with the highest survival rates were also those with the greatest height growth potential (assessed under fungicide treatment).

Some features of our experiment may explain such absence of a negative correlation between growth and disease resistance. First, only 15 mother trees were sampled on a small spatial scale in one local population, thus limiting the phenotypic variation that could be observed. It has to be noted however that genomic diversity was high, in the range of values, or greater than He values reported with the same type of markers (i.e., SNPs) for various *Q. robur* stands in Europe (Blanc-Jolivet et al. 2020, Degen et al. 2021a, b). Trade-offs between traits (including disease resistance) may be easier to detect when considering phenotypic variation across a wider spatial range, in relation to differing selection pressures and evolutionary strategies of populations (McKnown et al. 2014; Heckman et al. 2019). In addition, the expression of growth-defense trade-offs can be context dependent (Karasov et al. 2017), and it is usually stronger in environments where the level of resource acquisition is limited by shade or abiotic stresses (van Noordwijk and de Jong 1986; de Jong 1995).

Finally, the detection of growth-defense trade-offs may depend on the choice of the traits that are assessed. In our study, with height as the growth variable, we considered trade-off in a very general sense, encompassing processes linked to both acquisition and allocation of resources (Laskowski et al. 2021).

Our results could also suggest that tolerance was more important than resistance to explain mean survival differences of progenies across mother oak trees under high disease pressure. Plants use different lines of defense to respond to pathogens, including resistance sensu stricto and tolerance (Desprez-Loustau et al. 2016; Pagan and Garcia-Arenal 2020). Resistance sensu stricto relates to mechanisms that limit pathogen development within the plant. The variable corresponding to percent leaf area infected in our monitoring can be considered as inversely related to resistance. By contrast, tolerance relates to mechanisms with no direct effect on the pathogen but that limit the negative impact of infection on plant fitness (Jeger et al. 2006).

We previously demonstrated that mechanisms such as increased polycyclism and compensatory growth are likely involved in the response of oak seedlings to powdery mildew (Desprez-Loustau et al. 2014). In our experiment, mean survival across families in plots naturally exposed to powdery mildew, i.e., one component of tree fitness, was not correlated with mean leaf area infection but significantly correlated with mean progeny height in protected plots (height potential). We can thus hypothesize that such height potential is related to tolerance mechanisms. Parker and Gilbert (2018) also reported that the impact of infection (tolerance) on 17 closely related clover species was less negative on fast-growing species, possibly because of their better ability to acquire resources in the environment and compensate for damage (de Jong 1995). Moreover, some authors suggested that tolerance could be especially advantageous for long-lived species (Roy et al. 2000). Although tolerance has been far less investigated than resistance, there is ample evidence of its occurrence in crops and wild plants (Pagan and Garcia-Arenal 2020), and more in-depth molecular approaches would probably be needed for unraveling the cascades of metabolic pathways behind tolerance and its correlation with growth-related traits (Monson et al. 2021, 2022).

4.3 Increased powdery mildew pressure had no equalizing effect on the relative contribution of mother trees to the next generation

The surviving population was slightly less diverse in terms of family composition under high than under low powdery mildew pressure. This pattern may be explained by previous results showing that the advantage of the fast-growing families over the slow-growing families in terms of survival was not suppressed under pathogen exposure. On the contrary, fast-growth might be associated with higher tolerance to infection damage. Parker and Gilbert (2018) obtained very similar patterns with closely related species instead of families and suggested that greater tolerance in fast-growing species may limit rather than promote species coexistence. Similarly, at the infra-specific level, Mundt et al. (2008) showed that the absolute fitness advantage of the more competitive genotype in absence of disease increased in the presence of disease.

However, differences in mean final height among families were reduced under disease pressure, as well as the variance within families. During the time frame of our experiment, this did not affect the family ranking for survival between disease exposures but maybe in a longer term, or with greater disease pressure, powdery mildew could have an equalizing effect on family and individual performances (survival).

4.4 Impact of powdery mildew on genetic diversity

We did not observe any changes in genetic diversity (estimated by the mean H_e across a large number of SNPs) or any

significant genetic differentiation (estimated by F_{ST}) between the initial and the surviving oak populations in both exposures, with mortality rates as high as 60% in some naturally infected plots at the end of the monitoring period. Few studies have addressed the impact of disease on the genetic diversity of natural plant populations contrary to animal populations (McKnight et al. 2017). One of the best studied wild plant pathosystem in a long time series is the interaction between *Linum marginale* A.Cunn. and *Melampsora lini*, which showed temporal patterns of genetic change in the host and pathogen at local scale, consistent with coevolutionary dynamics (Thrall et al. 2012). However, these changes were associated with a gene-for-gene model, i.e., the existence of matching genes for resistance in the host and virulence in the pathogen (Flor 1971), which is not characterized for the oak-powdery mildew interaction. One possible explanation to the lack of any observed change in our study is that the powdery mildew pressure was not strong enough to have significantly affected the very high background diversity revealed across the oak genome (Plomion et al. 2018). Also, few if any powdery mildew infection causative or linked variants are probably included in our SNP sets due to a very low background linkage disequilibrium across the genome (Lang et al. 2021). A similar argument can be invoked for variants involved in the genetic determinism of traits linked to growth and survival that are most probably multigenic, which means that one single allelic variant explains a very small part of the total variation (see below in Sect. 4.5).

We also did not observe a significant increase of the mean individual heterozygosity in surviving populations compared to the initial ones or between surviving populations between exposures. An increase of this index could have been linked to an overall Heterozygosity-fitness correlation (HFC), associated with a deleterious effect of inbreeding (Slate et al. 2004). We can notice, however, that the seedlings with the lowest heterozygosity values (PHt inferior to 0.235) were often dead at the end of the experiment, leading to a slight increase of this statistic at the end of the experiment for most families in both exposures. Although a slight effect of multilocus heterozygosity at SSRs was detected on growth traits in *Q. robur* (Vranckx et al. 2014b, less than 5% of total variation explained), and in other species with different reproductive strategies (Cole et al. 2016 in Aspen; Stilwell et al. 2003 in *Castanea dentata* Sudw.), a better resistance or tolerance to pathogen infection for heterozygotes has not been established for plants, contrary to animals (e.g., Budischak et al. 2023). Specifically, oaks have generally large populations with a low inbreeding of their seedlings (Gerzabek et al. 2020), a genetic context for which HFC is not expected (Slate et al. 2004). HFC may also be more easily detected in stressful environments (Mopper et al. 1991, and see references above). In addition, opposite correlations between heterozygosity, growth, and mechanisms of resistance against pathogens were reported in Cole et al. (2016). Antagonistic effects of heterozygosity on different biological traits could occur in oak seedlings, thus masking possible HFC. As explained above (Sect. 4.2), tolerance to oak powdery mildew could be a composite process involving different physiological functions that would lead to moderate optimizing selection effects on genotypes and thus the maintenance of a mean diversity level (Walsh and Lynch 2018).

4.5 Few genetic associations identified

Our design with well-characterized phenotypic traits and individuals genotyped at several hundred SNPs was suitable to test for genetic associations. Overall, in conservative statistical testing conditions, very few significant associations were observed: zero, one or two per trait for survival, mean powdery mildew infection and height, and up to seven for acorn weight, across both exposures. We did detect one SNP significantly associated with seedling survival in the natural exposure, which differed from the one detected in the disease-protected exposure. This SNP was not associated with powdery mildew infection, and the allele with a beneficial effect on survival was at a very low frequency. If true, this beneficial effect might be counterbalanced by pleiotropic effects, and the advantage detected in our study might be related to particular conditions. Indeed, this SNP was located in a gene coding for a putative histone H4, a type of protein involved in the structure of chromatin that has been linked to survival strategy against drought in plants (Kim et al. 2017). The second SNP associated with survival but under-protected exposure was located in a gene coding for a family of proteins involved in organelle biogenesis (O'Toole et al. 2008).

Genetic association studies of resistance to plant diseases are common in pathosystems of agronomic interest (e.g., maize (Zhao et al. 2022, reviewed in Shrestha et al. 2019), wheat (Du et al. 2021) or soya bean (Wen et al. 2018)). They are far less common in natural pathosystems. The genetic architecture of resistance (sensu lato) to powdery mildew in oak species is still poorly understood, although QTLs have been detected and some candidate genes have been suggested (Bartholomé et al. 2020). The present study points to another possible candidate gene identified by the GWAS approach, a gene coding for a putative ethylene response factor C3. Interestingly, a similar type of protein has been linked to pathogen resistance in cotton (Guo et al. 2016). Despite a measurable effect on tree mortality from oak powdery mildew in our experimental setup, the only SNP associated with susceptibility to powdery mildew was not associated with the survival trait. This is probably related to the complex and partly indirect nature of the effect of powdery mildew on oak mortality, as evidenced by the SEM model results.

Appendix

Fig. 11 Pictures of the experimental setup located in the INRAE experimental domain in Pierroton, Cestas, France (44.747, – 0.767, WGS84). The pictures were taken in September 2011. The top two photos show the two fences installed to protect the experimental setup from herbivores. The bottom left and middle photos show the C3 unit plot with natural exposure to powdery mildew from the front and profile, respectively. The bottom right photo shows pedunculate oak seedlings in more detail

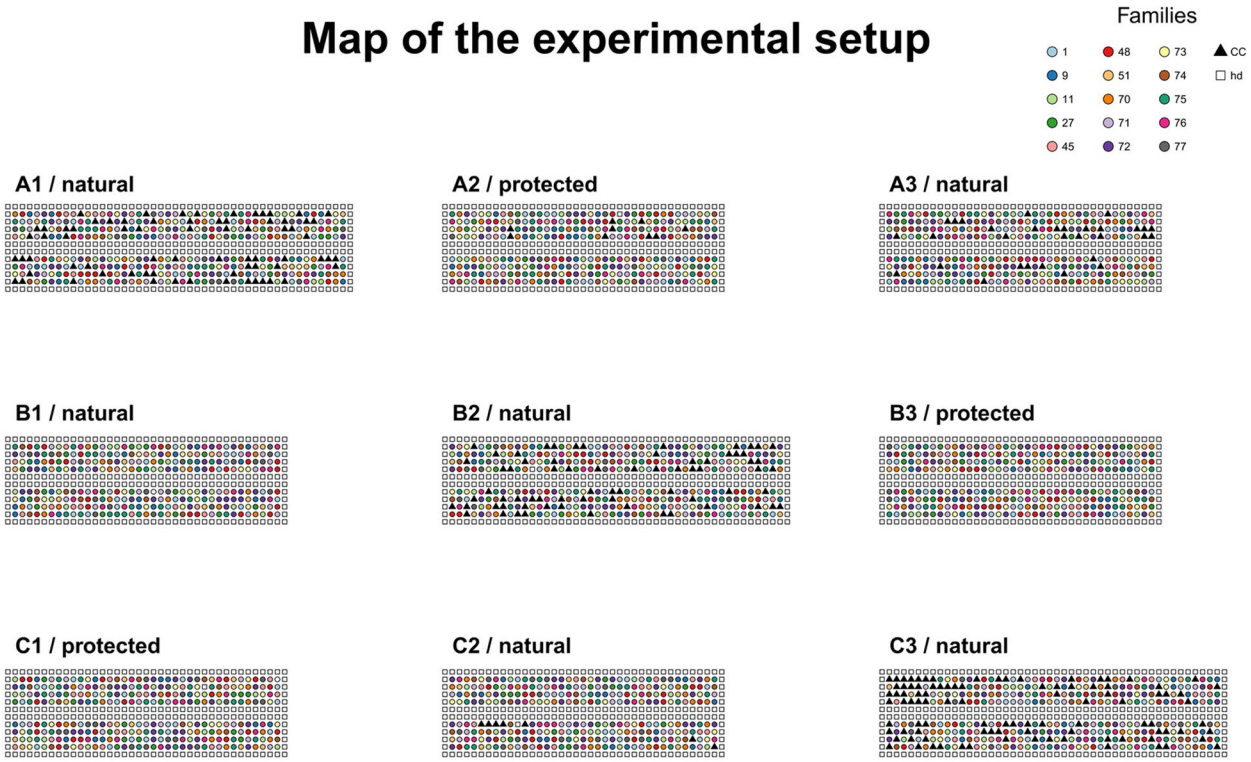

Fig. 12 Map of the implantation of the 15 progenies within the experimental setup organized in two powdery mildew exposures (Natural and Protected with a fungicide) and three blocks A, B, and C. Individuals marked "hd" and "CC" belong to borders and a control crossing, respectively. Therefore, they were not included in this study

Table 1 Definition of analyzed phenotypic variables at individual tree level

Name of the variable (*units of measurement*)	**Assessment**
Acorn weight (*g*)	Acorn weight was measured just before sowing.
Survival (2017) (*Y/N*)	Measured once a year, at the end of the growing season in 2009–2010–2011–2012, the in spring (after budburst, in May) in 2014–2015–2016–2017. Survival in spring 2017 was used as "final" survival, which was the analyzed variable.
Height (*cm*)	Height of the highest living bud, measured at survival assessment each year. Height at the end of the first growing season (in 2009) was used for a generalized linear model ("Height in 2009", Model 3), and Height at the end of 2012 was used in the SEM and GWAS analyses ("Height (late 2012)").
Mean infection (2009–2013) (*%*)	The percentage of total leaf area infected by powdery mildew was assessed at tree level by trained observers at several times in each year (except no assessment in 2014–2015). Only one value per year was then retained, corresponding to the highest value (not necessarily the latest since defoliation of highly infected leaves may occur). The analyzed variable in the different models (including SEM) was the mean disease severity in the first five years, calculated as the mean of the 2009–2010–2011–2012–2013 values of percentage of total leaf area infected.
Frost damage (2013) (*Y/N*)	Presence or absence of significant damage (necrosed leaves) in May 2013, when a major frost episode took place.

Table 2 Logistic models of individual "Survival (2017)" (= dependent variable). The explaining variables are either quantitative variables (Q), or categorical variables (C). Block effects were tested in a first step but then removed since non-significant

Model ID	**Explaining variables**	**Type of variables**
Model 1	1. Acorn weight	Q
	2. Powdery mildew exposure	C (Natural or Protected)
Model 2	1. Acorn weight	Q
	2. Mean infection (2009–2013)	Q
Model 3	1. Height in 2009	Q
	2. Powdery mildew exposure	C (Natural or Protected)
Model 4	1. Acorn weight	Q
	2. Powdery mildew exposure	C (Natural or Protected)
	3. Frost damage	C
Model 5	1. Acorn weight	Q
	2. Powdery mildew exposure	C (Natural or Protected)
	3. Frost damage (2013)	C
	4. Family	C (15 levels)
	5. Family:Powdery mildew exposure	C
Model 6	1. Acorn weight	Q
	2. Powdery mildew exposure	C (Natural or Protected)
	3. Frost damage (2013)	C
	4. Family	C (15 levels)
	5. PHt	Q

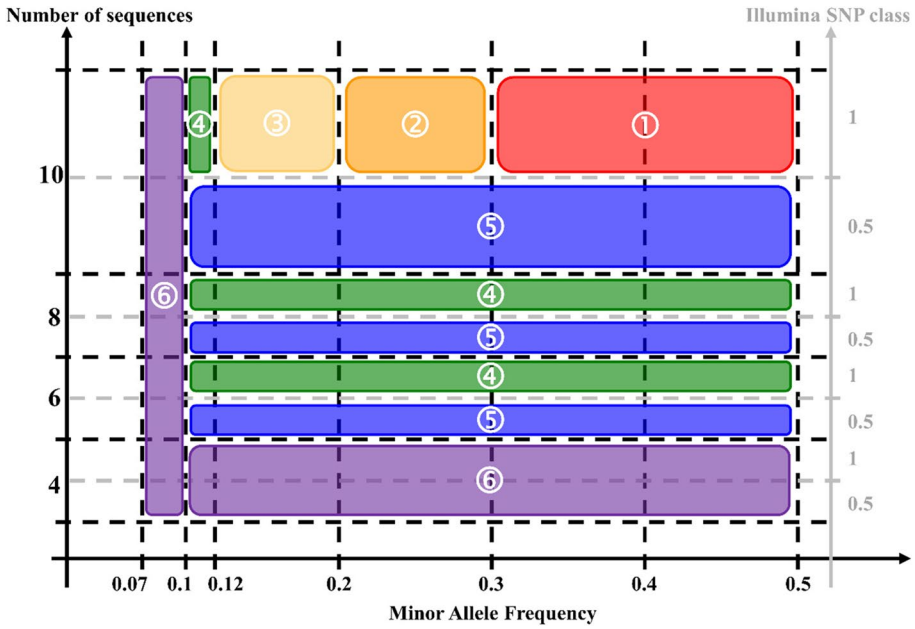

Fig. 13 Definition of the different *a priori* quality categories of SNP for genotyping, based on three criteria. The first criterion defines two "Illumina SNP class" values: 0.5 if the score obtained by using the Assay Design Tool (ADT) software is between 0.4 and 0.6, and 1 for an ADT score greater than 0.6. The second criterion is the depth of sequence alignment used to define the SNP. The third criterion is the 'Minor Allele Frequency' which is the frequency of the rarest allele. SNP quality categories range from 1 (best quality, in red) to 6 (worst quality, in purple)

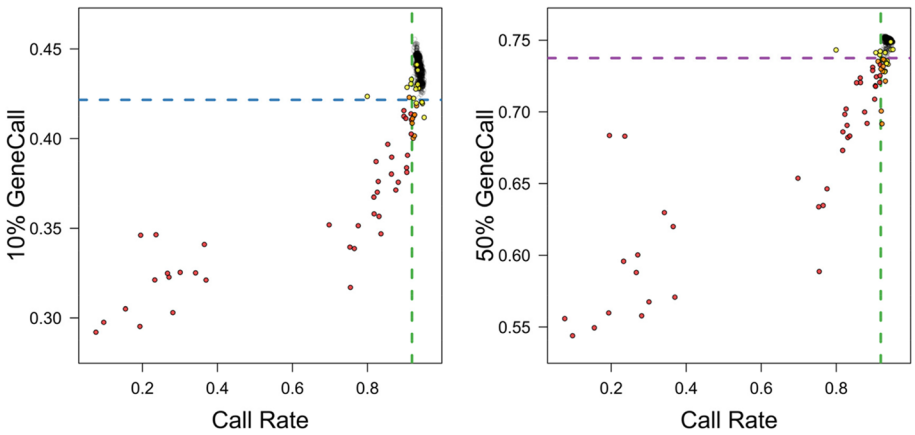

Fig. 14 Scatter plots of 10% GeneCall and 50% GeneCall scores against Call Rates of samples. Each point represents an individual. The GeneCall scores decrease in value the further a sample data point is from the center of the cluster. Poorly performing samples are outliers from the majority of samples. Thresholds were fixed as follows: 10% GeneCall mean minus 0.015 (dotted blue line), 50% GeneCall mean minus 0.01 (dotted purple line) and Call Rate mean minus 0.015 (dotted green line). Individuals behind one of these thresholds were excluded from the study (yellow, orange, and red points for individuals performing poorly on one, two, or three criteria)

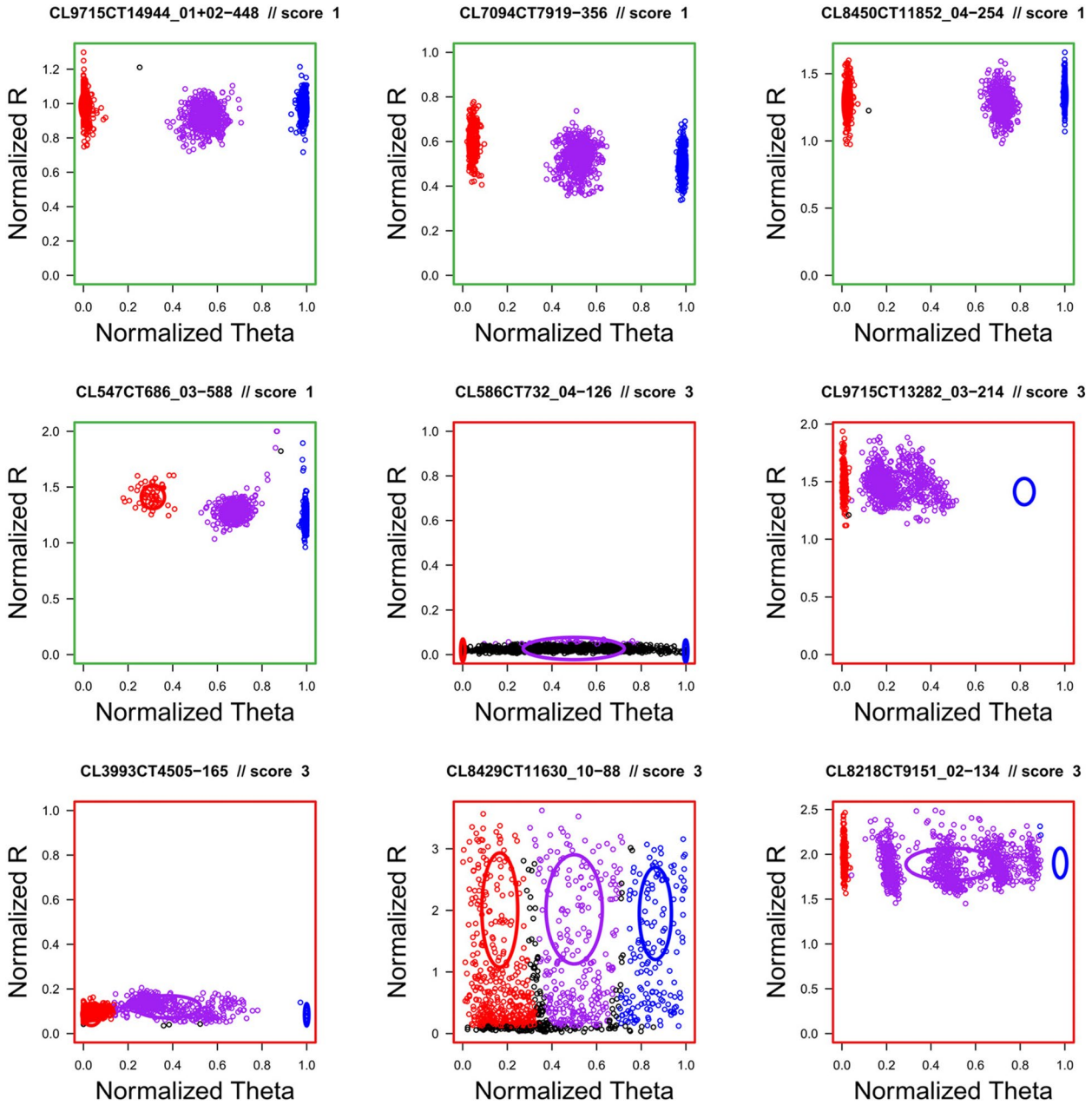

Fig. 15 Examples of good (green boxes, score 1) and bad (red boxes, score 3) quality SNP markers. In each panel, a scatterplot of genotype of individuals for a SNP marker in normalized polar coordinates is represented. The *x*-axis is the normalized Theta (i.e., the normalized fraction of bases that are genotyped as the variant allele) and the *y*-axis is the normalized intensity of fluorescence of the probe. AA genotype, AB genotype, and BB genotype are depicted in red, purple, and blue, respectively. Panels from left to right and top to bottom: a good quality SNP marker; a good quality SNP marker with a slightly low fluorescence intensity; a good quality SNP marker with a slightly high fluorescence intensity; a good quality SNP marker with moderately compressed but separated clusters; a bad quality SNP marker with a very low calling rate because of low fluorescence intensity; a bad quality SNP marker with very compressed and overlapping clusters; a bad quality SNP marker with too low fluorescence intensity; a bad quality SNP marker with random fluorescence intensity and no cluster; a bad quality SNP marker with multiple and overlapping clusters

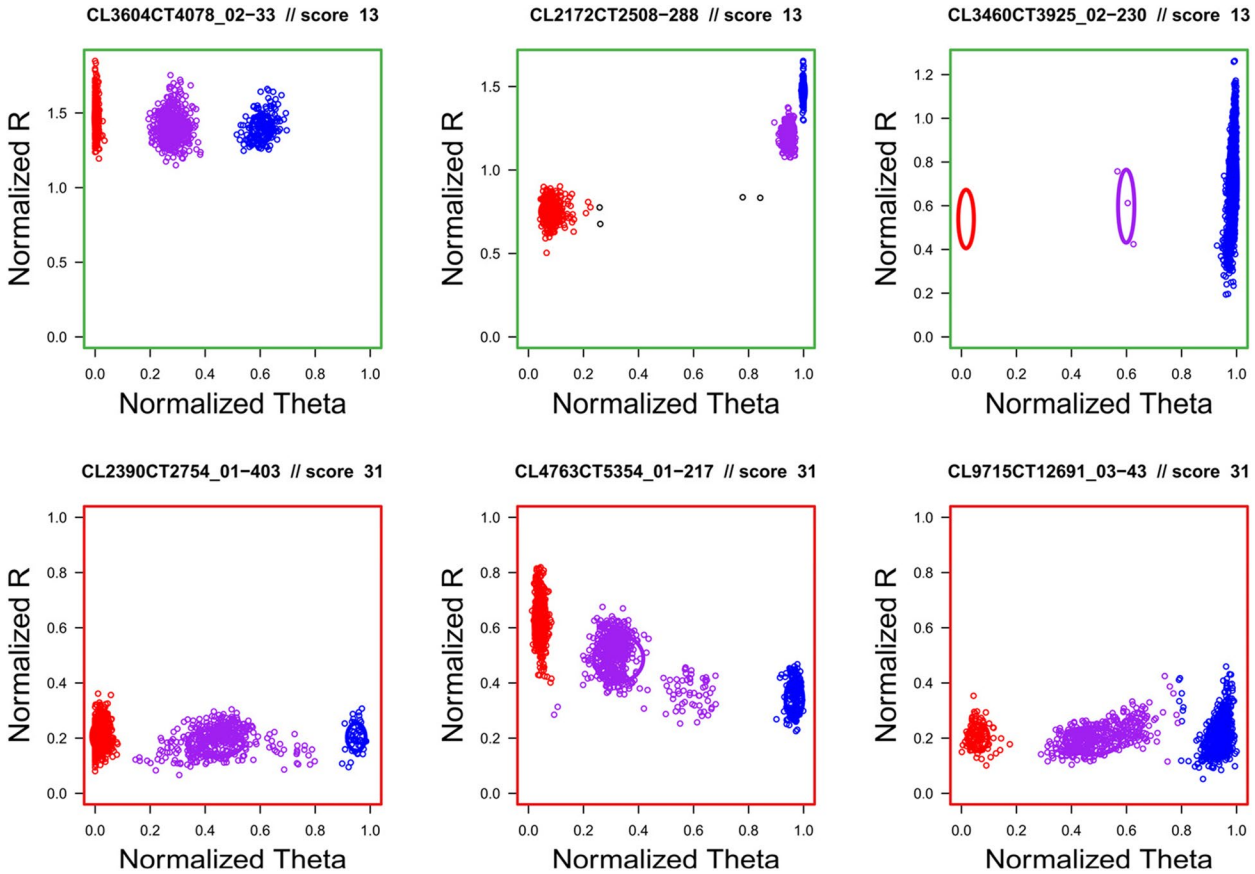

Fig. 16 Examples of SNP markers included (first line with green boxes, score 13) or excluded (second line with red boxes, score 31) after visual inspection. In each panel, a scatterplot of the genotype of individuals for a SNP marker in normalized polar coordinates is represented. The *x*-axis is the normalized Theta (i.e., the normalized fraction of bases that are genotyped as the variant allele) and the y-axis is the normalized intensity of fluorescence of the probe. AA genotype, AB genotype, and BB genotype are depicted in red, purple, and blue, respectively. Panels from left to right and top to bottom: one SNP marker included despite cluster compression as clusters remain clearly distinct from each other; a SNP marker included despite a shift of the heterozygote cluster to the right but still distinct from the BB cluster; a SNP marker included despite a very low frequency of one of the allele but with clusters at expected position; a SNP marker excluded because of a low fluorescence intensity; a SNP marker excluded because of more than three distinct clusters; a SNP marker excluded because of ambiguity of the calling of some individuals and low fluorescence intensity

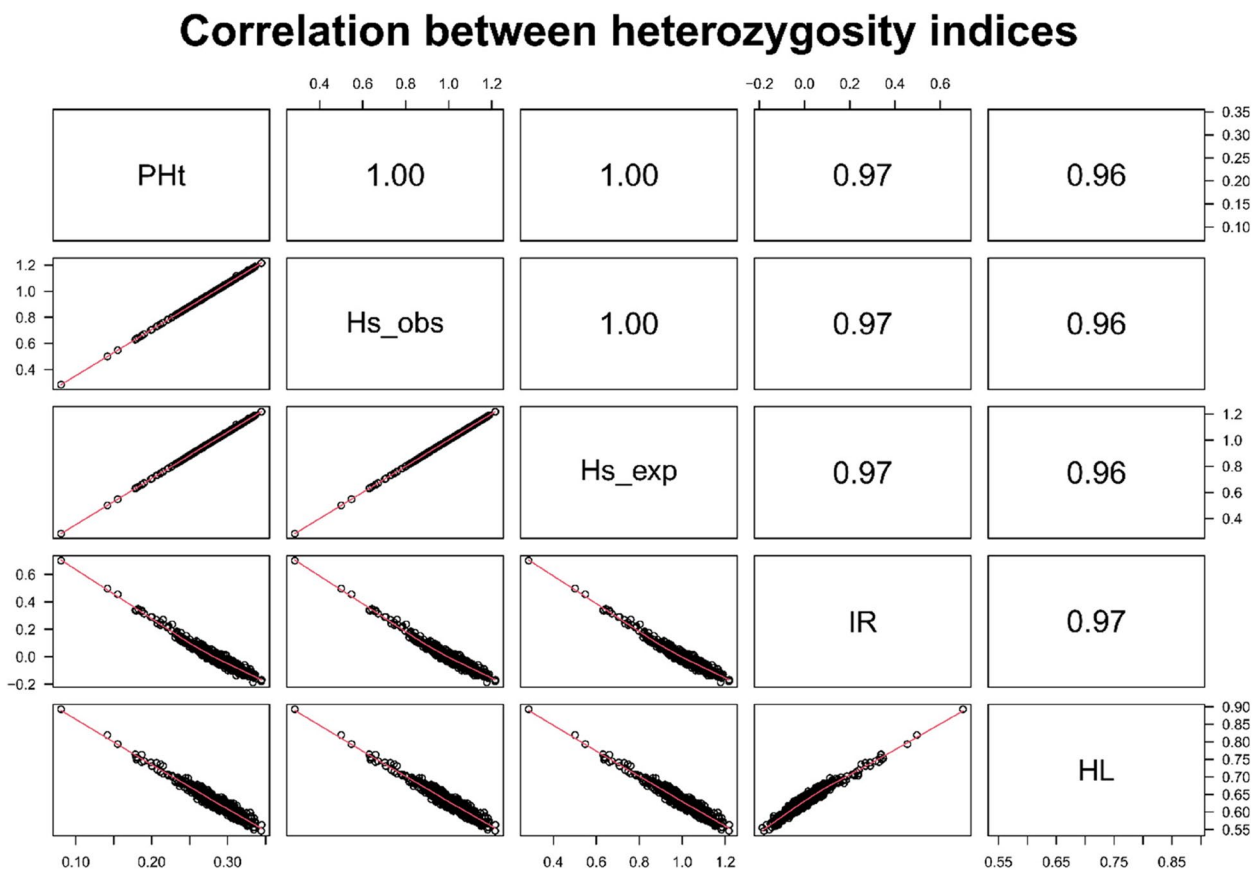

Fig. 17 Matrix of scatterplot and correlation between the five indices of heterozygosity. The lower left panel shows the scatterplot and the upper right panel indicates the absolute Spearman's rank correlation coefficient. PHt, proportion of heterozygous loci; Hs_obs, standardized heterozygosity based on the mean observed heterozygosity; Hs_exp, standardized heterozygosity based on the mean expected heterozygosity; IR, internal relatedness; HL, homozygosity by locus

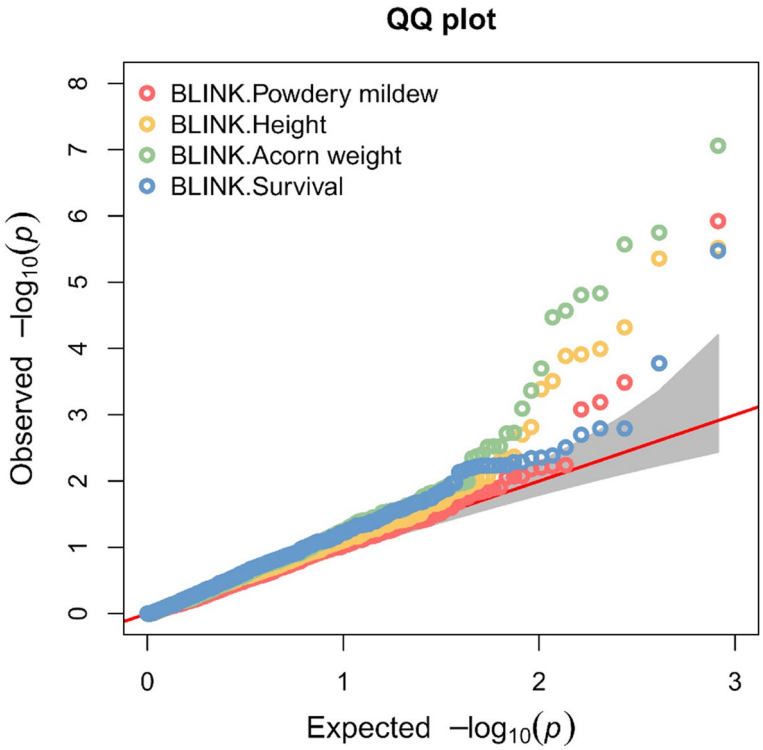

Fig. 18 QQ-plot of the GWAS analysis on the individuals of the Natural exposure for the four phenotypic traits investigated. The probability distribution Observed for each of the 819 tested SNPs is plotted against the probability distribution Expected under the null model. Outliers points are SNPs with observed *P* values more significant than expected under the null hypothesis for the investigated trait

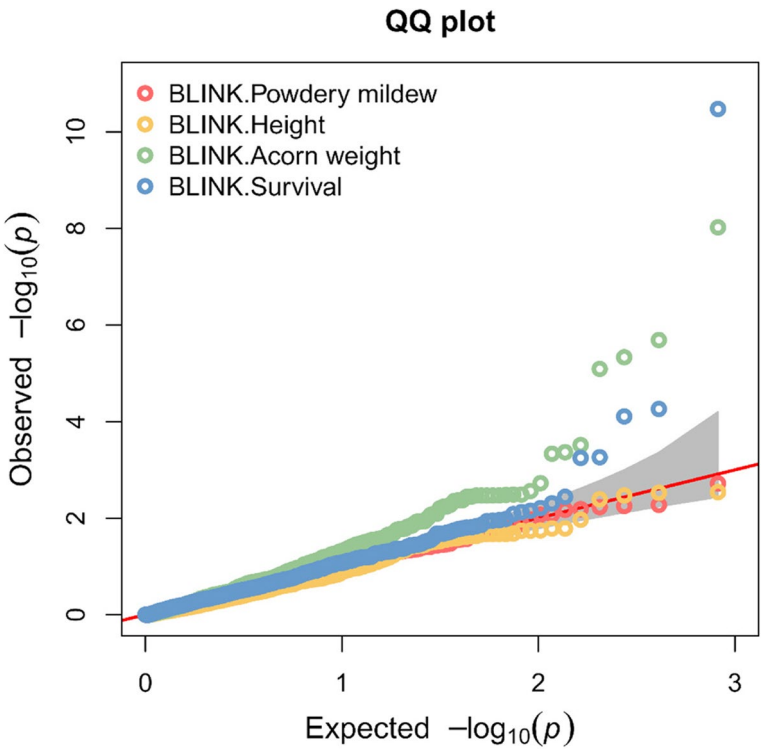

Fig. 19 QQ-plot of the GWAS analysis on the individuals of the Protected exposure for the four phenotypic traits investigated. The probability distribution Observed for each of the 819 tested SNPs is plotted against the probability distribution Expected under the null model. Outliers points are SNPs with observed *P* values more significant than expected under the null hypothesis for the investigated trait

Table 3 Results of the logistic analysis of seedling survival in 2017 with "Acorn weight" and "Powdery mildew exposure" as explanatory variables (Model 1 in Appendix Table 2). The parameters were estimated by maximum likelihood

Dependent: Survival (2017)					
Variables	**Degree of freedom**	**Estimate**	**Standard Error**	**Wald Khi-2**	***P***
(Intercept)	1	−0.4778	0.2225	4.6117	0.0318
Powdery mildew exposure	1	0.4450	0.2225	4.0003	0.0455
Acorn weight	1	0.2349	0.0451	27.1465	< .0001
Acorn weight:Powdery mildew exposure	1	0.0520	0.0451	1.3314	0.2486

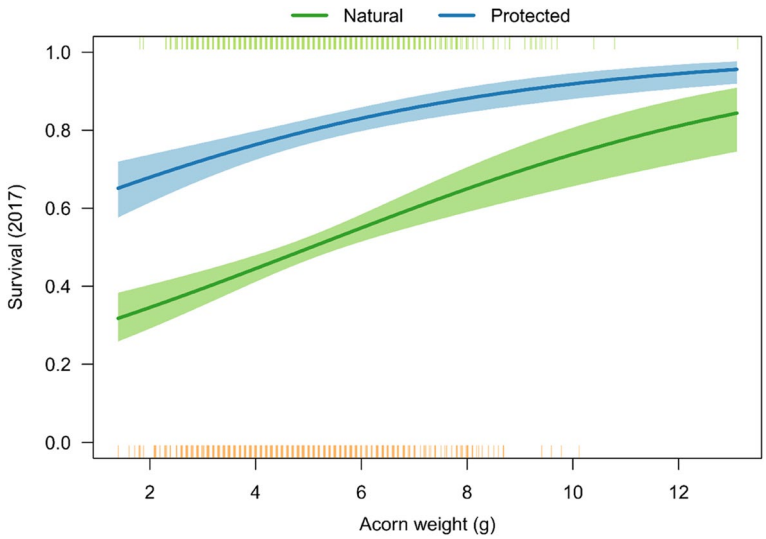

Fig. 20 Predicted effects of "Acorn weight" and "Powdery mildew exposure" on juvenile survival. The fraction of surviving juveniles is indicated on the *y*-axis. Envelops around the lines represent the 95% confidence interval

Table 4 Results of the logistic analysis of seedling survival in 2017 with "Acorn weight" and "Mean infection (2009–2013)" as explanatory variables (Model 2 in Appendix Table 2)

Testing global null hypothesis: BETA = 0			
Test	**Khi-2**	**Degree of freedom**	***P***
Likelihood ratio	150.9885	2	< .0001
Score	145.3046	2	< .0001
Wald	133.8057	2	< .0001

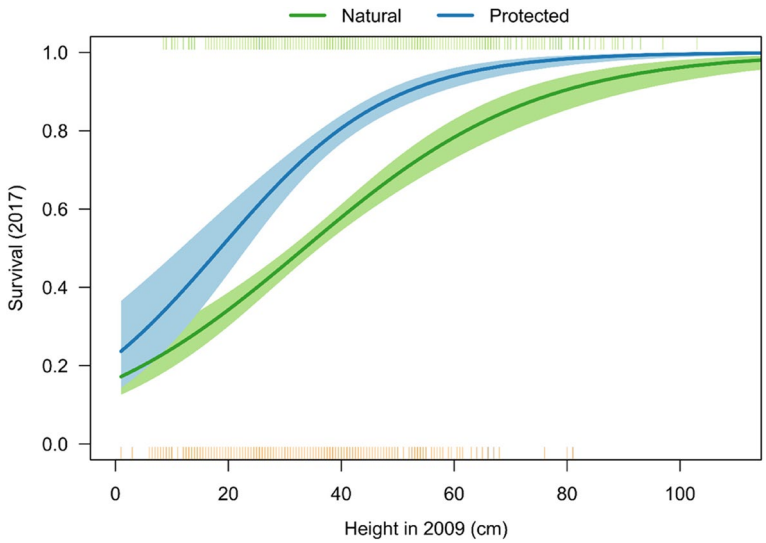

Fig. 21 Predicted effects of height at the end of the first growing season ("Height in 2009") and "Powdery mildew exposure" on seedling survival in 2017 (Model 3 in Appendix Table 2). The fraction of surviving juveniles is indicated on the *y*-axis. Envelops around the lines represent the 95% confidence interval

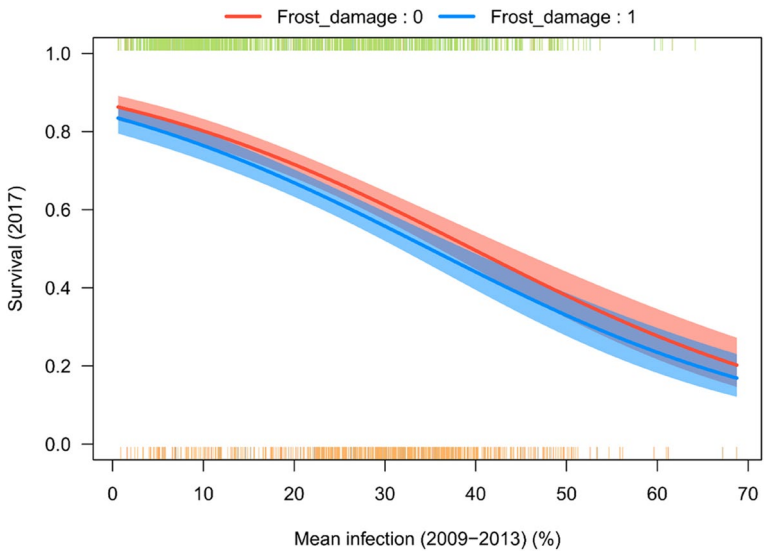

Fig. 22 Predicted effects of "Powdery mildew exposure" and "Frost damage" in 2013 on seedling survival in 2017 (at a given Height in 2009 = 15.64 cm). The fraction of surviving juveniles is indicated on the *y*-axis. Envelops around the lines represent the 95% confidence interval

Table 5 Results of the full logistic model of seedling survival in 2017 with block effects, "Powdery mildew exposure", "Acorn weight", "Frost damage (2013)", "Family" and "Family:Powdery mildew exposure" as explanatory variables (Model 5 in Appendix Table 2)

Dependent: Survival (2017); type III effect analysis			
Variables	**Degree of freedom**	**Wald Khi-2**	***P***
Block	2	4.4526	0.1079
Powdery mildew exposure	1	112.8216	< .0001
Family	14	126.6772	< .0001
Powdery mildew exposure:Family	14	8.6690	0.8517
Acorn weight	1	17.8265	< .0001
Frost damage (2013)	1	5.9965	0.0143

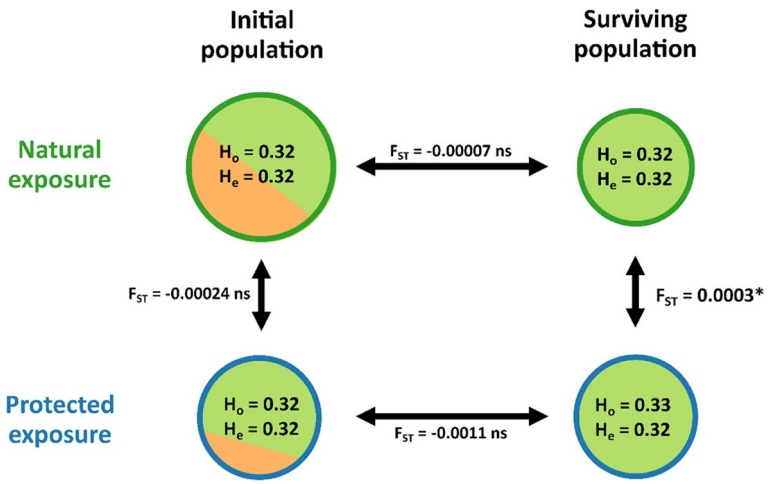

Fig. 23 Observed and expected heterozygosity for initial and surviving populations for both disease exposures. Differentiation between populations was assessed by F_{ST} and is indicated near the arrows. ns: not significant; *: $P < 0.05$

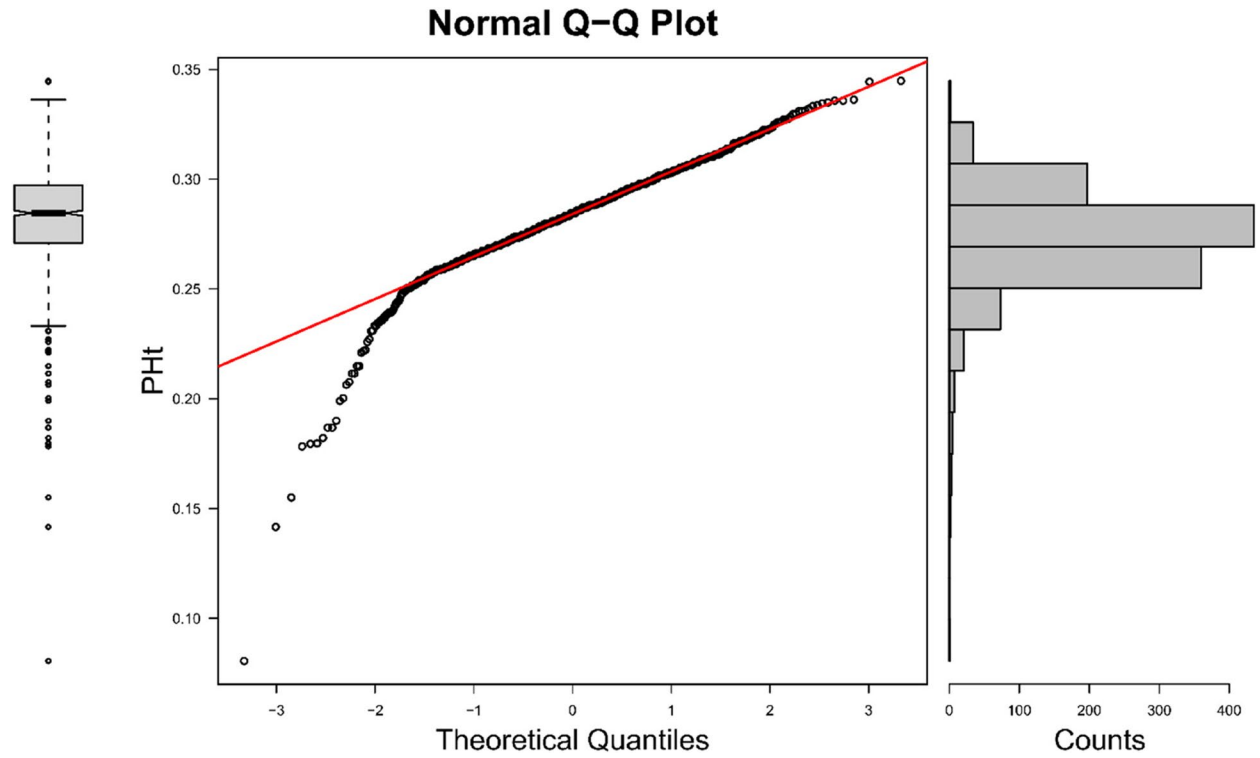

Fig. 24 Distribution and probability curve of individual PHt values

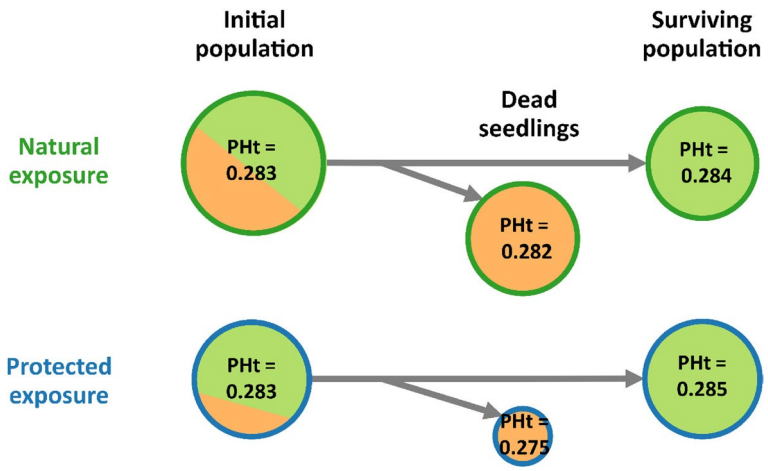

Fig. 25 Evolution of the PHt for the two disease exposures between the initial oak population and the final populations separated between dead and surviving seedlings

Table 6 Results of the GLM model for PHt with block, "Powdery mildew exposure", "Family", and "Family:Powdery mildew exposure" as explanatory variables. The model was applied to the initial population (first table) and the surviving population (second table)

Variables	Degree of freedom	Sum of square (type III)	Quadratic mean	*F* value	*P*
Initial population/dependent: PHt					
Block	2	0.0003560	0.0001780	0.36	0.6949
Powdery mildew exposure	1	0.0000577	0.0000577	0.12	0.7312
Family	14	0.0532739	0.0038053	7.78	< .0001
Powdery mildew exposure:Family	14	0.0053766	0.000384	0.79	0.6858
Surviving population/dependent: PHt					
Block	2	0.0004039	0.0002019	0.59	0.5568
Powdery mildew exposure	1	0.0000053	0.0000053	0.02	0.9015
Family	14	0.0313628	0.0022402	6.50	< .0001
Powdery mildew exposure:Family	14	0.0080025	0.0005716	1.66	0.0598

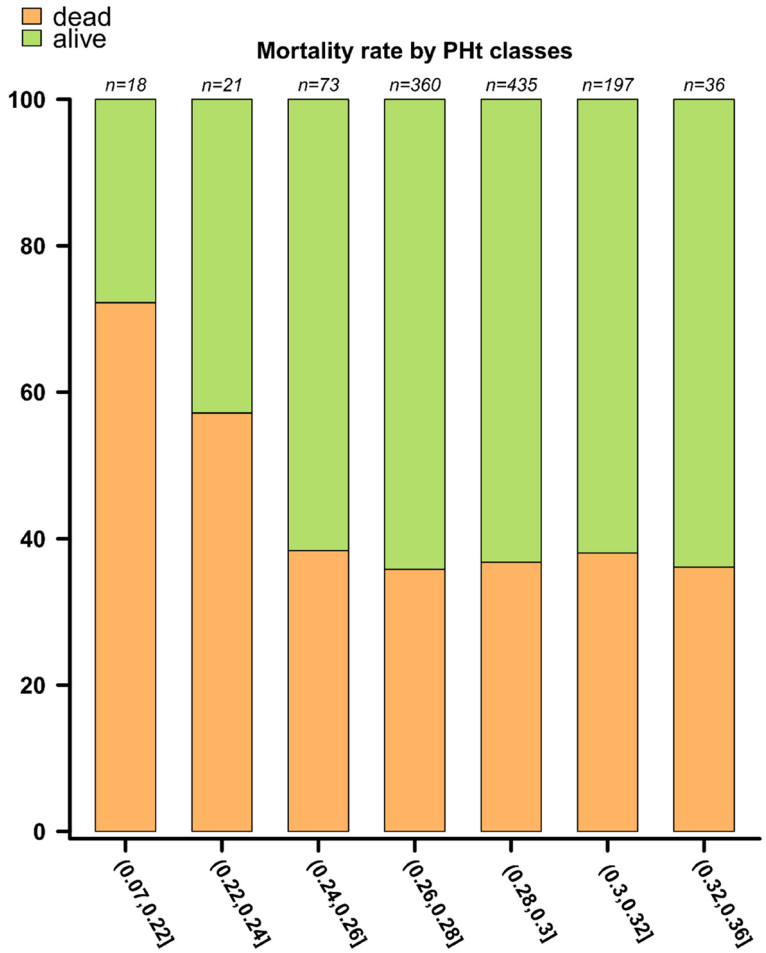

Fig. 26 Mortality rate as a function of individual heterozygosity classes. The number of individuals in each class is indicated at the top of each barplot

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Code availability
The custom code generated during the current study is available in the Zenodo repository, https://doi.org/10.5281/zenodo.7517641.

Authors' contributions
Conceptualization: Marie-Laure Desprez-Loustau, Cyril Dutech, Pauline Garnier-Géré. Funding acquisition: Marie-Laure Desprez-Loustau, Cyril Dutech, Pauline Garnier-Géré. Investigation: Benoit Barrès, Gilles Saint-Jean, Marie-Laure Desprez-Loustau. Data curation: Benoit Barrès, Marie-Laure Desprez-Loustau, Gilles Saint-Jean. Formal analysis: Benoit Barrès, Marie-Laure Desprez-Loustau, Cyril Dutech, Camille Lepoittevin, Catherine Bodénès, Christian Burban. Visualization: Benoit Barrès, Marie-Laure Desprez-Loustau. Writing - original draft preparation: Marie-Laure Desprez-Loustau, Benoit Barrès, Cyril Dutech. Writing - review and editing: Benoit Barrès, Marie-Laure Desprez-Loustau, Cyril Dutech, Pauline Garnier-Géré, Camille Lepoittevin, Catherine Bodénès, Christian Burban, Virgil Fiévet. Project administration: Marie-Laure Desprez-Loustau. Supervision: Marie-Laure Desprez-Loustau. The authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.7517641.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors gave their informed consent to this publication and its content.

Competing interests
The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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