

Editor's comments

This manuscript addresses the very important question of the interactions between a widely disseminated pathogen (powdery mildew, *Erysiphe alphitoides* and a related species) recently (beginning of the twentieth century) introduced in Europe and english oak (*Quercus robur*) progenies, checking for differences in mortality among genotypes and in growth trends of seedlings with respect to infection. The results are important and contribute to a clarification of the interactions, which will be quite useful for understanding the biology behind these infestations, and for the management of oak stands in their very young stage (seedlings and saplings).

Two reviewers have produced very detailed assessments of the manuscript. They were both very positive about the content, the results, and their analysis. They also converged in underlying a few shortcomings in the presentation of the methods, of the actual species of mildew, and a few further details that need revision and some attention.

At some point, I wondered, given the numerous results presented (and the frequent reference to supplementary material) whether the manuscript did not address two complementary but rather independent groups of questions: that of the interactions between fitness and powdery mildew infection (questions 1-3), and that about the genetic analysis of the sensitivity to powdery mildew (questions 4 and 5). This could have justified two different and more concise manuscripts, with a shorter and easier-to-follow presentation of the results. I very seldom make such a statement, given that most authors are rather inclined to salami publication. Anyway, the choice remains that of the authors, and it is also very positive to have a unique manuscript addressing a very complete range of questions based on a single and long-term experiment (over 10 years!) in a unique albeit quite long manuscript.

We sincerely thank you for accepting to be a recommender and for your work and positive comments on our manuscript. We agree that there are many data, but we thought it was interesting to present and analyze phenotypic and genotypic data together in order to address the general question of the impact of powdery mildew on oaks, at individual and population level. In that respect, question 4 is tightly related to question 3. Also, please note that following a comment from one of the reviewers, question 4 has been reworded. We acknowledge that the last question (5) dealing with genetic markers associated with oak susceptibility is slightly apart the general question on impacts of powdery mildew. We simply took advantage of our phenotypic and genetic data to present preliminary results on putative associations. The set of genetic markers that we used was amply sufficient for population genetic studies, *e.g.* assessing diversity, but an in-depth analysis of the genetic determinants of susceptibility using GWAS would require much more markers.

As recommender I have a few more mostly minor suggestions.

1. Like the reviewers, I would like to see the full species names (with authorities) quite early in the abstract, introduction, and possibly the title, and authorities associated with every species name at first occurrence.

This was done (see response to reviewer 1). For powdery mildew, the difficulty is that it is a complex of cryptic species (*i.e.* that cannot be distinguished to the naked eye). From various samplings in the experimental field, in different years, we can say that the dominant species was *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam, but *Erysiphe quercicola* S. Takam. & U. Braun was also often present in mixture.

2. I wondered why the data were not deposited in the French [recherche.data.gouv](https://recherche.data.gouv.fr/) repository, which has more interesting features for data visibility and organization than Zenodo, and is more compliant with the FAIR criteria. A switch to the former repository is not required at this stage, but I urge the authors to consider this in the future.

Thank you for the advice. The main reason why the code, data and supplementary material were deposited in zenodo is that we were not aware at the time of this (pretty new) data repository solution availability. In addition, we use github for script development and versioning. The integration between github and zenodo is seamless, with the ability to release updated version of the code in zenodo very easily. Nevertheless, the “recherche.data.gouv” is a very interesting alternative to the use of zenodo and we will consider it for future project.

3. I also suggest avoiding the use of supplementary material and would recommend adding all supplementary material either to the data set (which is the case here) or as an annex associated with the main text (which would be justified for a reader not willing to search through the Zenodo database. A few figures should be available as an annex to the main text.

Data sets that were associated with R or SAS scripts are provided separately because it is much easier for a reader that want to rerun the analyses to be able to download ready to use data files (instead of copy/pasting text from a pdf file for example). We think it should stay that way for reasons of practicality.

For similar reason, the flanking sequences data file was provided as a separated file. But it wasn't included with the other data and script files because there was no script associated with it. It was also considered that this was an additional dataset that was not essential for the core paper analyses. But on second thought, we agree with the recommender that it makes more sense that all data files be grouped within the same data repository. Therefore, this data set is now included to the same downloadable file of the *Data, scripts and codes availability* section.

Regarding the transfer of some of the supplementary material within an “Annex” section, we can, of course, move figures and tables from the supplementary material file to an annex section if this is better suited to the PCI Wood & Forest format. However, it is not clear to us which figures and tables should be affected by this move.

4. A few more details of the mode of action of the fungicide used would be helpful to guess the degree of protection provided: is it a systemic fungicide, and what is its specificity. Similarly, the procedure used to assess the degree of infection would also need some more details.

We added the name of the active ingredient “(myclobutanil, a systemic fungicide which inhibits the ergosterol biosynthesis of the fungus)” in the methods section (lines 169-170). More details about the fungicide were given in Desprez-Loustau et al 2014, referred to at the beginning of the Methods section.

5. Please check Figure 2C: does it really represent the total height of the seedlings, or is it the annual increment? A stability of total height after year 3 at 30-50 cm would be surprising; if yes, this requires a specific comment and discussion. The answer comes later L442: the final height of the seedlings reached values from 17 to 53 cm after almost 10 years of growth. The apparent stability over time is surprising: were annual growth flushes that small? or did the tallest seedlings die over time?

Actually, this is a mean value. But, as indicated, the variance largely increased, *i.e.* some seedlings still grew in height, whereas many remained small. In many cases also, height growth could be negative due to bud mortality on the leader shoot. Many plants eventually showed a bushy architecture. The impact of powdery mildew on oak growth patterns was more thoroughly analyzed in Desprez-Loustau et al 2014. It is also noteworthy that we used a high density of sowing for acorns and that the soil in this area is very sandy thus very poor (acidic), which may explain overall bad conditions for oak growth.

6. The results of the models should be at least partly shown in the main text and not in the annex (and even less in supplementary material).

We included the main results of the different models in the main text, while keeping the references to the supplementary material so that the reader can have a more complete picture without making the text too cluttered.

Lines 391-392: “(Wald Khi2 = 4.00, P = 0.045 and Wald Khi2 = 27.1, P < 0.0001, respectively; Supplementary material, Table S4 and Figure S10)”

Lines 418-419: “(Wald Khi2 = 5.99, P = 0.014, odds ratio = 0.804; C.I. of 0.649 to 0.996)”

Lines 420-421: “(Wald Khi2 = 17.8, P < 0.0001 and Wald Khi2 = 112.8, P < 0.0001, respectively; Supplementary material, Figure S12)”

8. The caption of Figure 7 should make clear that the index is computed in the 9 different plots (6 exposed and 3 protected). Similarly, the caption of Figure 9 should provide more details and clearly identify the significant associations for people who are not familiar with Manhattan plots.

One sentence was added to Figure 7 legend: “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.”

The following details were added in the Figure 9 legend: “Each dot represents a SNP. The negative logarithm of the association *p*-value corrected for multiple tests is displayed on the vertical axis. SNP with a significant association with a trait are indicated with yellow, orange and red dots.”

9. The first paragraph of the discussion sounds like a summary of the results; while this is relevant given the large array of complementary results, it should be made clear in the results section or significantly reduced in length. The structure of the rest of the discussion is excellent and follows the different questions raised at the end of the discussion, and I have no particular comment for the discussion which is very clear and precise.

In agreement with the recommender's comment, the first paragraph of the discussion has been significantly shortened. We deleted the «summary part» (original lines number 537-545) since main results were already underlined in the result and abstract sections. The sentence (now lines 563-564) was slightly modified as follow: “This powerful design enabled us to demonstrate various effects of powdery mildew at demographic and genetic levels in early stages of an oak cohort.”

Review by anonymous reviewer 1

We thank you very much for accepting this review and for your time in carefully reading our manuscript. Thank you for your positive comments and valuable suggestions.

l. 80 it would be good to describe the causal agents of powdery mildew in the introduction somewhere more specifically. In the first mention I recommend stating the genus or species scientific name.

We agree that this was missing. We added one sentence in the *Introduction* section lines 83-88 and one in the *Methods* section lines 186-189 to address this comment.

In the *Introduction* section:

“In Europe, powdery mildew was shown to be associated with a complex of cryptic (morphologically similar) species, of which *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam is nowadays the most prevalent throughout Europe, often in mixture with *Erysiphe quercicola* in southern Europe S. Takam. & U. Braun and with *Erysiphe hypophylla* (Nevod.) U. Braun & Cunningt. in northern Europe (Mougou et al 2008; Desprez-Loustau et al 2018; Gross et al 2021).”

In the *Methods* section

“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.”

l. 127 to me the maternal conditions high vs low disease pressure come here as a surprise. I would recommend using a small paragraph earlier in introduction for transgenerational effects of disease. There is also a recent publication on maternal effects in powdery mildew resistance in *Plantago*: Höckerstedt et al. *J Ecol.* 2021; 109: 1439–1451. <https://doi.org/10.1111/1365->

This is an interesting comment but, in this manuscript, the focus was on differences in susceptibility to powdery mildew between oak families, and not on transgenerational effects of disease. We have no data on powdery mildew infection of mother trees (*i.e.* trees under which acorns were collected). Family effects may include true genetic effects but also maternal effects such as those related to acorn weight, which was accounted for in statistical analyses. Consequently, we do not think it is necessary to introduce the transgenerational effects of plant diseases topic in an already lengthy manuscript.

l. 141-142 Please, be more specific.

l. 144 under which stress?

Question 4 was re-written (lines 142-148), hoping this is clearer now:

“Does powdery mildew impact the genetic diversity of the oak population, not only in terms of family composition? In order to address this specific question, a large number of emerging seedlings were genotyped at several hundred SNP and heterozygosity statistics were estimated. 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)?”

1. 149 A brief description of the genetics and biology of *Q. robur* is needed here. E.g. is it diploid or what? What powdery mildew species might infect it? What other stresses are important in its ecology?

Quercus robur is indeed a diploid, monoecious, wind-pollinated tree, 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). This information was added lines 156-159. Information on the powdery mildew species infecting this oak species was added lines 83-88 and 186-189. Competition between seedlings at the first stages and herbivory are among the largest selective pressures for this species. No significant impact of herbivory was observed in our field trial.

1. 151 Please, describe here how infected the mother trees were and for how long was that assessed? How far the mother trees were from each other? How is this species pollinated? How is it known that the seeds are half sibs?

Acorns were collected under isolated trees (*i.e.* with no overlapping canopies) in autumn. This information was added lines 155-156. The mother trees were distant from a few tens to a few hundreds of meters. The assignment of the individuals to their half-sib families was checked by genetic analyses using nine microsatellites (Guichoux et al 2011) as was indicated in the manuscript lines 259-260 (now lines 275-276). Unfortunately, the infection of mother trees could not be studied. A few of them died or were cut during the study. Furthermore, a precise, quantitative assessment of powdery mildew infection in a big tree is quite difficult. However, this did not prevent us from studying the issues addressed in this manuscript.

1. 154 when and where the trees were sown? It would be good to state here the duration of the study.

This information was given in Desprez-Loustau et al 2016, referred to at the beginning of the paragraph. We however added this information lines 161 and 163-164.

1. 168 please, provide details of the annual disease scoring for each year. Was the scoring performed every year? How many times?

Yes, the disease scoring was carried out several times each year. However, to assess disease severity, we only used one scoring per year corresponding to the highest infection, usually at the end of the season (September or October), but sometimes earlier in case of defoliation. We slightly modified the text to explain that (lines 179-182):

“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).”

1.270 How the 7 % minor allele frequency threshold was chosen?

The 7% threshold is used to exclude alleles that have only been observed once and could therefore be the result of a sequencing error. For the oak species, which is diploid, and since a minimum number of 8 sequenced individuals is retained, we have a minimum frequency of $(1/(2*8))*100=6.25\%$. We agree

with the reviewer that this was not clear in the manuscript. Therefore, the phrasing was changed from “(i.e., excluding singletons)” to “(allowing exclusion of variants found only once for the minimum of 8 diploid individuals sequenced)” (line 287)

1. 671 it would be good to explain the underlying genetics of powdery mildew-oak system somewhere.

As was indicated in the discussion (formerly lines 714-716, now lines 737-739), very little is known about the underlying genetics of the powdery mildew-oak system, contrary to some other well-studied plant-pathogen systems (especially in annual plants). To date, we can only refer to our previous study about QTL (Bartholome et al 2020).

Minor

1.23 it would be good to mention also scientific name for the pathogens causing powdery mildew in the abstract.

Because powdery mildew could be associated with a complex of species, listing these species in the abstract would be, in our opinion, too long. However, the scientific names of the main species are listed in the keywords. We also added information on the pathogens species involved lines 83-88 and 186-189

1. 89-90 specialized feeding structures that penetrate in living cells?

Yes, *Erysiphe haustoria* penetrates in living cells. The sentence was slightly reworded (lines 94-96) as follow:

“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 & Ayres 1976).”

1. 94 replace little with rarely

Done

1 96. what kind of importance? Economic, cultural, ecological or what?

Oaks are indeed especially important species in Europe, for economic, cultural and economic reasons. This was added (lines 101-102):

“...owing to the ecologic, cultural and economic importance of these species...”

1. 97-100 this sentence is unclear and needs clarification.

The sentence was re-written to make it clearer (lines 103-105):

“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).”

l. 105-108, this sentence could be simplified.

This was done (lines 110-111):

“Genetic changes in plant populations under pathogen pressure have been reported in a few pathosystems (Thrall et al 2012).”

l.113-115 could the authors place the citation to refer the study in question.

This was done.

l. 339 something weird in this sentence

The sentence was simplified (lines 356-358):

“This confirmed that the 819 SNPs were scattered across the whole genome, on all chromosomes, in 426 different genic regions (see Table S3), with an average distance between regions of 2.11 Mb (ranging from 0.0029 to 15.3 Mb).”

Fig. 5. This is very informative way of presenting the results! There seems to be error in the direction of] in the infection rate legend.

Thank you. No, it is not an error. The $]xxx]$ denotation is a left-open interval. It is a mathematical convention which means that the lower value is not included in the interval, contrary to the upper value.

l. 591 see also Susi et al. Journal of Ecology 105, no. 5 (2017): 1399-1412.

Thank you. This is indeed a nice additional reference on the flax rust pathosystem. It was added line 612

We thank you very much for accepting this review and for your time in carefully reading our manuscript. Thank you for your positive comments, interesting questions and valuable suggestions.

1. **In the Discussion, the authors state that the study of a single population sampled locally may not have allowed for the detection of trade-offs between traits, and suggest that sampling across a wider spatial range might have yielded different results (L613-617). Yet, in the Introduction, the authors state that high genetic diversity is present within oak populations (L97-98; L115-116). So, if the level of genetic diversity found within the Cestas population is comparable to the average level found in populations of *Q. robur* throughout its range, the problem associated with sampling only a local population may not be so important after all. On the other hand, if the Cestas population has an unusually low level of genetic diversity, the possibility that methodological constraints prevented the detection of trade-offs between traits becomes higher. How does the level of genetic/genomic diversity of the Cestas population compare with that of other *Q. robur* populations?**

Thank you for this interesting comment. First, we now included the values of Heterozygosity (used as genetic diversity indices) obtained in our study in the main text (they were previously only given in Supplementary material).

Lines 493-495:

“Observed and Expected heterozygosity did not vary between initial and surviving populations in both disease exposures, with values of H_o and H_e of 0.32-0.33 in all cases (Supplementary material, Figure S13)”

These values are in the range of or greater than H_e values reported for other *Q. robur* stands in various regions of Europe with the same type of genetic markers, *i.e.* SNPs (the value depends on the type of markers thus our results cannot be compared with older studies using microsatellites or other markers). A sentence was added in the discussion to emphasize this result, *i.e.* that only phenotypic variation was potentially affected by the local sampling and not the overall genetic variation. Indeed, as explained in the following sentence, trade-offs are expected to be more easily detected when considering populations with differing selection pressures and evolutionary strategies.

Lines 633-639:

“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 H_e values reported with the same type of markers (*i.e.* SNPs) for various *Q. robur* stands in Europe (Blanc-Jolivet et al 2021, Degen et al 2021 a and 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...”

2. **In their description of the experimental design, the authors state that there were two levels of exposure to powdery mildew: either Natural or Protected, with protection provided by the application of the fungicide Myclobutanil in the latter case. In their 2014 Annals of Forest Science paper based on the same experimental design, Desprez-Lousteau et al. indicate there were three levels of exposure (low, medium, and high) to powdery mildew.**

The low level corresponded to repeated applications of Myclobutanil. The medium level corresponded to natural inoculation, whereas the high level was obtained by blowing spores from highly sporulating leaves above the seedlings, using a portable air compression device. My understanding is that the new study included only plots with medium (Natural) and low (Protected) exposures. If this is indeed the case, I suggest mentioning this in the Materials and Methods section.

You are right. This should be explained. Actually, the Medium and High level disease treatments described in Desprez-Loustau et al 2014 did not differ much in seedling infection and, eventually, in mortality. Inoculations in the «High» treatment were performed only during the first three years of the ten-year experiment. These two treatments were therefore pooled for all analyses presented in this manuscript. This is why there were 6 plots in the «natural infection» treatment and three in the «protected» treatment. We added this information lines 166-170 as follow:

“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.”

- 3. The identity of the causal agents of powdery mildew is not mentioned in the text, except for the mention of *Erysiphe quercicola* and *E. alphitoides* in the Keywords. I suggest including at least one sentence on the identity and specificity of the pathogens responsible for powdery mildew on *Q. robur*, as was done in the 2014 Annals of Forest Science paper.**

This information was added, in the introduction, and in the method section (with some results of molecular analyses) lines 83-88 and lines 186-189.

- 4. The caption of Figure 3 should include information on the meaning of the small vertical green and beige/light brown bars at the top and bottom of the graph, respectively.**

The legend of Figure 3 has been completed by adding the following sentence:

“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.”

- 5. The caption of Figure 10 should include information on the acronyms for the genotypic classes C/C, T/C, and T/T.**

The legend of Figure 10 was modified as follow:

“Genotypic classes are named according to the IUPAC nucleotide symbol convention: A= adenine, C=cytosine, and T=thymine.”

I have indicated a few typos on the annotated PDF copy of the manuscript.

The typos you have pinpointed were taken into account.