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PROCEEDINGS B

Assessing the effects of quantitative host resistance on the life-history traits of sporulating parasites with growing lesions

Melen Leclerc, Julie A. J. Clément, Didier Andrivon and Frédéric M. Hamelin

Article citation details
Proc. R. Soc. B **286**: 20191244.
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Review timeline

Original submission:	28 May 2019
1st revised submission:	26 July 2019
2nd revised submission:	3 September 2019
Final acceptance:	11 September 2019

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-1244.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
In this manuscript, the authors consider a detailed life-history analysis of host-pathogen interactions in a system designed to only contain quantitative forms of resistance. The analysis is very interesting and I largely have some suggestions for improving the link for the generalist biology reader.

This work nicely shows that each life-history trait is affected by quantitative resistance, possibly via different processes given the differences in the genetic interactions. This affects the literature by showing that more detailed analysis will provide a better interrogation of quantitative resistance while also showing that host x genotype interactions are not likely solely a germination issue. The paper largely covers the first of these results but should also comment on the later given the growing issue of some in the community assuming that quantitative resistance is all just germination.

In the discussion, it would be helpful for the authors to discuss the difficulty in conducting this detailed analysis on the large genetic populations with either 100s of plant or pathogen genotypes that are required for this work. I agree that the models are not as scary as a generalist biologist may perceive but it does feel like the authors may be underestimating the difficulties in generating the necessary time scales on populations requiring 1000s of independent innoculations just to conduct a single biorep on all host x pathogen interactions. Similarly, some estimation of computational time and minimal biological replication to ensure model convergence would also be a boost for the generalist biologist who had an interest in applying this to their system.

In the introduction and discussion, the authors often discuss fitness. However, in most pathogen studies, the measurement is either on biomass of the pathogen or other aspects of the developing lesion and not directly on fitness. The authors should be careful to comment on the lack of knowledge about how these traits correlate with fitness in the field. For example, in viruses enhanced virulence is often linked to lower long term fitness. In this instance, the authors are directly measuring sporulation but in the vast majority of papers on this topic that is not the case.

Similarly, in the introduction, it should also be clear that not all host/parasite interactions involve large-effect qualitative loci. In my reading of the literature, there are likely as many if not more interactions that have no evidence of qualitative loci and are solely governed by quantitative loci. This addresses the assumption that quantitative resistance is some fall-back position when it may be that the qualitative loci are the outliers.
On line 113- what is the evidential support for the assumption that lesion growth is elliptical at the start rather than radial or some other shape? I understand how this likely simplifies the modelling but it does seem to imply that the pathogen has "measured" the leaf prior to growth and grows accordingly. It would help to discuss this assumption and how it may or may not influence the results. The observational data on line 122-126 would suggest that a better fit is obtained by having R1=R2.

In other pathogens there is evidence that the relationship of R1 to R2 in the lesion growth is linked to tracking the primary vasculature and as such there could be genetic variation in how the isolates behave with regards to this model. Do the authors have any evidence for or against this or if this possibility would influence their germination estimates.

Do the authors have any empirical measures on the pathogens germination to compare to the model estimates? I understand that it is not uniformally possible to compare germination on media to that on the host but it would be helpful to have some empirical measures to assess if the media germination provides any assistance to the model. For example, is t0 more driven by the time to germination of 50% of the spores or is it more linked to the shape of the curve as some pathogens show a log-shaped germination curve while others can show a linear curve linking time to germination percentage.

In Figure 1c, where the authors able to identify any block or technical effects that are linked to the extreme outliers from the linear slope. The data seems to be extremely long tail biased.

Is it safe to assume a log-function for in host growth between the second and third time point? There is evidence for this with single cell pathogens but I am less clear on the evidence for this in tip growing filamentous pathogens.

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

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Is it adequate?
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Comments to the Author
The paper addresses a question that is rarely taken into account in plant pathology and helps understanding how a fungus develops at the lesion scale by analysing both the growth kinetics and the sporulation kinetics. This paper brings together modeling and experimental approaches. It is helpful to understand how quantitative resistance reduces the disease.
I suggest to improve the following points :

Introduction
L67. clarify the links between epidemiological processes and life history traits. Life history traits are part of the epidemiological processes. I don't understand the point of view of the authors that their paper « enables the disentanglement of the epidemiological processes and the estimation of key life history traits »
L70. replace « on pathogen sporulation » by on « pathogen growth and sporulation »

M&M
L93. replace « too » by « to » ; replace « and then kept in clear plastic boxes and stored… » by « and kept in clear plastic boxes stored… »
L108. add information on the tresholds (minimum and maximum sizes of particles) chosen for the coulter
L141. replace « fig 2a-b » by « fig 2b »
L146. check that ref 3 is suitable here
L165-166. « distinct shapes ». It is not in agreement with the hypothesis stated in L110 that the leaflets are « ellipse-shaped, that seems to be reasonable in the particular case of this pathosystem ». The authors should explain what they mean by « distinct shapes ». It is also unclear why « distinct shapes » are a reason to « visually assess the adequacy of the models by looking at the raw residuals ».

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L184. replace « between 72.4 and 121.4 » by between « 68.2 and 121.4 »

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Tables and figures

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Table 2. change the order of the parameters to be the same as in the text : t0, t1, t0-t1, ρ, …

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Fig2a. The term « susceptible » is not adequate here. Replace by « non infected » ?
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Legend (d) : replace « cumulated number produced by the lesion» by « cumulated number of spores produced by the lesion »

Fig3d. Not useful to have two nested figures. I supposed that it was done to distinguish two of the curves that are very close. Maybe add this in the legend instead of adding the figure with the 0-25 y scale.
legend (c). the end of the sentence seems to be missing.

Decision letter (RSPB-2019-1244.R0)

22-Jul-2019

Dear Dr Leclerc:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. It is important to note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

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If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Victoria Braithwaite

==========
Professor V A Braithwaite
mailto: proceedingsb@royalsociety.org
==========

Associate Editor, Comments to Author:
Your paper has been seen by two expert reviewers, who were on the whole positive. The reviewers made some suggestions how the paper can be improved in a revision. In my view your paper has very nicely integrated an experimental approach with modelling, and therefore has the potential to reach audiences both of a theoretical and empirical background. the paper is well written, but is perhaps not an easy read for all. The reviewers suggestions for revision will help making the paper more accessible to those with a more empirical background, and will therefore increase the potential impact of the paper. Could you please revise your paper in line with the suggestions made by the reviewers.

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Reviewers' Comments to Author:

Referee: 1

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===

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Fig3d. Not useful to have two nested figures. I supposed that it was done to distinguish two of the curves that are very close. Maybe add this in the legend instead of adding the figure with the 0-25 y scale.
legend (c). the end of the sentence seems to be missing.

Author's Response to Decision Letter for (RSPB-2019-1244.R0)

See Appendix A.

Decision letter (RSPB-2019-1244.R1)

05-Aug-2019

Dear Dr Leclerc:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The comments from the Associate Editor are included at the end of this email for your reference. As you will see, the Editor still has some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We urge you to make every effort to fully address all of the comments. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with

Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online

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Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Victoria Braithwaite

Professor V A Braithwaite
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor, Comments to Author:

Thank you for a careful revision, which has very much improved the manuscript.

I have a few small points that I thiink you should look into, to make it even better

L177-178 "The adequacy of the models to the data were assessed visually by looking at the raw residuals." It is good that you checked, but given the rogour you apply elsewhere, this seems a handwaving solution. Can you do a goodness of fit test?

L352-353 "A common difficulty that arises when trying to combine modelling and experimentation is
the design of experiments, which has to be thought differently than for non-modelling purpose" Neither the grammar nor the meaning of this sentence is clear to me. Do you mean " "When trying to combine modelling and experimentation the experimental design is often different for modeling and non-modelling purposes.""

Is this really the case? It seems to me that this depends on the question that one is asking, not on whether a model is involved or not. Could you please think through carefully what you are saying here. See also the point below.

Reviewer 1 raised the point, "In the discussion, it would be helpful for the authors to discuss the difficulty in conducting this detailed analysis on the large genetic populations with either 100s of

plant or pathogen genotypes that are required for this work. .... Similarly, some estimation of computational time and minimal biological replication to ensure model convergence would also be a boost for the generalist biologist who had an interest in applying this to their system."
You have changed the last paragraph in response, by agreeing with the concerns raised. However, your response does not really answer the question. It seems that you answer is that it is important, and perhaps possible, but you don't help those who would consider using your model as a basis for experimentation much. Could you please reconsider your last paragraph (or address this point at another place in the discussion) how, in practice, this could be done.

It seems to me that the reviewer is a little bit pessimistic in outlook, and that with well-chosen experiments, and by applying appropriate statistical methods (model selection and a Bayesian approach is the way to go here, I would say) this can be done. However, it will require careful design of experiments so that these can indeed yield the information that is needed. Some elaboration of what that careful design entails would be helpful. You could do this by outlining a particular question, and the possible experimental approach.

In my view, addressing this question (rather than just saying that it is important, and that it is possible in principle) and reaching out to the empirical community (even without being able to give an exact answer) will add to your manuscript and strengthen it.

Please do this in a minor, and hopefully last, revision.

Author's Response to Decision Letter for (RSPB-2019-1244.R1)

See Appendix B.

Decision letter (RSPB-2019-1244.R2)

11-Sep-2019

Dear Dr Leclerc

I am pleased to inform you that your manuscript entitled "Assessing the effects of quantitative host resistance on the life-history traits of sporulating parasites with growing lesions" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Victoria Braithwaite

Professor V A Braithwaite
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:

Thank you for addressing these last points. I look forward to seeing this published!

Appendix A

Dear Editor,

Thank you for your positive comments on our article « Assessing the effects of quantitative host resistance on the life-history traits of sporulating parasites with growing lesions » submitted in Proceedings of the Royal Society B. We thank the reviewers for their constructive comments that were useful for improving the initial version of the manuscript and clarify some important points. We have revised the paper in line with their suggestions and hope that the accessibility to readers with an empirical background is improved. The revisions and the answer to reviewers are addressed below. We also submitted our data with a R code for fitting the models to Dryad.

Yours sincerely,

Melen Leclerc

Associate Editor, Comments to Author:
Your paper has been seen by two expert reviewers, who were on the whole positive. The reviewers made some suggestions how the paper can be improved in a revision. In my view your paper has very nicely integrated an experimental approach with modelling, and therefore has the potential to reach audiences both of a theoretical and empirical background. the paper is well written, but is perhaps not an easy read for all. The reviewers suggestions for revision will help making the paper more accessible to those with a more empirical background, and will therefore increase the potential impact of the paper. Could you please revise your paper in line with the suggestions made by the reviewers.

====

Reviewers' Comments to Author:

Referee: 1

In this manuscript, the authors consider a detailed life-history analysis of host-pathogen interactions in a system designed to only contain quantitative forms of resistance. The analysis is very interesting and I largely have some suggestions for improving the link for the generalist biology reader.

This work nicely shows that each life-history trait is affected by quantitative resistance, possibly via different processes given the differences in the genetic interactions. This affects the literature by showing that more detailed analysis will provide a better interrogation of quantitative resistance while also showing that host x genotype interactions are not likely solely a germination issue. The paper largely covers the first of these results but should also comment on the later given the growing issue of some in the community assuming that quantitative resistance is all just germination.

We thank the reviewer for their feedback. We added the following sentence in the introduction: “In filamentous plant pathogens, quantitative resistance applies not only to spore germination and infection, but also to within-host growth and spore production (Niks et al, 2015).”

Niks, R. E., Qi, X., & Marcel, T. C. (2015). Quantitative resistance to biotrophic filamentous

plant pathogens: concepts, misconceptions, and mechanisms. Annual Review of Phytopathology, 53, 445-470.

In the discussion, it would be helpful for the authors to discuss the difficulty in conducting this detailed analysis on the large genetic populations with either 100s of plant or pathogen genotypes that are required for this work. I agree that the models are not as scary as a generalist biologist may perceive but it does feel like the authors may be underestimating the difficulties in generating the necessary time scales on populations requiring 1000s of independent innoculations just to conduct a single biorep on all host x pathogen interactions. Similarly, some estimation of computational time and minimal biological replication to ensure model convergence would also be a boost for the generalist biologist who had an interest in applying this to their system.

We thank the reviewer for this important point. It is in line with the last paragraph of the discussion (i.e. “it would be relevant to see whether we could reduce the number of inoculations in further similar experiments. This question is particularly important because assessing the variability of phenotypes among plant and pathogen populations requires a large numbers of samples.”), that has been expanded to improve the discussion on the difficulty of generating such phenotypic data for testing models or assessing the genetic architecture of traits.

In the introduction and discussion, the authors often discuss fitness. However, in most pathogen studies, the measurement is either on biomass of the pathogen or other aspects of the developing lesion and not directly on fitness. The authors should be careful to comment on the lack of knowledge about how these traits correlate with fitness in the field. For example, in viruses enhanced virulence is often linked to lower long term fitness. In this instance, the authors are directly measuring sporulation but in the vast majority of papers on this topic that is not the case.

The terms “fitness” occurs only once in the manuscript (in the first sentence of the second paragraph of the introduction). We now cite Gilchrist el al (2006) who properly derived an explicit fitness metric for spore producing fungi: it is the expected lifetime spore production of the fungal patch/lesion. Fitness expresses as an integral formula involving the latent period and a sporulation function, very much in line with our sporulation model (Eq. 3).

Gilchrist, M. A., Sulsky, D. L., & Pringle, A. (2006). Identifying fitness and optimal life- history strategies for an asexual filamentous fungus. Evolution, 60(5), 970-979.

This reference/definition was indeed missing, thank you.

Similarly, in the introduction, it should also be clear that not all host/parasite interactions involve large-effect qualitative loci. In my reading of the literature, there are likely as many if not more interactions that have no evidence of qualitative loci and are solely governed by quantitative loci. This addresses the assumption that quantitative resistance is some fall-back position when it may be that the qualitative loci are the outliers.

We agree that it was not explicit that Quantitative resistance can occur with or without qualitative resistance. In line with the suggestion of the referee, we included the sentence “When it is present, Quantitative host resistance to disease can occur alone or in combination with qualitative resistance.” at the beginning of the second paragraph.

On line 113- what is the evidential support for the assumption that lesion growth is elliptical at the start rather than radial or some other shape?
I understand how this likely simplifies the modelling but it does seem to imply that the pathogen has “measured” the leaf prior to growth and grows accordingly. It would help to discuss this assumption and how it may or may not influence the results. The observational data on line 122-126 would suggest that a better fit is obtained by having R1=R2.

In fact, we indeed assume that lesion growth is radial at the start, and it becomes elliptical once the lesion reaches an edge. We summarised this as an overall elliptic expansion (since a circle is a particular kind of ellipse) but your feedback made us realise that this description was indeed too compact. We now write:

“the lesion […] expands as a circle until reaching an edge; then it expands as an ellipse up to completely recovering the surface of the leaf (Fig. 1).”

Thanks again for a good point.

In other pathogens there is evidence that the relationship of R1 to R2 in the lesion growth is linked to tracking the primary vasculature and as such there could be genetic variation in how the isolates behave with regards to this model. Do the authors have any evidence for or against this or if this possibility would influence their germination estimates.

We agree that the vascular system can be an important driver of the spatial expansion of several pathogens such as vascular bacteria. We included this idea in the discussion « iii) introduce the leaf vein structure that can be crucial to predict the spatial expansion of vascular pathogens ». However, we do not have evidence that observed effects of quantitative host resistance on radial growth rate can be explained by some differences in the vascular system, nor how such traits can be explained genetically. This would be an interesting point to be further investigated at a finer spatial scale and would probably require a different experimental set-up.

Do the authors have any empirical measures on the pathogens germination to compare to the model estimates? I understand that it is not uniformally possible to compare germination on media to that on the host but it would be helpful to have some empirical measures to assess if the media germination provides any assistance to the model. For example, is t0 more driven by the time to germination of 50% of the spores or is it more linked to the shape of the curve as some pathogens show a log-shaped germination curve while others can show a linear curve linking time to germination percentage.

We agree with the reviewer that a pathogen germination, or infection efficiency, is an important component of partial resistance and a crucial variable for predicting pathogen development as small lesions induced by each spore quickly coalesce (fungal mycelium may also merge). We also agree that life-history traits estimated at the lesion scale can be influenced by the level of spores that actually infect host tissues. As in this study the level of inoculum was standardised we believe that we can compare host-interactions and assess the effects of host-quantitative resistance. However, as indicated in the discussion (lines 218-223) we could not estimate infection efficiency. This would be very interesting for further studies but would require experimental development for this pathosystem.

In Figure 1c, where the authors able to identify any block or technical effects that are linked to the extreme outliers from the linear slope. The data seems to be extremely long

tail biased.

The data actually exhibits outliers. We were able to test the effect of cultivars on the slope (see responses to reviewer 2) but unfortunately we cannot test block or technical effects for these data. These outliers may be explained by the variability of potato leaflets that might be affected by several factors such as the position the plant in the greenhouse or the age of the leaflet that might not have the time to fulfil its development.

Is it safe to assume a log-function for in host growth between the second and third time point? There is evidence for this with single cell pathogens but I am less clear on the evidence for this in tip growing filamentous pathogens.

We thank the reviewer for raising this point. We added the following sentence: “Tip growing filamentous pathogens often show a constant radial growth rate in a homogeneous medium (Pirt 1967, Edelstein 1982,).” However, as discussed above, the heterogeneity of the host (e.g. its vasculature) may slightly challenge this assumption.

Pirt, S. J. (1967). A kinetic study of the mode of growth of surface colonies of bacteria and fungi. Microbiology, 47(2), 181-197.

Edelstein, L. (1982). The propagation of fungal colonies: a model for tissue growth. Journal of Theoretical Biology, 98(4), 679-701.

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Referee: 2

The paper addresses a question that is rarely taken into account in plant pathology and helps understanding how a fungus develops at the lesion scale by analysing both the growth kinetics and the sporulation kinetics. This paper brings together modeling and experimental approaches. It is helpful to understand how quantitative resistance reduces the disease.
I suggest to improve the following points :

Introduction
L67. clarify the links between epidemiological processes and life history traits. Life history traits are part of the epidemiological processes. I don’t understand the point of view of the authors that their paper « enables the disentanglement of the epidemiological processes and the estimation of key life history traits »

We thank the reviewer for their feedback. The term “epidemiological” was indeed misleading as we implicitly referred to sort of within-host epidemic (SEIR) models. Rather, our paper enables the disentanglement of the sporulation and within-host growth processes, which enables the estimation of key life history traits. We rephrased the introduction accordingly in a few places (lines 45, 64, and 67).

L70. replace « on pathogen sporulation » by on « pathogen growth and sporulation »
M&M
L93. replace « too » by « to » ; replace « and then kept in clear plastic boxes and stored… » by « and kept in clear plastic boxes stored… »

We thank the reviewer for pointing out these typos. We made the appropriate

modifications in the main text.

L108. add information on the tresholds (minimum and maximum sizes of particles) chosen for the coulter

We included the lower and upper thresholds used for the coulter in the main text.

L141. replace « fig 2a-b » by « fig 2b »

We thank the reviewer for highlighting this mistake. As suggested by the reviewer (see below) we have modified and splitted figure 1. References to figures have therefore been changed along the main text.

L146. check that ref 3 is suitable here

It is actually a reference to the convolution equation (3) and not a reference to the article [3] of the bibliography. Thus, we believe that this reference remains suitable here.

L165-166. « distinct shapes ». It is not in agreement with the hypothesis stated in L110 that the leaflets are « ellipse-shaped, that seems to be reasonable in the particular case of this pathosystem ». The authors should explain what they mean by « distinct shapes ». It is also unclear why « distinct shapes » are a reason to « visually assess the adequacy of the models by looking at the raw residuals ».

We thank the reviewer for pointing out this point. We actually meant sizes (described here by minor and major radii) instead of shapes. The reason to « visually assess the adequacy of the models by looking at the raw residuals » is that as leaflets had distinct minor and major radii, for an identical growth rate the predicted times at which the lesion reaches the edges (end of phase 2 and phase 4) are also different and the graphical display of the fitted model against the raw data is difficult to read. As investigating the raw residual is a standard method for assessing the adequacy of a fitted model to raw data and to prevent any misunderstanding we simplified the text and only left the sentence: « The adequacy of the models to the data were assessed visually by looking at the raw residuals. »

Results
L184. replace « between 72.4 and 121.4 » by between « 68.2 and 121.4 »

We thank the reviewer for their careful reading. We corrected this mistake in the manuscript.

Discussion

L241. see also Caffier, V., et al. (2014). "Erosion of quantitative host resistance in the apple - Venturia inaequalis pathosystem." Infection, Genetics and Evolution 27: 481-489.

We included this relevant reference in the manuscript.

L264. why is it expected that symptomatic lesion match with necrotic area ? More information should be given in M&M L105 to explain what is considered as a lesion and what are the limits of the lesion that are measured.

We thank the reviewer for this point and agree that this point had to be clarified in the

M&M. We added a sentence to explain what was actually measured in the 2.1.3 Measurements section of the manuscript.

L276. delete « and »

We corrected this typo in the main text. Thank you.

L324. « decrease the discrepancy between the model and the data ». This discrepancy should be presented in the result part.

This discrepancy is actually presented through raw residuals (Figs. S1 & S2) and exposed in the first paragraph of the results. We believe that revisions performed in line with comments above would prevent this misunderstanding.

L345-346. Progress is currently done concerning the use of imaging in plant diseases. See for instance D. Rousseau & T. Boureau references.

We thank the reviewer for this suggestion. We modified this part of the discussion and included one of these references. (i.e. Belin, Étienne, et al. "Thermography versus chlorophyll fluorescence imaging for detection and quantification of apple scab." *Computers and electronics in agriculture* 90 (2013): 159-163.)

Tables and figures

Table 1. replace « mode the sporulation function » by « mode of the sporulation function »
Table 2. change the order of the parameters to be the same as in the text : t0, t1, t0-t1, ρ,

We modified both Tables 1 & 2 in line with the suggestion of the reviewer.

Fig 1. I suggest to split in 2 figures, because fig 1c is not on the same domain than fig 1a and 1b.
Then fig 1a and 1b could be aligned based on the differents phases Ph1 to Ph4 in a similar t scale. Add a representation of t0 in fig 2b.
For Fig 1c, the R2 value should be given for each cultivar separately. It should also be necessary to explain how the manual annotation of images were done to assess the surface of the leaf (use of imageJ ?).

We thank the reviewer for these suggestions on figure 1. We thus splitted figure 1 in two figures and combined Fig1a and Fig2b. For Fig1c (now figure 2 in the revised manuscript) we included supplementary information on leaf annotation and differences between cultivars in the main text. As we were not able to detect a significant cultivar effect on the slope with an ANCOVA with did not introduced separate R^2 values.

Fig2a. The term « susceptible » is not adequate here. Replace by « non infected » ?
Legend (a) : precise that the illustration presents two different times (during Ph3 and Ph4)
Legend (c) : the end of the sentence seems to be missing.
Legend (d) : replace « cumulated number produced by the lesion» by « cumulated number of spores produced by the lesion »

We thank the reviewer for these comments and suggestions. We integrated it in the caption and changed the term « susceptible » to « uninfected » in the figure.

Fig3d. Not useful to have two nested figures. I supposed that it was done to distinguish two of the curves that are very close. Maybe add this in the legend instead of adding the figure with the 0-25 y scale.
legend (c). the end of the sentence seems to be missing.

Indeed the nested figures were originally made for distinguishing the two close curves. Following the reviewer's suggestions, we only kept the 0-60 scale figure and explained in the caption that two curves overlap.

Appendix B

Dear Editor,

Thank you for your constructive feedback on our manuscript. We have revised the paper in line with your suggestions and hope you will find it improved. The revisions and the answer to your comments are addressed below.

Yours sincerely,

Melen Leclerc

L177-178 "The adequacy of the models to the data were assessed visually by looking at the raw residuals." It is good that you checked, but given the rogour you apply elsewhere, this seems a handwaving solution. Can you do a goodness of fit test?

→ Thank you for raising this point. Plotting the data against the fitted model is indeed a qualitative assessment of the goodness-of-fit. We followed your suggestion and included a standard goodness-of-fit test for least-square estimation. The GoF tests support the hypothesis of a good fit for the two models on all datasets. The test is presented in the Model fitting and statistical analyses subsection (lines 177-181), the results are introduced in the Result section (lines 189-190) and detailled are given in a new section of the Appendix.

L352-353 "A common difficulty that arises when trying to combine modelling and experimentation is
the design of experiments, which has to be thought differently than for non-modelling purpose"
Neither the grammar nor the meaning of this sentence is clear to me. Do you mean " "When trying to combine modelling and experimentation the experimental design is often different for modeling and non-modelling purposes.""

Is this really the case? It seems to me that this depends on the question that one is asking, not on whether a model is involved or not. Could you please think through carefully what you are saying here. See also the point below.

→ We agree that the experimental design depends on the question and not on whether a model is involved. Our sentence was actually in agreement with it. However, the study of dynamic processes, and by extension the use of models, remains seldom integrated in plant pathology where most studies consist in comparing treatments, isolates, genotypes… Thereofore, the time is sometimes considered as a treatment (dates are modalities) and the experiments are often designed for comparing dates instead of capturing a temporal process. We deleted this sentence, and improved two paragraphs of the discussion (see below).

Reviewer 1 raised the point, "In the discussion, it would be helpful for the authors to discuss the difficulty in conducting this detailed analysis on the large genetic populations with either 100s of plant or pathogen genotypes that are required for this work. .... Similarly, some estimation of computational time and minimal biological replication to ensure model convergence would also be a boost for the generalist biologist who had an interest in applying this to their system."

You have changed the last paragraph in response, by agreeing with the concerns raised. However, your response does not really answer the question. It seems that you answer is that it is important, and perhaps possible, but you don't help those who would consider using your model as a basis for experimentation much.

Could you please reconsider your last paragraph (or address this point at another place in the discussion) how, in practice, this could be done.

It seems to me that the reviewer is a little bit pessimistic in outlook, and that with well-chosen experiments, and by applying appropriate statistical methods (model selection and a Bayesian approach is the way to go here, I would say) this can be done. However, it will require careful design of experiments so that these can indeed yield the information that is needed. Some elaboration of what that careful design entails would be helpful. You could do this by outlining a particular question, and the possible experimental approach.

In my view, addressing this question (rather than just saying that it is important, and that it is possible in principle) and reaching out to the empirical community (even without being able to give an exact answer) will add to your manuscript and strengthen it.

→ Thank you for pointing out this lack of explanation regarding i) how our models could be used in furthers studies by non-modellers and ii) the perspectives we had in mind regarding the design of experiments.

We first modified the 5th paragraph of the discussion giving more details on the reutilisation of our modelling framework for further studies, as well as order of magnitudes for the computational time required for parameter estimation (that was indeed overestimated by reviewer 1). We now write :

« Our models are quite generic and can be used to estimate life-history traits of several sporulating pathogens with growing lesions. The R code attached to the manuscriptenables one to fit the models against temporal data and may help non-modellers to apply the framework on their specific datasets. As long as the temporal data cover the dynamics of both lesion spread and sporulation, the implemented Bayesian procedure should provide estimates of the parameters, even with fewer replicates than we had. Furthermore, the implemented estimation procedure is relatively fast (e.g. about respectively 1 & 3 minutes for fitting models (1) & (3) with 100000 MCMC iterations on a Intel R Xeon R E5 with 32 Go of RAM) and, from a computational time point of view, its application to large datasets may be reasonable. »

We also modified the last paragraph in line with the comment of reviewer 1 on the issue of collecting data for fitting models in genetic studies, for which the experimental cost is already high. We agree that a Bayesian framework can handle small data set and enable fewer measurements. However, we believe that the application of methods from the Optimal Design of Experiments (which works with both frequentist and Bayesian frameworks) is a good way to tackle this very important issue for promoting the use of models with empirical data, at least in phytopathology. We also included to relevant references on this topic that remains poorly considered by modellers. We now write :

To finish with, mathematical modelling offers a mean to improve plant disease phenotyping by allowing a finer quantification of traits. Thus, it would be relevant to promote model-based phenotyping, especially for assessing the genetic architecture of traits, either for the plant or the pathogen. However, generating data for modelling purpose in genetic studies can increase the, already substantial, experimental cost. This experimental bottleneck might be partially overcome by using methods from the Optimal Design of Experiments \citep{ryan2016,walter1990}. This field of statistics provides methods for designing experiments (e.g. size of the experiment, times of observation, number of replicates) that optimise the information on the processes for parameter estimation or model selection. In this study, the experiment was rather designed based on our knowledge on the time scale of pathogen development, and our experimental constraints. While this empirical space-filling design allowed us to fit the models, it would be interesting to improve our modelling framework by defining optimal experimental strategies that enable the proper estimation of life-history traits with the minimal number of lesion-scale data \citep{cook2008}.

Cook, A. R., Gibson, G. J., & Gilligan, C. A. (2008). Optimal observation times in experimental epidemic processes. *Biometrics*, *64*(3), 860-868.

Ryan, E. G., Drovandi, C. C., McGree, J. M., & Pettitt, A. N. (2016). A review of modern computational algorithms for Bayesian optimal design. *International Statistical Review*, *84*(1), 128-154.