Our answer to reviewers (in green)

Revision needed before a recommendation is possible
by Benoit Moury, 2017-09-02 00:13
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Decision & reviews

After evaluation by two reviewers, there are a number of points that should be addressed before a recommendation is possible.

The most important issue, perceived somewhat differently by the two reviewers, is the lack of highly significant effect when the pathogenicity of Z. tritici isolates is compared at the intra-annual scale (p=0.087), whereas this effect is emphasized and deeply discussed and interpreted (overinterpreted ?).

While the p=0.05 significance threshold can be considered somewhat arbitrary, it is widely accepted in the scientific community. This effect should therefore be interpreted as a trend rather than a definitively established effect and its interpretation in the Discussion should be taken with more caution and be given less importance. **Done. Se our first answer to the second reviewer.** You can for example argue that the trend observed during a single year results from differential selection effects that would have needed to be maintained during several years to reveal more significant effects. **Done.**

Please consider also the additional reviewers' comments.

Reviewed by Benoit Moury (first reviewer), 2017-08-04 10:45

The manuscript « Trade-off between intra- and interannual scales in the evolution of agressiveness in a local plant pathogen population » by Suffert et al. shows an analysis of the evolution of agressiveness of a fungal pathogen, Zymoseptoria tritici, in a wheat cultivar with a medium resistance level. To do so, Z. tritici isolates were collected in field plots and their agressiveness evaluated in controlled laboratory conditions on the same wheat cultivar plus another one representing an allopatric host.
The evolution of quantitative life history traits of plant parasites exposed to host plants which themselves harbor quantitative resistance factors is poorly understood and poorly studied. Notably, the speed at which parasite adaptation takes place in these conditions has rarely been determined. The important advance of Suffert et al. is to help determine the appropriate timescale to measure the gain of adaptation of the parasite. Indeed, in this study, Suffert et al. compared Z. tritici isolates (i) at the annual scale, i.e. before and after they had been exposed to the host plants during one growing season only and (ii) at the interannual scale, i.e. before and after six years of exposure. Importantly, if comparisons at the intra- and inter-annual scales lead to opposite conclusions about the capacity of the parasite to adapt to its host, then multiple timescales should be more systematically taken into account for such studies and particular caution should be taken in interpreting data focusing on only one particular timescale. Indeed, considering the « local » host cultivar on which the parasite most probably evolved, a gain of adaptation (shorter latent period, though the effect was marginal with p=0.087) was observed at the intra-annual scale, whereas no adaptation change was observed at the inter-annual scale.
The experiments in Suffert et al. are well designed and described. My only regret is that the populations Ci-2009 and Cf-2010, compared to study adaptation at the intra-annual scale, were not tested on the « allopatric » cultivar Apache. This may have allowed to check if adaptation to the allopatric host could also occur during this timeframe. I understand that this test can be considered as missing. **The test was not performed mainly because host adaptation was here not a central question, but also because of the lack of human and technical resources. The cultivar effect was tested and reported in the current study because it was required to interpret our results correctly: the host exerts a selection pressure that can alter phenotype, and therefore phenotypic differences can be used for highlighting evolutionary dynamics. The specific question of adaptation to allopatric vs. sympatric host was investigated by Morais et al. 2016. The priority was given to isolates sampled early and late over the time course of the experiment.** In addition, I would have liked some more details about the representativity about the 15 isolates in each population, if such data exist. Can we exclude any strong bias between the 15 isolates and the overall epidemic parasite population? This is particularly true for populations Cf-2010, Ai-2009 and Af-2015 for which we have no details about sampling. Maybe the information is available in other publications by the same group, but it would be worth providing brief elements here. **The question is sounded. Much more details about the sampling scheme and the genetic structure of the subpopulations Ci-2009 and Cf-2010 are given in Suffert et al. (2015). The subpopulations Ai-2009 and Af-2015 were randomly selected in a larger collection consisting in several isolates collected from debris collected in five points in the field as described in Suffert & Sache (2011). There is no reason that the two pairs of subpopulations would not have been representative of the local pathogen diversity considering the experimental design and the epidemiological knowledge of Z. tritici.**

I have several more specific points that may help clarify the manuscript and for which I would like a revision.

Introduction.
-page 2, column 1, « Alternatively, selection may be negligible relative to other antagonistic selective forces (gene flow…». As the word 'selection/selective' is in the two parts of the sentence, the idea is not clear. Moreover, I consider gene flow and selection to be quite distinct evolutionary forces. **"antagonistic selective forces" was changed to "antagonistic forces".**

Materials and Methods.
-For lesion size, I did not get which variable was analyzed (size at final date, all dates, AUDPC…). Can you be more precise? **The variable analyzed was the "maximum lesion size" (i.e., usually the lesion size assessed at final date or a previous date when assessment was not possible at final date). This precision now appears in the M&M section.**
-Which software was used for data analyses ? **ANOVA were performed with the S-PLUS 6.0 software (Lucent Technologies, Inc.) and permutation tests with the R software. These precisions now appears in the M&M section.**

Results.
-Table 1 : Can you indicate which r values are significant ? **Done.**

-page 7, column 1 : « …consistent with of the generally… » -> replace with « …consistent with the generally … » **Done.**
-Figure 5 : Can you compare statistically the two distributions ? **Because of the low proportion of statistically significant coefficients of correlation, no statistical test was suitable for comparing the two distributions.**
-page 7, column 2 : « …with an early attack following moderate winter… »-> replace with « …with an early attack followed by moderate winter… ». **Done.**
-Lesion growth curves that did not fit with the Gompertz curves were excluded from analysis. However, the Gompertz parameters were used only to determine the latent period. You could have used these data for the analysis of the lesion size. Does it affect the results ? **In several cases the lack of model convergence was due to a failure of inoculation: no symptoms, or minute symptoms that appeared relatively late. For this reason, the risk of underestimating the actual "maximum lesion size" was high and we preferred to exclud all the disease variables obtained from the same leaf (correlation between disease variable can be high, as established in Suffert et al., 2013).**
-page 8, column 1 : I would prefer « isolate - cultivar combination » to « isolate x cultivar interaction » here. **Done.**

Discussion.
-page 10, column 2 : Is it two pairs or one pair of Z. tritici populations here ? I understand that the cultivar effect could be tested only with the Ci-2009 and Cf-2010 populations. **The sentence was changed by "The significant effect of cultivar on the latent period of one of the two pairs of *Z. tritici* subpopulations may reflect the difference in resistance between the two cultivars."**
-page 10, column 2 to page 11, column 1 : I do not completely agree with the interpretation of the data as maladaptation to the allopatric host, Apache. I find that it depends on the point of view and the statement may be modulated. Considering the latent period, it is true that populations Ai-2009 and Af-2015 perform better on the sympatric than on the allopatric host. However, on the allopatric host, the Af-2015 population induces larger lesions than Ai-2009, hence, in my vision, it gained adaptation to the allopatric host along the six years of exposure to the sympatric host.
The results given in Table 4 confirm that the Af-2015 population induces larger lesions than Ai-2009, only on the allopatric host. This part of the discussion was changed and the term "maladaptation" was deleted.
-page 11, column 1 : « A host shift alters the environment… » : I did not understand that sentence. **"Immigrant" was changed to "resident".**
-page 11, column 1 : « …at least partly due to evolutionary forces (as opposed to chance)… »->replace with « …at least partly due to deterministic evolutionary forces (as opposed to chance)… ». **Done.**
-page 11, column 2 : « … the effects of selection are much smaller than those of diversity… » : I do not understand the idea here. **The sentence was changed to "Moreover, sample size was too small to allow the detection a change in the frequency of pathogen genotypes: the probability of detecting the same clone several times is very low with respect to the high population diversity."**
-page 11 column 2 : « Phenotyping isolates on adult plants, … (Morais et al., 2016a) » : I do not understand how such phenotyping allows to draw that conclusion. **The sentences were changed to: "The absence of change from 2009 to 2015 may have been concealed by the impact of immigrant strains (clouds of ascospores released from distant debris) exhibiting an evolutionary trajectory different from that of the resident strains. A previous comparison of the aggressiveness of resident and immigrant strains on adult plants, as in this study, however, demonstrated that most of the primary inoculum originated within the field (Morais et al., 2016a)."**
-page 12, column 1 : I do not really understand how the local host maladaptation (that may be discussed as mentioned above) and the temporal continuity of pathogen pressure « demonstrate the robustness of this analysis ». Could you explain more ? **This part was deleted. The explanation is given just before: "Finally, the overall temporal continuity in disease development over the six-year period and the evidence of local host adaptation provide two arguments supporting that the increase in aggressiveness at the annual scale and the stability of aggressiveness at the pluriannual scale, are due – at least partly – to deterministic evolutionary forces (as opposed to chance), such as selection acting on quantitative traits."**
-page 12, column 1 : « The trade-off between the capacity to overcome… » : among the traits compared, one belongs to the parasite and the other to the host ; thus I do not see any trade-off here. The first part of the sentence was changed to "A trade-off between capacity to overcome the QTL and capacity to keep in *V. inaequalis* populations over years was suggested". This is exactly the sentence used by Caffier et al (2016) in their discussion.

Second reviewer (anonymous)

This is a very interesting manuscript that sets out to test the evolution of aggressiveness traits (latent period and lesion size) in Z. tritici at two temporal scales – within an growing season i.e., over a number of asexual cycles of infection versus across several years with several different epidemics, punctuated by sexual recombination. The authors (claim to) show evidence for significant evolution within seasons but less or none across seasons, and invoke a "trade-off" in the evolution of aggressiveness within versus among epidemics. It is a very interesting story, but I am rather perplexed by a number of things. I agree that the authors find a significant decrease in phenotypic variation in latent period between the beginning and the end of the 2009-2010 epidemic cycle. Mean latent period also decreased, but not significantly so, between the beginning and the end of the 2009-2010 epidemic cycle. So there was loss of variation but no significant change in mean phenotype. For lesion size there was evidence neither for loss of variation nor for shift in phenotype. So, if I understand correctly, there was not really any significant evolution within the epidemic cycle. However, the authors argue, on Page 11 of the discussion, that "A difference in aggressiveness was observed between the initial and final conidial subpopulations (Ci-2009, Cf-2010)…". Perhaps I really missed something, but I do not see on what they can base this claim.
Your sounded interpretation, based on a conservative use of statistics, convinced us to nuance our conclusion.
The observed decrease in latent period should be presented as a trend (p = 0.08) and not as a fact, as suggested also by the first reviewer. The different observed "trends" (e.g. the intra-epidemic effect on latent period) form together as "a body of inter-related evidence" when combined. Accordingly, this part of the discussion was changed to: "A difference in aggressiveness was observed between the initial and final conidial subpopulations (Ci-2009, Cf-2010) expressed on the sympatric host cv. Soissons over the annual time-scale. The dynamics of lesion development were different. On average, the latent period was shorter for the final conidial subpopulation than for the initial one. The difference, however, was not statistically significant at p = 0.05 (p = 0.087). While there was no difference in lesion size when assessed on adult plants at 18.1°C (spring conditions), lesion size was larger for the isolates of the final conidial subpopulation than for those of the initial conidial subpopulation when assessed on seedlings at 8.9°C (winter conditions) (Suffert et al., 2015). The trend observed during a single year results from differential selection effects that would have needed to be maintained during several years to reveal more significant effects. These results suggest that aggressiveness increased over the course of a single annual epidemic, although we could not demonstrate it formally. This evolution reflects a pattern of adaptation, interpreted as the outcome of short-term selection driven by seasonal environmental conditions. Our interpretation is

strongly supported by the significant decrease in the between-isolate variance for latent period at the intra-annual scale, compared to the stability of this variance at the interannual scale."

Given that I have doubts about the basis of the claim of the intra-epidemic patterns, I have difficulty with the arguments that set out to explain the difference between the intra- and inter-epidemic patterns, which also showed no significant changes in aggressiveness phenotypes.
We agree that we have to consider several inter-related evidence to conclude about the intra-epidemic pattern. However, the results are clear concerning the inter-epidemic pattern: no decrease in diversity (p = 0.442 for latent period), no increase in aggressiveness either (see curves of Ai-2009 and Af-2015 for Soissons in Figure 7a and corresponding statistics in Table 4).

In addition to this very general objection (which pains me, because this is such a good idea, but I just do not see that the data uphold the claims, but really, perhaps I have not understood something properly) I have a number of more precise suggestions for clarifying and improving the presentation.

I list several things that I would like to see clarified or corrected. Some are details of wording that could be improved to enhance readability and understanding. Some are more fundamental. I provide them in the order they appear in the manuscript.

There is some use of terminology that has me puzzled and I think this must be clarified or corrected.
1) Intro, par 2. It appears that the authors are equating "pathogen fitness" with "pathogenicity", and defining "fitness" as "a pathogen's ability to infect a plant host and grow". I agree that this may be a good enough definition of "pathogenicity" (though I expect "pathogenicity" to include some pathogenic effects, i.e., damage to the host) but I do not AT ALL see that this is a good definition of "pathogen fitness". Surely infection and growth is not sufficient unless there is also reproduction or production of propagules. Pathogens may, in some cases, infect, grow and cause damage to the host without being able to successfully reproduce. They may have high pathogenicity but zero fitness.
Of course. We have dropped the term "fitness", which is not relevant here. "Pathogenicity is defined as the ability of a plant pathogen to cause disease, i.e. to infect a plant host and grow"

2) Intro, par 3-5. The authors appear to confuse "selection" with "evolution". These are not the same thing. Indeed, selection CAN lead to evolution, i.e., a change in allele frequencies over time. On the other hand, there can be selection without evolution if the selected phenotypic variation is not heritable. Perhaps non-heritable variation for virulence is unimaginable for a plant pathologist, so the authors feel no need to distinguish between selection and evolution. However, for the more general reader please be more rigorous in your wording. Par 3: host resistance decreases because of the evolution of pathogen populations, with an increase in the frequency of virulent or aggressive strains. **Done.** Par 4: I think you mean "… In several cases, evolution of greater aggressiveness was independent of host …" **Done.** Par 5: "gene flow" is not a "selective force". It IS an "evolutionary force". Here again, please distinguish clearly between selection, which is one of several evolutionary forces, and other evolutionary forces that are non-selective (e.g., mutation, drift, gene flow…). **Done.**

There are also some aspects of structure of the writing that puzzle me.
Paragraph 5 of the Intro presents a lot of different ideas. It starts by presenting reasons why there may be no change or no detectible change in aggressiveness over the course of an annual epidemic (1) the change is too small, 2) selection is to small to cause evolution, 3) selection is less powerful than migration (or I suppose, drift or mutation … why not?), 4) there may be competition within hosts (but the more aggressive may not always be the more competitive). Then it switches to talking about adaptation to host types, via serial passage or specialisation. This paragraph needs to be reorganised and structured to present a coherent message.
The paragraph was rewritten to remove confusion between selection and evolution. The structure was maintained because it corresponds to the message with want to give.

On the other hand, the next 2 paragraphs (6 and 7) both present the idea that the intra- versus inter-season or –epidemic processes may counteract each other. Perhaps this could also be better organised and structured.

Intro, last paragraph. This is mostly fine, but "remote dates" is not very clear. Perhaps try "at the temporal extremes" or simply "early and late over the time course of the experiment".
Done.

Also, "sympatric and allopatric" hosts is surprising here. It is not explained, so it is rather incomprehensible. Perhaps describe more directly what you did. On the other hand, it is not so clear to me that Apache can be considered "allopatric" since it was grown during the entire experimental period over rather large areas within the broad geographical area of the study. You even state, in the Methods, on page 4, column 2, top of the page, that you consider that Apache was a likely source of the local pathogen subpopulations… The terms sympatric and allopatric were also used for the sake of simplicity, as in Morias et al (2016).
This part was changed to "In our study, Soissons was referred to as "sympatric" host cultivar for the tested pathogen isolates because they were directly sourced from it. As a once-predominant cultivar now in decline, Soissons is assumed to have played a major role in the overall evolutionary trajectory of pathogen populations in France. Soissons played a specific role in the experimental study area because it was grown there in monoculture for eight years. Apache was considered to be an "allopatric" host cultivar. It partially replaced Soissons as the predominant cultivar in France, but probably played a less important role than Soissons in the evolutionary trajectory of the local pathogen population, with potentially some isolates immigrating from commercial fields located around the field plot. We therefore considered the most likely origins of the local pathogen subpopulations to be, firstly, cv. Soissons, and, secondly, cv. Apache."

Figure 3. The circled numbers are not explained in the legend. We learn about then on pages 7 and 8… They need some sort of explanation here.

The legend was completed by: "The circled numbers corresponds to discontinuities in pathogen pressure (change of at least 0.5 on a standardized scale, i.e. half the maximal amplitude observed during the 2008-2016 period for each variable normalized in the range 0-1) between two disease variables successive in time and positively correlated during the 2008-2016 period."

Methods: The spores for the Ai-2009 subpopulations were 6 years old when tested and compared with the (fresh) Af-2015 spores. Is this a problem? Should it be explicitly mentioned? What problems could this pose?
To our knowledge and our experience this is not a problem. The inoculum was obtained for each isolate from stock conidial suspensions stored at -80°C in glycerol-water. Certain stocks (e.g. Ai-2009) were older than others, but we (and also several colleagues) did not observe differences in growth in vitro or pathogenicity on plants after a few years of storage.

Methods, Page 6 2nd paragraph: What does "Three stems per plant were kept." mean? I have never worked with wheat. I understand that it tillers. Does this mean that you trimmed or clipped the plants so they had exactly three tillers? At 7 weeks of age? This seems strange to me but perhaps that is what you did. Please explain it more completely.
This is a standard procedure to growth adult wheat plants. After tillering, only three main stems are kept while all the others are cut in order to obtain three heads. This mimics competition between plants observed in fields.

Methods, Data analysis: This is not completely clear. Do you mean that the aggressiveness traits of each of the two pairs of subpopulations were assessed in a separate greenhouse trial, i.e., one trial for each pair of subpopulations? Or do you mean that for each of the two pairs of subpopulations there were two, independent greenhouse trials, i.e., two independent trials for each pair of subpopulations?
The first option is the right. The sentence was changed to: "The aggressiveness traits of each pair of pathogen subpopulations were assessed in two a separate greenhouse trial, by two different people."

Results Figure 6: The figure legend is not clear or complete. Please state the subpopulation from which the two "representative" isolates was drawn. Please state, for the test of I47 (which I recognise must be an ascospore isolate, whether the test was on cv. Soisson or Apache. And why do you present "representative" isolates instead of a central tendency and confidence intervals? Is this because you want to stress the variation observed for the different replicates?
This figure is just an example to represent the type and the amount of data which was used, and which were summarized in figure 7. This particular figure was chosen for the sake of simplicity and readability. The term "representative" was deleted, accordingly.

In the text, halfway down the first column of page 8 you state that you present "A representative example … for two Cf-2012 and Af-2015 isolates". You mean ""A representative example … for two isolates, one from Cf-2012 and one from Af-2015…"
The change was done.

Page 8 column 2 end of first paragraph: I do not really like the formulation "maladapted to their allopatric host". I have a bit of difficulty with the term "maladapted" in general. I know what "adaptation" is. It is the product ("an adaptation") or the process ("adaptation") of natural selection, which we generally consider leads to an increase in performance or fitness. I am less clear about what "maladapted" means, particularly in the context of allopatry. Does "maladapted" refer to a response to natural selection that did not lead to an increase in performance or fitness, or even one that leads to a decrease in performance or fitness? This can happen of course, and there are examples, but is that what you mean? On the other hand, if Apache is really "allopatric" (though I rather doubt that, since it is planted in the cultivation area) then there is no (or little) natural selection occurring on Apache, so there is no adaptive process if there is no selection. So perhaps you mean "maladapted" as a total absence of adaptation, because there is no natural selection? But then you should rather state that the ascospore subpopulations were not adapted to the allopatric host (which is what you would expect, if it were allopatric (unless you assume that adaptation to one host (Soissons) also generates adaptation to the other host (Apache), i.e., a general scheme of adaptation rather than a specific one. What I am trying to say is that I think it is inappropriate to talk about "adaptation" (mal- or otherwise) in the absence of selection. You can say they are not adapted … of course, because there has been no selection… There will be more on this below when we get to the Discussion.
The distinction between "adaptation" and "maladaptation" was introduced in the case of Z. tritici from experimental results presented in Morais et al. (2016): "Pathogen populations are usually considered to be locally adapted if they perform better on local (sympatric) hosts than on foreign (allopatric) hosts (Gandon and Van Zandt 1998; Kaltz and Shykoff 1998; Kawecki and Ebert 2004; Laine 2007). In contrast, pathogen populations are considered to be locally maladapted if they perform worse on sympatric than on allopatric hosts (Kaltz et al. 1999)." For the sake or clarity, "maladaptation" was deleted, except in one sentence (see answer to another remark below): "Rather, it may indicate local host adaptation (better performance on the "local" vs. "foreign" host): considering the latent period, after several years of monoculture the resident pathogen population becomes more adapted to sympatric host. This is consistent with the differential adaptation of resident and immigrant Z. tritici subpopulations to wheat cultivars previously established in the same study area (Morais et al., 2016) and elsewhere for the same pathosystem (Ahmed et al.; 1995, 1996), and, more generally, with evolutionary concepts (Kaltz & Shykoff, 1998; Gandon & Van Zandt, 1998; Kawecki & Ebert, 2004; Tack et al., 2012). Considering the lesion size, this conclusion must be however qualified by the gain of adaptation observed to the allopatric host (only) over the six-year period, which can be consider as an evidence of maladaptation (worse performance on the "local" vs. "foreign" host; Kaltz et al. 1999). This result is also consistent with those obtained by Morais et al. (2016) from the comparison of the latent period of the immigrant and resident subpopulations on the two cultivars"

Page 9, first column: "data not shown), but there was NO significant …"
Done.

Page 9, second column: The "The aggressiveness of isolates depended on the cultivar on which it was assessed" implies a significant interaction. Perhaps mention this and refer to the interaction.
The sentence was completed: "The aggressiveness of isolates depended on the cultivar on which it was assessed, as shown by the significant interactions C×I(P) ($p < 0.05$)."

Table 3: Did you test the CxP interactions? Indeed, I am not really clear on what the CxI(P) interaction means. A

significant I(P) term means that isolates within one or both of the subpopulations are significantly heterogeneous for the phenotype measured/tested. A significant interaction with cultivar implies that the degree of heterogeneity differs between the cultivars, I think, but does not necessarily imply that the means across isolates differ between the cultivars (which is what is suggested by the text of the results I copied above ("The aggressiveness of isolates depended on the cultivar on which it was assessed"). For example, you would find a significant CxI(P) interaction if there were strong heterogeneity on Apache but little heterogeneity on Soissons, even if the means were similar on the two cultivars… I think…
We are not sure we understand your point. All interactions were tested. In ANOVA presented in Table 3, only the CxI(P) interaction was significant. This is basically the CxI interaction, but I was noted I(P) because in the ANOVA a nested effect should be considered.

Table 3. Please, in the note in the legend, call this "lesion size" instead of "sporulation area" (if it is the same thing) for consistency.
Done.

The second paragraph of the discussion is rather difficult. I here once again have trouble with some of the ideas expressed. Top of Page 11 you suggest that "pathogens "become maladapted" to allopatric hosts following repeated cycles on a sympatric host." There is nothing really wrong with this, and there are plenty of examples (all the serial passage experiments that work to attenuate diseases), but there are several implicit, unstated assumptions behind this idea. Indeed, IF repeated cycles of selection on host A leads to an evolutionary response and IF this evolutionary response leads to a change in the frequency of alleles that WOULD BE relevant to performance on host B (either because there is a trade-off and alleles relevant to performance on host A have negative pleiotropic effects on host B or simply because, in absence of selection on host B alleles that WOULD BE relevant to performance on host B are lost by drift), then yes, repeated cycles on host A will lead to loss of performance on host B. And I guess I would accept this being called "become maladapted" because indeed, it is natural selection on host A that leads to a change in genotype frequencies and the phenotype of performance on host B. So after objecting initially to this statement, I have to admit that it's all right, given the implicit, unstated assumptions.
See answer to a previous related comment.

However, I find the paragraph after the list of citations ("…When variation in fitness across different spatiotemporal…) quite confusing:
First, "local adaptation" can only occur under certain conditions of selection differential and migration, i.e., only when migration does not counterbalance selection. Hence there are some spatiotemporal scales at which selection is not adequate to generate local adaptation.
Second, a host shift may alter the current selection environment, but it cannot "alter the environment in which immigrant strains underwent preadaptation". You appear to be suggesting some retroactive effect, whereby the initial selective conditions are modified by what happens subsequently… This cannot be what you mean.
The next sentence: I suggest: "Immigrant strains may perform poorly on their new hosts." It is clearer and simpler.
Done.

The next sentence (Several studies have shown how…) is really unclear. I think that, again, "selection" is used where "evolution" is meant. Perhaps you mean: "Several studies have shown that virulence and aggressiveness increase over time on predominant host genotypes…"
Done.

And the last "trade-off" sentence is not really clear to me either. What causes the "selection for higher levels of aggressiveness on susceptible versus moderately resistant cultivars"?
The sentence was completed: "However, the results of Ahmed et al. (1996) and Cowger et al. (2002) concerning selection for higher levels of aggressiveness on susceptible vs. moderately resistant wheat cultivars were inconsistent, possibly caused by an artifact due to a genetic trade-off between virulence and aggressiveness (Zhan et al., 2002)."

Next paragraph: What is the "evidence for local host (mal)adaptation? Please explain this.
"Maladaptation" was changed to "adaptation".

Page 11 column 2: First paragraph: I just have a bit of trouble with the statement "… the effects of selection are much smaller than those of diversity and the evolutionary forces responsible for its (whose?) maintenance, so the probability of detecting the same clone several times is very low." Perhaps just delete this?
Done

I am also perplexed by your use of the term "trade-off". For me, a trade-off implies either some sort of pleiotropy, with alleles that improve one aspect decreasing another, or a common resource base that can be spent on one function or another. Here what is suggested is that the selective advantage within the season limits access to mating partners, because the successful isolates find themselves alone without a compatible partner in the upper parts of the plants, such that ascospores are only produced in the debris bearing the earlier infections, where selection had not already sorted out the genotypes and reduced the genetic variation. Here, indeed, successful isolates end up in the upper regions of the host plants in non-mating situations, more or less as victims of their own success, but they do not lose mating opportunities in the older infections where they are represented, but so are the other, less intra-epidemically successful isolates. What I am trying to say is that the more aggressive isolates do not lose, they simply fail to win, i.e., the less successful ones have similar mating chances, and hence inter-epidemic transmission chances. For me this is not a trade-off. I find the Allee effect argument convincing and compelling, but I would not call it a trade-off, i.e., I do not think that all "self-limiting" processes imply an underlying trade-off.
"Trade-off" is often used a generic, shortened version of the term "genetic trade-off". We identified a "compromise" or an "antagonism" which is not a "genetic trade-off" but an "epidemiological trade-off". Although not common, "epidemiological trade-

off" is appropriate to disambiguate the meaning of "trade-off", as used by Fels & Kaltz (2006) ["We found a temperature-dependent, epidemiological trade-off between transmission and latency"] and Alizon & Lion (2011) ["Evolutionary trade-offs at the epidemiological level have a crucial effect on virulence evolution…"]. So, I suggest to change the title (and the other occurrence in the manuscript) "Epidemiological trade-off between… ". We also used "trade-off relationship" in the text.