

We are beyond grateful for your assistance in the improvement of our manuscript to be considered for the PCI in Ecology. We have attended the remarks of this second revision round. We hope that this new version of our manuscript fulfills the requirements to be recommended by PCI in Ecology. We are highly motivated to perform the proposed experiment. We will be cheerful to share with you our future findings.

Warmest thanks,

Sergio Gabriel Olvera Vazquez on behalf of all the co-authors

by Eric Petit,

Minor revisions

All reviewers agree upon the fact that you satisfactorily addressed their main comments and questions. Please however consider the following additional comments before I can recommend your preprint:

- your reference list has not been updated so that many papers you cite in the revised version of your preprint cannot be found in the reference list; it seems also that some of the papers that are cited in the reference list do not correspond to citations (different years for instance);

-We appreciate your observation. We have updated the reference list.

- lines 150-155: please rephrase to make your argument easier to follow. The rosy apple aphid is widely distributed all across the range of its domesticated host in Eurasia, also covering the distribution of its wild contributors (*M. sieversii* and *M. sylvestris*). Your population genetic study of the aphid, though quite extensive, did not address the question of its relationships with *M. sieversii*. It seems thus difficult to exclude that this parasite did not follow the domestication of apple trees before they were introgressed with *M. sylvestris* and other wild relatives (see Cornille et al. 2012 Plos Genet);

-We would like to thank you for your suggestion to improve the clarity of our manuscript. We have rephrased our statements in lines 149-153.

- line 296: do you really mean "conformed"? See also "by12";

-We agreed with your suggestion, we have modified the text in lines 294-295.

- Table 3: please make column headers correspond to the columns of the Table (3 column headers for 4 columns). Fig. 1: the red dot is not placed on Asturia, the green

one is not close to Angers, and the blue one is in France, not in Belgium;Fig. 3: the legend and the figure overlap.

- We have modified Table 3, Figure 1, and Figure 3 based on your remarks.

Reviewed by Alex Stemmelen

Dear Dr. Petit, dear Dr. Olvera-Vazquez,

I have reviewed the second version of the manuscript “Experimental test for local adaptation of the rosy apple aphid (*Dysaphis plantaginea*) to its host (*Malus domestica*) and to its climate in Europe.” and the authors have satisfyingly answered to every points I rose in the first round of review.

The first version of this protocol was already well-thought and detailed and this revised version appears clearer and more robust. Pitfall in the data analysis plan have also been addressed.

The pre-registration for this experiment seems suited for a recommendation by PCI Ecology. The experiment appears very promising and I look forward to see the results!

-We would like to thank you for your priceless remarks. Your suggestions have been key to improve our experiment. We will be happy to share with you our future findings.

Reviewed by Sharon Zytynska,

I have read through the author responses to all reviewer comments, and the updated version. The authors have clearly taken consideration of all comments made and also performed preliminary tests to ensure their methods will lead to the strongest output. I have no further comments and wish them well for the experiment.

-We really appreciate your invaluable feedback; it has been key for the improvement of our experimental design.

Reviewed by anonymous reviewer

After the first round of reviewing by PCI ecology of the experimental project “Experimental test for local adaptation of the rosy apple aphid (*Dysaphis plantaginea*) during its recent rapid colonization on its cultivated apple host (*Malus domestica*) in Europe.”, authors have answered accordingly and adequately the main questions that

I was asking and issues that I pointed. Also authors modified figures and added figures and text for clarification of the different approaches and methodologies.

More description on the fitness value acquisition was provided: two different absolute fitness values will be used as i/ colony growth rate, ii/ the aphid sizes for the three categories (larvae, winged &apterous) and will be run separately in the models as different fitness dependent variables. Also fitness value measurements were described more precisely and seem adequate to me as the local variability will be accounted for and standardized with referent susceptible Golden Delicious genotype behaviors.

The tree selection was mainly done on the two geographic/genotypic groups corresponding to Belgium (same as west France) vs Spain. No wild Eastern French genotypes were included (Cornille et al 2015).

However more genotyping data are being analyzed for each tree genotype and will be integrated in the result. Additionally, genomes of the 28 genotypes are to be sequenced and will be available for all the tree genotypes and R genes will be scanned. Possibly presence of R genes could be further described and accounted for in the model.

Tree ecophysiological traits will not be evaluated anymore as they supposedly do not bring more support and valuable information to the authors. I believe this is not completely true (and may be of very good use for other types of studies more on the trees adaptation) but regarding the size and ambition of the experiment and the work to be done, it makes sense to decrease the volume and number of modalities.

As for the possible systemic response of trees resulting from the multiple infection of the eight parasite genotypes, authors propose to add a leaf effect into the model to account partly for this variability. However I do not understand this claim: “each aphid genotype will be randomly infested on each leaf of each tree, at least eight times, we will therefore control for this systemic infestation”. How is it possible to control for systemic infestation: if a tree genotype shows a particular resistance toward one aphid genotype amongst eight wherever they are inoculated, it might also increase the overall resistance to the other seven.

- We agree with the reviewer. We were not clear enough. We cannot control the systemic response of the trees. However, the treatment (i.e., nine infestations per tree) is the same for all the trees, and we have multiple repeats of the treatment per apple genotype. We consider that, if the systemic response of the tree is differential depending on the part of the tree infested (upper, middle, and lower), we could statistically control for this differential systemic response. This is the reason why we will consider this leaf-level effect in our statistical model. We have modified our statement in lines 468-472. That is said, the systemic response of the apple tree to the aphids is not well understood, so we tried to adapt as much as possible the design to the current knowledge. Note as well that apple trees are infested by multiple pathogens and aphid species along the season (Ahmedi and Beliën, 2016; Miñarro and Dapena, 2007; Tan et al., 2021), so our design is not so far from the “natural setting”. We will discuss these points regarding systemic apple response in the full manuscript.

In conclusion, overall I believe that the author propose a sufficiently robust study to be recommended on PCI ecology. So I recommend this Study Information on PCI ecology and I am looking forward to having a look at the results.

- We thank you most warmly for recommended our manuscript to be considered for the PCI Ecology. As well as your invaluable remarks have helped us to improve our manuscript. We will be thrilled to share with you our future findings.

References

Alhmedi, A., & Beliën, T. (2016). Indirect competition in aphid community and biological control: Towards a new approach of aphid management in apple orchards.

Miñarro, M., & Dapena, E. (2007). Resistance of apple cultivars to *Dysaphis plantaginea* (Hemiptera: Aphididae): Role of tree phenology in infestation avoidance. *Environmental Entomology*, *36*(5), 1206-1211.

Tan, X. M., Yang, Z. S., Zhou, H., Yang, Q. M., & Zhou, H. X. (2021). Resistance performance of four principal apple cultivars to woolly apple aphid, *Eriosoma lanigerum* (Hemiptera: Pemphigidae), by simulated seasonal temperature in northern China. *Arthropod-Plant Interactions*, *15*(1), 59-69.