

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

This experiment is an interesting study about local adaptation of aphids to its host, the apple tree, in three European countries (*Spain, France and Belgium*). The protocol is well thought and well detailed. It is appreciable that the authors planned to use of a large number of trees in each common garden, which ensure that the results will be robust. Most pitfalls have been anticipated through randomization and bias mitigation.

It prove to be a difficult task to review the protocol with only limited background information on the scientific context and model species, therefore, we only made some general comments, followed by some advice in the data analysis process. Most of our comments are mainly meant to encourage further thinking and prevent future problems that could arise during the future writing of the article.

## General comments

The authors should be careful when referring to an effect of the **climate**, when they actually test for an effect of the **site**. If aphids from Spain show higher fitness in the Spain's common garden, that can be a sign of adaptation to climate but also soil type, predation by natural enemies, etc. More broadly, the term climate is not mentioned anymore in the models written later, while it is present in the title of the manuscript. Maybe climate will be accounted for through the use of local climatic variables as covariates, but this information was missing.

It could be helpful to add some clarification/definition of confusing terms such as **genotype/variety/cultivar**, mentioned several times in the protocol, in order not to confuse the reader.

Authors presents in the question 1 to 3 a null hypothesis H0 and multiple alternate hypotheses. This is surely correct and of course it is appreciable that the authors have anticipated what could be the response(s) of their studied system. However, usually only one alternate hypothesis H1 is formulated, based on current knowledge of aphid biology and aphid-host interaction. Please note that this remark is not a criticism of the planned work, just a general comment on what could be the last paragraph of the introduction of the paper to come.

Question 4 mentioned wild and cultivated apple trees. Do we have information on how the domestication of apple tree could have altered resistance to aphid infestation? For example, plant domestication can lead to a decrease in constitutive and induced chemical defences, which could increase the growth and performance of aphid on cultivated apple tree (see [Moreira et al. 2018, Scientific Reports](#) for example). Such information could help refine or precise the hypotheses made in the question 4.

Rosy aphid colonies are reared and maintained on Jonagold cultivar before being synchronized on Golden Delicious cultivar, and before being transferred onto several cultivars in the different common gardens. While the synchronization steps is defined as being done with parthenogenic female - thus preventing adaptation to the Golden Delicious cultivar – could it be possible that the maintenance phase will lead to an adaptation of the aphid genotype to Jonagold cultivar? If so, could we expect an increased fitness in apple genotypes closer to Jonagold cultivar? In other word, could we anticipate that the results could be different should the synchronization phase be done on any other cultivar?

The protocol mentioned the acquisition of several types of data on the local climate (*Temperature, humidity*) and apple tree physiology (*polyphenol content, chlorophyll*) but no information on how these data will be used is reported. While these data could probably be used *a posteriori* in the study (or in a companion paper), it is unclear how they will help answer the original question about local adaptation. Maybe it is not necessary to include them in the protocol if the authors don't plan on using them later in their analyses.

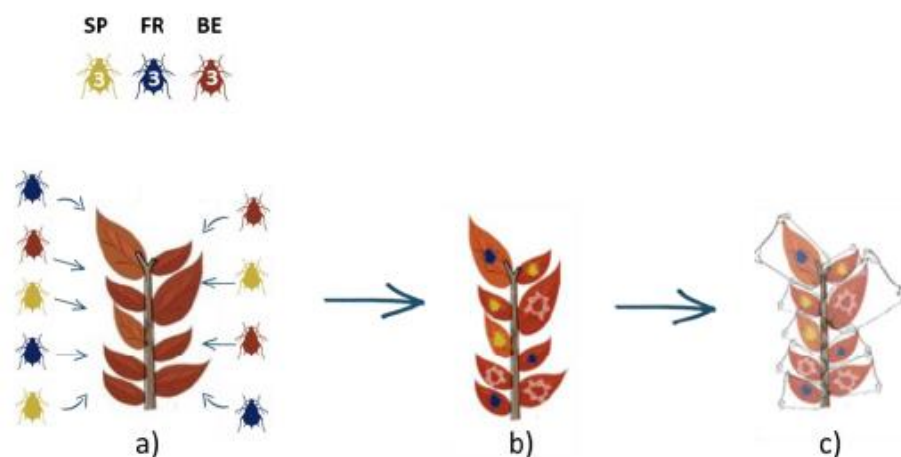
Cross-infestation will begin in Spring 2021. Does the start of the experiment will be similar in each location? Climatic condition could be different between Spain and Belgium for example and could lead to different fitness between location. Will this be taken into account in the modelling approach?

## Data analyses

Authors planned to make repeated measurements of aphid fitness in each apple tree in each common garden, as explain in the Fig. 4 below. If the authors conduct repeated measurements by using nine aphid genotypes on each tree, they have to make sure to include a parameter assessing the variability in aphid fitness attributable to the apple tree identity. The equation would then look like this:

$$W_{hijklmnttz} = \mu_w + \text{aphid\_origin}_h + \text{apple\_origin}_i + \text{site}_j + \text{site}_j(\text{block}_k) + Gh_l(\text{leaf}_m(\text{Gp}_n)) + \text{day\_of\_infestation}_t + \text{hour\_of\_infestation}_{t_2} + \text{Tree\_ID}_u + \text{aphid\_origin}_h * \text{site}_j + \text{aphid\_origin}_h * \text{apple\_origin}_i + \text{aphid\_origin}_h * \text{apple\_origin}_i * \text{site}_j + \epsilon_{hijklmnttz}$$

With  $\text{Tree\_ID}_u$  being random and a single ID given to each apple tree across all common garden, to assess the part of variability attributable to the apple tree identity.



Representation of rosy apple aphid infestation on the different apple genotypes. a) Nine aphid genotypes from different origins (three from Belgium, three from France, and three from Spain) will be used to infest a cultivated apple tree. b) A single synchronized adult female aphid from each of the nine aphid genotypes will be randomly infested on nine leaves of a tree. c) Each infestation will be protected with a cellophane bag and sealed with a stapler. BE = Belgium, FR=France, SP=Spain.

Following this issue, it is said that “aphid *genotype<sub>n</sub>* is nested within *leaf ID<sub>m</sub>*, and *leaf ID<sub>m</sub>* is nested within apple genotype *Gh<sub>l</sub>*, and they were added to the models as random-effect terms.”

However, each apple tree will be infested with 9 aphid genotypes on 9 different leaves or leaf clusters but there won't be more than one of each aphid genotype per apple tree, hence wouldn't the random effect  $leaf\ ID_m$  be confounded by the aphid  $genotype_n$  effect? You probably don't need to include the random  $leaf\ ID_m$  effect, as it would lead to potential overparametrization of your model.

During the cross-infestation experiment and if only one block is infected each day, caution should be taken that the random effect  $day\_of\_infestation_t$  isn't confounded with the random  $block_k$  effect.

Data will be transformed to fit linear model assumption. We suggest trying Generalized Linear Mixed-Models with suitable family (*Poisson or Gaussian for example*), depending on the data distribution, to avoid unnecessary transformation of the data.

It is possible that some aphid colonies will not establish, such that aphid count can be zero (i.e., less than the originally  $n = 1$  aphid per leaf). It is therefore possible that the dataset will contain a large amount of zeros, which may be a problem. Maybe this won't be the case, but if so it would be nice to know how the authors will deal with the data distribution. It is for instance possible to use zero-inflated poisson distribution in GLM(M)s. Alternatively, should the number of zeros be large, one option is to use a two-steps modeling approach, starting with a binomial response (1 = presence of an aphid colony ; 0 = No colony), followed by the analysis of aphid count data limited to the subset of non-zero outcomes.