Study Information

1.- Title

1.1. Provide the working title of your study. It may be the same title that you submit for publication of your final manuscript, but it is not a requirement.

Title: 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.

2.- Authors

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Abstract

Understanding the extent of local adaptation in natural populations and the mechanisms enabling populations to adapt to their environment is a major avenue in ecology research. Host-parasite interaction is widely seen as a major driver of local adaptation and has therefore been a study model to dissect the evolutionary processes at work during local adaptation. However, to date, the relative contributions of species interactions (*i.e.*, biotic factor) and abiotic factors to local adaptation are still unclear. Addressing these issues is more than a simple academic exercise. Understanding local adaptation processes in host-parasite interactions will also help to tackle pressing issues, such as the ways in which environmental changes alter the emergence of pathogens leading to host extinction, how to promote sustainability of agroecosystems in the face of emerging crop diseases or in guiding public health practices as more human pathogens and their vectors expand their ranges. Here, we propose to investigate whether local adaptation occurred during the recent rapid colonization of cultivated apple (*Malus domestica*) by *Dysaphis plantaginea*, the major aphid pest of cultivated apple orchards in Europe. We will experimentally test whether different populations, from Belgium, France, and Spain, of the aphid *D. plantaginea* show fitness differences in three common garden orchards located in Belgium, France, and Spain, comprised each of a panel of wild and cultivated apple genotypes from Belgium, France, and Spain, as well as previously reported tolerant and susceptible apple genotypes. This experiment will start in the Spring of 2021 and will generate original results adding to our understanding of how the biotic (the host) and abiotic conditions can shape local adaptation in a parasite.

Key words: local adaptation, aphid, fruit trees, apple, common garden, G*G*E interaction, host-parasite interaction, domestication.

3.- Research questions

3.1. Please list each research question included in this study.

4.- Hypotheses

4.1. For each of the research questions listed in the previous section, provide one or multiple specific and testable hypotheses. Please state if the hypotheses are directional or non-directional. If directional, state the direction. A predicted effect is also appropriate here.

The general question that we would like to address is whether there is a pattern of local adaptation of the rosy apple aphid (*Dysaphis plantaginea* Passerini) to 1) its local environment and/or 2) its cultivated apple host (*Malus domestica* Borkh)? To that aim, we will answer several questions, outlined below.

Question 1 and hypotheses: Is there evidence of rosy apple aphid adaptation to the local environment? Note here that the local environment will be tested with the "site" effect (Equation 1), which includes abiotic (*i.e.*, soil or climate) and biotic (*i.e.*, other aphid species and parasites of the cultivated apple host) factors. However, the biotic effect of the local cultivated apple host will be tested separately in Question 2. Note also that we will record the temperature of each leaf before and after the infestation. This temperature record per leaf will be used for statistical analyses to specifically test whether temperature plays a role in aphid infestation success (see statistical analyses part).

Do the rosy apple aphid genotypes from three different origins (Belgium, France, and Spain) show higher fitness in their local environment (*i.e.*, Belgium, France, and Spain, respectively) and lower fitness in their foreign environment (Figure 1)?

Hypothesis 0: There are no differences among the aphid populations across the three common garden orchards (Belgium, France, and Spain).

Hypothesis 1: There are differences among the aphid populations across the three common gardens (Belgium, France, and Spain). A significantly higher aphid fitness in the local common garden, while lower elsewhere, will support the hypothesis of local adaptation of the rosy apple aphid to its local environment. A significantly lower aphid fitness in the local common garden, while higher elsewhere, will support the hypothesis of maladaptation (Capblancq et al., 2020). Local adaptation of parasites is not a universal phenomenon; maladaptation has been observed in some systems such as the obligate parasite *M. violaceum* on its host *Silene latifolia* Poir. (Kaltz et al., 1999), with higher resistance of sympatric hosts. For aphids, only a handful of studies have been performed to test for local adaptation of aphids, and only to their hosts (Smadja et al., 2012; Simon et al., 2015; Wolly et al., 2020).

Question 2 and hypotheses: Is there evidence of rosy apple aphid adaptation to the local cultivated apple host genotypes?

Do the rosy apple aphid genotypes from three different origins (*i.e.*, Belgium, France, and Spain) show higher fitness on their respective local apple host genotypes (*i.e.*, local Belgian, French, and Spanish apple genotypes, respectively) and lower fitness on their foreign apple genotypes (Figure 1)?

Hypothesis 0: There are no differences among the aphid populations infested on the different local apple genotypes from different origins (Belgium, France, and Spain).

Hypothesis 1: There are differences among the aphid populations infested on the different apple genotypes from different origins (Belgium, France, and Spain). A significantly higher aphid fitness on local apple genotypes, while lower on non-local apple genotypes, will support the hypothesis of local adaptation of the rosy apple aphid to its host. A significantly lower aphid fitness on local apple genotypes, while higher on all other apple genotypes, will support the hypothesis of maladaptation of the rosy apple aphid to its host.

Question 3 and hypotheses: Is there evidence of rosy apple aphid adaptation to the local cultivated apple host and the local environment?

Is the fitness of the rosy apple aphid genotypes from three different origins (Belgium, France, and Spain) higher on their respective local cultivated apple host (*i.e.*, local Belgian, French, and Spanish apple genotypes) and in their respective local environment (*i.e.*, local Belgian, French and Spanish), compared with the fitness of the different rosy apple aphid genotypes on foreign apple host genotypes and the foreign environment (Figure 1)?

Hypothesis 0: There are no differences among the aphid populations infested on the different local apple genotypes from different origins (Belgium, France, and Spain) and across the three common gardens (Belgium, France, and Spain).

Hypothesis 1: There are differences among the aphid populations infested on the different local apple genotypes from different origins (Belgium, France, and Spain) and across the three common gardens (Belgium, France, and Spain). A significantly higher aphid fitness on the local apple genotypes and at the local common garden, while lower elsewhere, will support the hypothesis of local adaptation of the rosy apple aphid to its environment and host. On the other hand, a significantly lower aphid fitness on the local apple genotypes, and at the local common garden, while higher elsewhere, will support the hypothesis of maladaptation.

Question 4 and hypotheses: Is the rosy apple aphid adapted to the cultivated apple or to the locally occurring wild apple in Europe?

Is the fitness of the rosy apple aphid higher on the cultivated apple host than on the European wild apple *Malus sylvestris* (L.) Mill?

Hypothesis 0: There are no differences among the aphid populations infested either on wild or cultivated apple genotypes.

Hypothesis 1: There are fitness differences among the aphid populations infested on wild apple genotypes and cultivated apple genotypes. A significantly higher aphid fitness on the wild apple genotypes will support the hypothesis that the rosy apple aphid is better adapted to the local wild apple. So far, there is no information on how the domestication of the apple tree could have altered resistance to aphid infestation, but we can suggest a hypothesis. The

European wild apple is the local wild apple in Europe and has been present there for at least the past 120,000 years. In contrast, the cultivated apple has been present in Europe for much less time; it was brought by the Romans and Greeks in Europe about 1,500 years ago (Cornille et al. 2014, 2019). A population genetics study (Olvera-Vazquez et al. 2020) and the geographic distribution of the rosy apple mainly in Europe and the Middle East suggest that the rosy apple aphid has likely not followed its cultivated apple host journey from Central Asia to Europe. Therefore, the rosy apple aphid has probably been associated with the European wild apple longer time than with the cultivated apple and therefore may have had more time to adapt.

Question 5 and hypotheses:

Is the fitness of the rosy apple aphid lower on apple genotypes known *a priori* to be tolerant (Pagliarani et al., 2016, Marchetti et al. 2018) to the rosy apple aphid?

Hypothesis 0: There are no differences among the aphid populations infested on apple genotypes known to be tolerant to the rosy apple aphid infestation and on other susceptible apple genotypes.

Hypothesis 1: Yes, there are fitness differences between aphids infested on the tolerant apple genotypes (*Malus floribunda* Siebold ex Van Houtte, *M. domestica* Florina, and *M. domestica* Priscila) and the susceptible apple genotype (*M. domestica* Golden Delicious). Previous studies suggested that the apple genotypes tolerant to the rosy apple aphid infestations induced lower fitness of the rosy apple aphid (Miñarro and Dapena, 2007; Pagliarani et al., 2016; Dall'Agata et al., 2018).

Sampling plan

In this section we ask you to describe how you plan to collect samples, as well as the number of samples you plan to collect and your rationale for this decision. Please keep in mind that the data described in this section should be the actual data used for

analysis, so if you are using a subset of a larger dataset, please describe the subset that will actually be used in your study.

5.- Existing data

5.1 Preregistration is designed to make clear the distinction between confirmatory tests, specified prior to seeing the data, and exploratory analyses conducted after observing the data. Therefore, creating a research plan in which existing data will be used presents unique challenges. Please select the description that best describes your situation. Please do not hesitate to contact us if you have questions about how to answer this question (prereg@cos.io).

5.1.1 Registration prior to creation of data: the data have not yet been collected, created, or realized. YES

5.1.2. Registration prior to any human observation of the data: As of the date of submission, the data exist but have not yet been quantified, constructed, observed, or reported by anyone - including individuals that are not associated with the proposed study. Examples include museum specimens that have not been measured and data that have been collected by non-human collectors and are inaccessible. **NA**

5.1.3. Registration prior to accessing the data: As of the date of submission, the data exist, but have not been accessed by you or your collaborators. Commonly, this includes data that has been collected by another researcher or institution. **NA**

5.1.4. Registration prior to analysis of the data: As of the date of submission, the data exist and you have accessed it, though no analysis has been conducted related to the research plan (including calculation of summary statistics). A common situation for this scenario when a large dataset exists that is used for many different studies over time, or when a data set is randomly split into a sample for exploratory analyses, and the other section of data is reserved for later confirmatory data analysis. **NA**

5.1.5. Registration following analysis of the data: As of the date of submission, you have accessed and analyzed some of the data relevant to the research plan. This includes preliminary analysis of variables, calculation of descriptive statistics, and observation of data distributions. Studies that fall into this category are ineligible for the Pre-Reg Challenge. Please contact us (prereg@cos.io) and we will be happy to help you. **NA**

6. Explanation of existing data

6.1. If you indicate that you will be using some data that already exist in this study, please describe the steps you have taken to assure that you are unaware of any patterns or summary statistics in the data. This may include an explanation of how access to the data has been limited, who has observed the data, or how you have avoided observing any analysis of the

specific data you will use in your study. The purpose of this question is to assure that the line between confirmatory and exploratory analysis is clear. **NA**

7. Data collection procedures.

7.1. Please describe the process by which you will collect your data. If you are using human subjects, this should include the population from which you obtain subjects, recruitment efforts, payment for participation, how subjects will be selected for eligibility from the initial pool (e.g. inclusion and exclusion rules), and your study timeline. For studies that don't include human subjects, include information about how you will collect samples, duration of data gathering efforts, source or location of samples, or batch numbers you will use.

Overall design

The experiment will be located at three common garden orchards at 1) **Sint-Truiden in Belgium** (50°48´0" N, 5° 11´0" E), presenting a mean annual temperature of 9.6°C and annual precipitation of 823 mm, 2) **Les Hauts d'Anjou in France** (47°28'57" N, 0°36'52" W), presenting a mean annual temperature of 11.4°C and annual precipitation of 675 mm annual precipitation, and 3) **Villaviciosa in Asturias in Spain** (43°28'45" N, 5° 26´32" W), presenting a mean annual temperature of 11.8°C and annual precipitation of 869 mm. The bioclimatic information was extracted from the WorldClim – Global Climate database https://www.worldclim.org/ (Fick et al., 2017) with the raster R package (Hijmans and van Etter, 2012). In the spring of 2021, we will perform an infestation experiment using nine aphid genotypes, each representing the clonal offspring of a single female (called hereafter matriline) that had been collected in Belgium, France, and Spain, with three matrilines from each country. Below we describe the material that will be used.

Apple trees

Each common garden is made of **28 apple genotypes** (Figure 2, Table 1). A total of **15 cultivated apple genotypes** (*M. domestica*) **comes from three countries**, with five local genotypes from each country. The selection of the local cultivated apple genotypes was based on several criteria. First, whenever possible the genotypes were chosen to be apple genotypes locally cultivated in the surrounding area of each common garden. In the cases of Spain and France, the local genotypes encompass traditional genotypes, while in Belgium, the

253 cultivation of apple encloses recent commercial genotypes. Second, we chose cultivated
254 genotypes inferred not to be the most genetically closely related based on microsatellite
255 genetic characterization (Cornille et al., 2012). Third, unpublished qualitative assessments of
256 *D. plantaginea* attacks onto several cultivated apple varieties allowed choosing five apple
257 varieties per locality that showed variability in their response to *D. plantaginea* infestation
258 (from susceptible to tolerant). We also added **nine wild apple genotypes** (*M. sylvestris*), six
259 from Belgium, and three from Spain. We obtained scions from mother trees maintained in a
260 conservation orchard in Belgium, and from sampling in a forest in Northern Spain. The
261 choice of the genotypes was based on previous studies that showed that Spanish and Belgian
262 wild apples belonged to genetically differentiated populations in Europe (Cornille et al. 2013,
263 2015). Note that we failed to obtain scions for French wild apple genotypes in the year of the
264 grafting. We also included **four apple genotypes with different susceptibility levels to**
265 **aphid infestations**: three tolerant apple genotypes (two *M. domestica* apple genotypes,
266 'Priscilla' and 'Florina' genotypes, and one genotype of the ornamental species *Malus*
267 *floribunda*), and one susceptible genotype, the *M. domestica* Golden Delicious genotype. We
268 selected these apple genotypes to have a range of tolerance to *D. plantaginea* infestation
269 (Miñarro and Dapena, 2007; Pagliarini et al., 2016). Note that the 28 apple genotypes used
270 in this experiment have been genetically characterized using 13 microsatellite markers (data
271 not shown), and we sequenced their genomes (Illumina sequencing), which will be analyzed
272 in 2021.

273 According to the availability of the scions at the beginning of the project in 2018, we
274 grafted 10 to 12 clonemates for each of the 28 apple genotypes (Figure 2, Table 1). Besides,
275 for the aphid rearing and synchronization steps that will be performed at each common
276 garden orchard (see method below), we also grafted 206 clonemates of the Golden Delicious
277 genotype (Table 1), to get at least 60 trees per locality available for the rearing. In total, 1,157
278 apple trees (Table 1, 951 for the infestation experiment and 206 for the rearing step) were
279 grafted in early 2019 on an M9 Pajam 2® apple rootstock and maintained for one year
280 (February 2019-2020) at an outdoor nursery at La Retuzière, Les Hauts d'Anjou, Angers,
281 France (47°28'57" N, 0°36'52" W). In early February 2020, the trees were transferred and
282 planted in the three common garden orchards (Figure 2). Each tree was sprayed with
283 Teppeki® (flonicamida 50%) insecticide, a Bordeaux mixture (20% cupper) fungicide,

DELFIN® (*Bacillus thuringiensis* sp. *kurstaki*) anti-lepidopterous, Essen'ciel (orange essential oil) insecticide and fungicide, Karate Zeon® (Lambda cihalotrin 1.5%) and Movento® (Spirotetramat 15% p/v OD) insecticides, and Sokalcarbio WP® (calcined kaolin), a mineral physical barrier between pest and plants. These treatments will be continued until the beginning of the experiment (March 2021). We will also apply an aphicide and fungicide treatment two weeks before the beginning of the aphid infestation experiment (Figure 2).

Rosy apple aphid genotypes

We collected 36 rosy apple aphid colonies on several cultivated apple trees at each common garden during the spring of 2020. The colonies were conformed by12 colonies from Belgium, eight colonies from France, and 16 colonies from Spain. The colonies were sent to the GQE-Le Moulon laboratory at University Paris-Saclay in France. The colonies, consisting of one to several genotypes, are currently being reared and maintained asexually in a climate chamber at 20°C, 60-65% of relative humidity, 16 hours of light, and 8 hours of dark) on *in vitro* apple plants (Jonagold genotype) provided by the CRA-W (Micropropagation laboratory, Biological Engineering Unit, Gembloux, Belgium), in preparation for the cross-infestation experiment. The apple genotype used for aphid rearing (this case the Jonagold) were chosen to be different from any cultivars that are in the infestation experiment of the Spring of 2021, to avoid any aphid acclimatization to a specific apple genotype.

Currently, we are isolating one female from each colony onto a new *in vitro* Jonagold apple plant to ensure that we will have "single-genotype" colonies (*i.e.*, matrilines) for the infestation in March 2021. Indeed, although the aphid colonies were collected to avoid mixing several clonal lineages, this can happen. Therefore, once grown up enough (about 30 individuals), we will utilize a single adult aphid to start a new colony. After the colony grows about 30-40 individuals, the colony will be genetically characterized using newly developed microsatellite markers (Olvera-Vazquez, 2020). This step will allow us to build a collection of at least three distinct matrilineages from each locality (*i.e.*, Belgium, France, Spain) that will be available for the infestation experiment in March 2021. Because some lines could be

lost, we will maintain more than three genotypes per locality until March of 2021 in controlled conditions. In the end, from our complete set of 36 rosy apple colonies, we will maintain at least nine matrilines from Belgium, France, and Spain. In March 2021, some progeny of each of the nine-matriline rosy apple aphid colonies will be sent to each local laboratory in Belgium, France, and Spain. Locally, each lab will rear and synchronize each of the nine colonies in a greenhouse onto Golden Delicious genotypes (63 trees in Belgium, 80 trees in France, and 63 trees for Spain; Table 1) for the infestation experiment that will be performed in March 2021.

8. Sample size

8.1. Describe the sample size of your study. How many units will be analyzed in the study? This could be the number of people, birds, classrooms, plots, interactions, or countries included. If the units are not individuals, then describe the size requirements for each unit. If you are using a clustered or multilevel design, how many units are you collecting at each level of the analysis?

Global design and sampling size

Each common garden orchard contains 10 to 12 clones of each of the 28 apple genotypes (Table 1). These are planted in 10 to 12 rows, each row comprised of the available genotypes placed at random (Figure 3). The experiment will be divided into two modalities (Figure 3):
-**modality 1:** apple genotypes infested by the rosy apple aphid genotypes from different origins; seven to nine replicates of the 28 genotypes.
-**modality 2:** apple genotypes free of rosy apple aphid infestations; three replicates of the 28 genotypes that will be used as non-infested controls.

In the spring of 2021, we will perform a cross-infestation experiment. At that time, the planted apple genotypes will be two years old, having acclimatized to their field conditions in the common garden for one year. Each of the nine rosy apple aphid genotypes will be placed on a different leaf on the same apple tree of each of the 28 different apple genotypes in the three common garden orchards (Figures 2, 3, and 4 and Tables 1 and 2). The infestation will be performed at the apple phenological stage E2 when the development of

the inflorescences occurs (Figure 4). Aphid genotypes will be placed on the leaves at random for each level of the tree (upper, middle, lower). Performing the infestation is delicate and time-consuming and will, therefore, require several days to be completed (we estimate 18 days per orchard, see Figure 3). Every day, we will record the date of initiation of each infestation and include these in the analyses as temporal blocks and the time within the days as a covariate.

In total, we plan to perform 6,408 aphid infestations on 712 apple trees across the three common gardens in Belgium, France, and Spain (Figure 3 and Table 1), with nine aphid genotypes per tree (three aphid genotypes per location, from Belgium, France, and Spain). On those trees, we will have 2,196 infestations on 244 apple trees in Belgium, 2,214 infestations on 246 trees in Spain, and 1,998 infestations on 222 trees in France (Tables 1 and 2). We expect all trees to survive, but tree sample sizes may be reduced at the start of the experiment if trees die during the fall of 2020. Overall, each aphid genotype will be confronted with 1) five cultivated apple genotypes from its native range, 2) 10 cultivated apple genotypes from two different non-native ranges, 3) nine wild apple genotypes, and 4) three apple genotypes tolerant to rosy apple aphid infestations (two *M. domestica* and one *M. floribunda*). In addition, each aphid genotype will experience the climatic conditions from its native origin and two different local environmental conditions (including abiotic and biotic factors, such as climate or soil composition, and attacks of local parasites, respectively). This will allow us to experimentally test the existence of local adaptation of the rosy apple aphid to the cultivated apple host and its local environment, as well as to compare aphid performance on wild apple (*M. sylvestris*) and on apple genotypes tolerant to rosy apple aphid infestations.

Aphid genotypes and preparation for infestation

In early March 2021, each colony will be sent from the GQE-Le Moulon laboratory to each local laboratory in Spain, France, and Belgium for aphid rearing and synchronization in local greenhouses at 20ºC and 60 to 65% of relative humidity. Each colony will be reared and maintained on Golden Delicious apple trees grafted onto an M9 Pajam2® rootstock. Those

Golden Delicious trees were produced at the same time as the trees used in the common gardens (*i.e.*, 2019, Table 1).

We will infest on each leaf of a tree a "mini-colony", including two adult females and five larvae, of each aphid genotype. Indeed, infesting only one female is too risky, several trials in the lab showed that infestation success is minimal with a single female. We, therefore, plan to synchronize the rearing of each aphid genotype to get enough "mini-colonies" every 2-3 days along the infestation experimental period. We will need at least 40 synchronized "mini-colonies" of each aphid genotype per day to perform the cross-infestation schedule (Figure 5).

For the synchronization, we will place each of the nine aphid genotypes on Golden Delicious trees grafted onto an M9 Pajam®. Note that we will be able to test for the effect of genetic proximity of the Golden Delicious cultivar used for the rearing to the other apple genotypes used for the experiment as we have sequenced the genomes of the apple genotypes used in this study. For the rearing, one Golden Delicious tree will host a given aphid genotype. After two weeks of colony growth, we will expect to have enough females to start the aphid synchronization for each genotype. Once we will get enough adult females (10-20), we will synchronize the rearing for each aphid genotype (Figure 5). The aphid synchronization aims to ensure the same developmental stage of the females and larvae that will be infested on a plant. Aphid synchronization will start mid-March 2021. Details of the synchronization procedure are described in Figure 5. For each aphid genotype (Figure 4), we will launch the aphid synchronization gradually every 2-3 days on different leaves of a Golden Delicious tree. Daily aphid synchronization is indeed challenging in such a large experimental design. We, therefore, plan to synchronize our rearing every three-four days.

Detailed of modalities 1 and 2

As previously explained, we will test two modalities for each common garden (Figure 3):

-modality 1: apple genotypes infested with rosy apple aphids from different origins; seven to nine replicates of the 28 genotypes.

-**modality 2:** apple genotypes free of rosy apple aphid infestations; three replicates of the 28 genotypes that will be used as non-infested controls.

Modality 1: infestation, no treatment against aphids.

This modality will consist of the infestation of a mini-colony (two females and five larvae) by each of the nine aphid genotypes on nine different leaves on each of the 28 apple genotypes. Each mini-colony will be isolated using a clip-cage. Note that preliminary tests in our lab show that these clip cages do not influence aphid behavior (Florencio-Ortiz et al., 2018). Each leaf will be infested with a single aphid genotype from either Belgium, France, or Spain (Figure 3). The infestation will be performed in early April 2021. Starting early April will allow us to avoid as much as possible attacks or colonization by natural enemies and other apple aphid species.

Because the aphid life cycle may vary with the climatic conditions among sites, at each site we will observe the duration of the aphid life cycle from adult to daughter-adult on a "time infestation control" cultivated apple genotype (Table 1), *i.e.*, a susceptible Golden Delicious genotype (Miñarro and Dapena, 2008). At the beginning of the cross-infestation experiment, for each of the seven to nine lines (Figure 3), a Golden Delicious apple tree will be first systematically infested with an adult female aphid. This "reference" Golden Delicious will allow us to determine what standard duration of aphid infestation will be taken for that site, *i.e.*, what will be the time to wait after an infestation to collect the colonies for each site. This duration is usually between nine to 12 days after initial infestation (Warneys et al., 2018). After this duration determined for each site, we will cut off each infested leaf together with the clip cage. Then, we will disassemble the clip cage to take the leaf with the aphid colony and transfer them into a Falcon tube previously filled with ethanol 96%. In the laboratory, we will count the number of adults and nymphs with the software ImageJ (Schneider et al., 2012).

Modality 2: control without infestation, treatment against aphids

This modality will consist of the same 28 apple genotypes, not infested (Figure 3), repeated three times (Figure 3). On this modality, we will record the flowering time and bursting time.

9. Sample size rationale

9.1. This could include a power analysis or an arbitrary constraint such as time, money, or personnel.

In this experiment, we have three common garden orchards located at three sites in Europe, each with five local and 10 foreign cultivated apple genotypes. Thus, we replicate local host conditions by using five independent cultivated apple genotypes from three different areas of apple cultivation. Similarly, we use three distinct aphid clone lineages from each area of origin that will be tested and selected for their genetic differences with neutral markers expected to reflect general differentiation across their genomes. This allows us to ensure that any findings consistent with local adaptation are robust. Altogether, we will have 216 sympatric combinations and 423 allopatric combinations, which provides adequate power for testing local adaptation (Kaltz and Shykoff, 1998; Kaltz et al., 1999): we will have 2/3 of allopatric comparisons (*i.e.,* aphid genotypes infested on their foreign apple genotypes and environments) against 1/3 sympatric comparisons (*i.e.,* aphid genotypes infested on their local apple genotypes and environments) (Table 2). Eventually, we will have 6,408 infestation spots (single aphid genotype on a single apple genotype leaf) in the three common gardens: 2,196 in Belgium on 244 apple trees, 1,998 in France on 222 trees, and 2,214 in Spain on 246 trees (Table 2).

We choose to perform all infestation treatments with all aphid genotypes on each apple tree. This minimizes the error variance associated with differences among trees due to their physical condition or microsite variation and therefore maximizes our power to detect differences among aphid genotypes, apple genotypes, and common garden orchards. We replicate the number of infestations as much as is logistically possible to maximize the reliability of our measures of aphid performance on a particular apple genotype at a particular site. This setup that maximizes the number of combinations, with a multigenotype test per single tree can induce a systemic response of apple trees that can impact the fitness of a given aphid genotype within each apple tree. To control for that effect, each aphid genotype will

be randomly infested on each leaf level of each tree. The leaf level effect will allow taking into account a specific systemic effect depending on the level of the leaf used for the infestation. Finally, we do replicate our common garden orchards within the different areas of origin, *i.e.*, Belgium, France, and Spain. Therefore, we can adequately test the existence of local adaptation.

10. Stopping rule

10.1. If your data collection procedures do not give you full control over your exact sample size, specify how you will decide when to terminate your data collection.

NA

Variables

In this section you can describe all variables (both manipulated and measured variables) that will later be used in your confirmatory analysis plan. In your analysis plan, you will have the opportunity to describe how each variable will be used. If you have variables which you are measuring for exploratory analyses, you are not required to list them, though you are permitted to do so.

11. Manipulated variables

11.1. Describe all variables you plan to manipulate and the levels or treatment arms of each variable. For observational studies and meta-analyses, simply state that this is not applicable.

We manipulate the species host, the genotype of the cultivated and wild apples, the origin of the rosy apple aphids, and the sites of origin of the common garden orchards.

Apples used in this study will be of either cultivated (*M. domestica*) or wild (*M. sylvestris*) apples, with different genotypes for each. The cultivated apple genotypes were selected to represents local genotypes genetically far from each other and showing variability in the response against rosy apple aphid attacks. For the wild apple genotypes, we chose them because of the already-characterized population genetic differentiation that has been observed in the European wild apple (Cornille et al 2015). We however acknowledge that the current experiment will give a first insight into the natural response of the wild apple genotypes to the attacks of the rosy apple aphid.

We will select three different rosy apple aphid genotypes from each common garden orchard (*i.e.*, Belgium, France, and Spain) once they will be genetically characterized. To that end, we will use recently developed microsatellite markers for *D. plantaginea* to select the aphid genotypes with contrasting alleles to use for the infestation experiment.

The sites chosen for settling the common garden orchards represent a European latitudinal gradient to test the effect of local environments on the rosy apple aphid adaptation.

12. Measured variables

12.1. Describe each variable that you will measure. This will include outcome measures, as well as any predictors or covariates that you will measure. You do not need to include any variables that you plan on collecting if they are not going to be included in the confirmatory analyses of this study.

Rosy apple aphid fitness: we will measure aphid fitness for each of the nine rosy apple aphid genotypes infested on the 28 apple genotypes. The aphid fitness (*W*) will be calculated as follows (Warneys et al., 2018):

$$W = \frac{n(nymphs\ at\ end\ of\ infestation) - naphid\ (nymphs\ at\ beginning\ of\ infestation)}{total\ number\ of\ day\ of\ infestation}$$

We will also, if possible, count the different insect life stages (*i.e.*, aphid larvae (L1 to L5), apterous adults, and winged forms (Angeli and Simoni, 2006)). This will be done by scaling the individuals into three categories: big (apterous females), small (larvae), winged.

Additional measurements: we will record the temperature and humidity during the experiment with a local meteorological station available next to each common garden. We will also record the temperature of each leaf, before, during, and after the infestation with Near-infrared Spectroscopy (NIRS).

13. Indices

13.1. If any measurements are going to be combined into an index (or even a mean), what measures will you use and how will they be combined? Include either a formula or a precise description of your method. If you are using a more complicated statistical method to

combine measures (e.g. a factor analysis), you can note that here but describe the exact method in the analysis plan section.

Design Plan

In this section, you will be asked to describe the overall design of your study. Remember that this research plan is designed to register a single study, so if you have multiple experimental designs, please complete a separate preregistration.

14. Study type

14.1. Experiment - A researcher randomly assigns treatments to study subjects; this includes field or lab experiments. This is also known as an intervention experiment and includes randomized controlled trials. YES, our design includes randomization, see above.

14.2. Observational Study - Data is collected from study subjects that are not randomly assigned to a treatment. This includes surveys, natural experiments, and regression discontinuity designs. **NA**

14.3. Meta-Analysis - A systematic review of published studies. **NA**

14.4. Other - please explain. **NA**

15. Blinding

15.1. Blinding describes who is aware of the experimental manipulations within a study. Mark all that apply. YES

15.1.1. No blinding is involved in this study. **NA**

15.1.2. For studies that involve human subjects, they will not know the treatment group to which they have been assigned. **NA**

15.1.3. Personnel who interact directly with the study subjects (either human or non-human subjects) will not be aware of the assigned treatments.

Three persons will be involved in the experiment at each common garden (Belgium, France, and Spain). Thus, people will be aware of our treatments. However, we randomized the experiment as most as possible: the infestation spot of the aphid genotype (leaf of apple genotype infested with a single aphid genotype) and the coordinates of the apple trees within each block were previously randomized. In addition, we have recorded the localization of each apple tree at each common garden orchard. Now that they are planted and growing, the initial labels attached to each tree will be removed. The trees will then have a genotype code

that will not reveal the provenance or species of the apple tree during data collection. We will control for the leaf stage and sampler effect in our statistical models, as well as the time (day and hour) of infestation.

15.1.4. Personnel who analyze the data collected from the study are not aware of the treatment applied to any given group.

People involved during the processing of the data will be aware of the treatments of our experiment. The design was randomized as much as possible and the recorder effect will be tested in the statistical models, if a recorder there will be, it will be added to the equations presented in section 15.1.3. Moreover, the trees will have a genotype code that will not reveal the provenance or species of the apple tree during data collection. Therefore, people infesting apple trees, counting aphids, and assessing leaf damage will not know which combination is sympatric *versus* allopatric.

16. Study design

16.1. Describe your study design. Examples include two-group, factorial, randomized block, and repeated measures. Is it a between (unpaired), within-subject (paired), or mixed design? Describe any counterbalancing required. Typical study designs for observation studies include cohort, cross sectional, and case-control studies.

We have already described this part above and see Figures 2, 3, and 4, and Tables 1 and 2.

17. Randomization

17.1. If you are doing a randomized study, how will you randomize, and at what level?

Yes, we will use replicated common gardens in three countries, Belgium, France, and Spain. Each of these experimental fields will be made of rows with randomized apple trees to prevent spatial autocorrelation of error variance from being confounded with genotypic effects. The global view of the aphid cross-infestation experiment is described in Figure 2.

18. Statistical models

18.1. What statistical model will you use to test each hypothesis? Please include the type of model (e.g. ANOVA, multiple regression, SEM, etc) and the specification of the model

(this includes each variable that will be included as predictors, outcomes, or covariates). Please specify any interactions that will be tested and remember that any test not included here must be noted as an exploratory test in your final article.

Analysis Plan

You may describe one or more confirmatory analysis in this preregistration. Please remember that all analyses specified below must be reported in the final article, and any additional analyses must be noted as exploratory or hypothesis generating.
A confirmatory analysis plan must state up front which variables are predictors (independent) and which are the outcomes (dependent), otherwise it is an exploratory analysis. You are allowed to describe any exploratory work here, but a clear confirmatory analysis is required.

Combining the data of the three common gardens, we will confront sympatric combinations (*i.e.*, aphid genotypes infested on apple genotypes and environments of the same origin: France, Belgium, or Spain) against allopatric combinations (*i.e*., aphid genotypes infested on apple genotypes and environments of a different origin: France, Belgium, and Spain). We will also consider that an aphid population is locally adapted to its host and environment if its fitness is the highest on its local host and environment (Figure 1).

Statistical models

We will use a generalized linear mixed model (GLMM) including different factors according to the question and hypothesis that we will aim to answer. In this GLMM, the aphid genotype and apple genotype will be used as random effects, as well as the day and hour of infestation and the leaf level effect. The other effects will be fixed (see below). Then, we will gradually remove interactions and effects according to their significance. In addition, we will evaluate the differences in the effect on aphid fitness using a contrast analysis.

To test the existence of local adaptation, we will partition the three-way interaction among sites (common garden orchards), apple origin, and aphid origin into sympatric *versus* allopatric comparisons. This sympatric versus allopatric contrast will also be performed within each locality, *i.e.*, separately for the three different common garden orchards in a similar way, in order to determine whether there is local adaptation at the different sites.

The linear mixed model that we will use to tackle each of our research questions and hypotheses are described below:

Question 1- ($G_{parasite}$* local environment): $aphid\_origin_h$*$site_j$

Question 2- ($G_{parasite}$ *G_{host}): $aphid\_origin_h$ * $apple\_origin_i$

Question 3 - ($G_{parasite}$ *G_{host}* local environment): $aphid\_origin_h$*$apple\_origin_i$*$site_j$

The following factors will be used

Equation 1

$$W_{hijklmnott2z} = \mu_W + aphid\_origin_h + apple\_origin_i + site_j + site_j(block_k) + Gh_l(leaf_m(Gp_n)) + day\_of\_infestation_t + hour\_of\_infestation_{t2} + leaf\_level_m + tree\_clone_o + aphid\_origin_h*site_j + aphid\_origin_h* apple\_origin_i + aphid\_origin_h*apple\_origin_i*site_j + \varepsilon_{hijklmnott2z.}$$

Mathematic equation:

$$W_{hijklmntt2z} = \alpha_h + \beta_i + \gamma_j + B_{jk} + P_{lmn} + \delta_t + \zeta_{t2} + \mu_m + \omega_o + \alpha_h* \gamma_j + \alpha_h*\beta_i + \alpha_h*\beta_i* \gamma_j + \varepsilon_{hijklmnott2z.}$$

Table 3 describes the indexes, terms, and the effect included in our proposed GLMM. Where $W_{hijklmntt2z}$ is the absolute fitness value of an aphid genotype *Gp* (*i.e.* parasite genotype) from the country of origin *n* infested on the apple genotype *l*, apple tree clone *o*, in block *k* on *leaf level m* and in the common garden *j* infested at day *t* and hour *t2*, $leaf\_level_m$ is the position of the infested leaf in the apple tree (upper, middle or lower), $tree\_clone_o$ is the clone o of the apple genotype *l*, μ_W is the mean absolute fitness, $site_j$ is the common garden location (Belgium, Spain, France), $block_k$ is the block effect within each site for modality 1, $aphid\_origin_h$ is the country of origin of the aphid (Spain, France, Belgium), $apple\_origin_i$ is the country of origin of the apple genotype (Spain, France, Belgium), Gh_l is the apple genotype (*i.e.*, apple cultivar name) and $\varepsilon_{hijklmnott2z}$ is the residual term. *Block* is random and nested within the site, and aphid $genotype_n$ is nested within $leaf\_level_m$, and $leaf\_level_m$ is nested within apple genotype Gh_l, and they were added to the models as random-effect terms.

The $leaf\_level_m$ effect is also added as a random factor alone to account for the global variability in aphid fitness that is explained by the levels at which each aphid colony was infested, whatever the apple genotypes. The *site* term measures the quality or suitability of the common garden locations, *aphid_origin* and *apple_origin* accounts for differences in fitness intrinsic to each local aphid genotype and apple genotype country of origin, $aphid\_origin_h*site_j$ accounts for differences in local adaptation to the environment among the three aphid origins, $aphid\_origin_h*\ apple\_origin_i$ account for differences in local adaptation to the host among the three aphid origins, $aphid\_origin_h*apple\_origin_i*site_j$ accounts for differences in local adaptation to the host and environment among the three aphid origins. The $day\_of\_infestation_t$ and the $hour\_of\_infestation_{t2}$ consider the effect of the infestation time of the aphid genotype *Gp* from the country of origin *n* on the apple genotype *l* in block *k* on *leaf m* and in the common garden *j*. We will run our proposed model using three different measures of absolute fitness (*W*): colony growth rate, and if possible, aphid sizes and aphid developmental stages. Note that we will measure the temperature of each apple leaf before and after aphid infestation. Temperature measured for each leaf will be first added as a fixed effect in a linear mixed model depicted in Equation 1 but without the site effect. If any effect is detected, the temperature will be added in Equation 1 as a covariance-variance matrix of a site random effect.

Question and hypothesis 4: **testing in the model the $aphid\_origin_h*\ crop\_wild\_status_i$ interaction.**

Equation 2

$$W_{hijklmnott2yz} = \mu_W + aphid\_origin_h + crop\_wild\_status_y + site_j + site_j(block_k) + Gh_l(leaf_m(Gp_n)) + day\_of\_infestation_t + hour\_of\_infestation_{t2} + leaf\_level_m + + tree\_clone_o + aphid\_origin_h*site_j + aphid\_origin_h*\ crop\_wild\_status_y + aphid\_origin_h*\ crop\_wild\_status_y\ *site_j + \varepsilon_{hijklmnott2yz}$$

Mathematic equation:

$$y_{hyjklmnott2yz} = \alpha_h + \eta_y + \gamma_j + B_{jk} + P_{lmn} + \delta_t + \zeta_{t2} + \mu_m + \omega_o + \alpha_h*\ \gamma_j + \alpha_h*\eta_y + \alpha_h*\eta_y*\ \gamma_j + \varepsilon_{hyjklmnott2yz}.$$

Question and hypothesis 5: testing in the model $aphid\_origin_h$*$tolerant\_status_i$*$site_j$ interaction

Equation 3

$$W_{hxjklmnott2xz} = \mu_W + aphid\_origin_h + tolerant\_status_x + site_j + leaf\_level_m + site_j(block_k) + Gh_l(leaf_m(Gp_n)) + day\_of\_infestation_t + hour\_of\_infestation_{t2} + tolerant\_status_x * site_j + + tree\_clone_o + aphid\_origin_h * tolerant\_status_x + aphid\_origin_h * tolerant\_status_x * site_j + \varepsilon_{hijklmnott2xz.}$$

Mathematic equation:

$$y_{hxjklmnott2xz} = \alpha_h + \kappa_x + \gamma_j + \omega_o + \mu_m + B_{jk} + P_{lmn} + \delta_t + \zeta_{t2} + \alpha_h * \gamma_j + \alpha_h * \kappa_x + \alpha_h * \kappa_x * \gamma_j + \varepsilon_{hxjklmnott2xz.}$$

19. Transformations

19.1. If you plan on transforming, centering, recoding the data, or will require a coding scheme for categorical variables, please describe that process.

We will transform our future data depending on the normality and dispersion of the residuals in our models.

20. Follow-up analyses

20.1. If not specified previously, will you be conducting any confirmatory analyses to follow up on effects in your statistical model, such as subgroup analyses, pairwise or complex contrasts, or follow-up tests from interactions. Remember that any analyses not specified in this research plan must be noted as exploratory. **NA**

21. Inference criteria

21.1. What criteria will you use to make inferences? Please describe the information you will use (e.g. p-values, Bayes factors, specific model fit indices), as well as cut-off criterion, where appropriate. Will you be using one or two tailed tests for each of your analyses? If you are comparing multiple conditions or testing multiple hypotheses, will you account for this?

As explained in section 18, we will consider multiple variables, factors, and interactions in our statistical models.

22. Data exclusion

22.1. How will you determine what data or samples, if any, to exclude from your analyses? How will outliers be handled?

We will not exclude data. We will transform our data to fit the homoscedasticity of the residuals. If there is an outlier, *e.g.*, one observation that looks vastly different from the other ones, we will first check whether there could have been any mistake. We will come back to the tubes in which each colony is conserved to count and check the number of aphids to control for mistakes. If the outlier is still valid, we will further investigate this number.

23. Missing data

23.1. How will you deal with incomplete or missing data?

The lack of aphids on a leaf will be a key-value, this will be counted as a true observation, *i.e.*, the absence of growth (*i.e.*, less than the original two aphid females and 5 larvae per leaf), death aphids. We will utilize Poisson, Gaussian distribution, or two-steps modeling approach with a binomial response (1 = aphid colony; 0 = absence of aphid colony) and the analysis of the aphid counting data depending on the subset of non-zero outcomes. Nevertheless, we will try to minimize recording zero in our data to avoid unnecessary data transformation. In particular, aphids are overly sensitive to any change in environmental conditions and some infestation might fail for a technical reason. Then, if after one day of infestation the female has died, we will consider that the infestation has failed. In the case of a technical issue, we will infest again the next day and we will note this re-infestation and take it into account for statistical analyses (section 18). We will check if the female aphid died because of a technical issue or for a biological reason.

24. Exploratory analysis (optional)

24.1. If you plan to explore your data set to look for unexpected differences or relationships, you may describe those tests here. An exploratory test is any test where a prediction is not made up front, or there are multiple possible tests that you are going to use. A statistically significant finding in an exploratory test is a great way to form a new confirmatory hypothesis, which could be registered at a later time. **NA**

Script (Optional)

The purpose of a fully commented analysis script is to unambiguously provide the responses to all of the questions raised in the analysis section. This step is not common, but we encourage you to try creating an analysis script, refine it using a modeled dataset, and use it in place of your written analysis plan. **NA**

25. Analysis scripts (Optional) **NA**

25.1. (Optional) Upload an analysis script with clear comments. This optional step is helpful in order to create a process that is completely transparent and increase the likelihood that your analysis can be replicated. We recommend that you run the code on a simulated dataset in order to check that it will run without errors. **NA**

Other

26. Other (Optional)

26.1. If there is any additional information that you feel needs to be included in your preregistration, please enter it here. **NA**

Table 1. Country of origin, species, name or identification ID, number of trees, assigned to each common garden. B = Belgium, F = France, and S = Spain. Each genotype has an identification including 1) the genotype name and 2) the accession ID.

Origin of the genotypes	ID	Common garden orchards			TOTAL	Origin of the genotypes	ID	Common garden orchards			TOTAL
		B	F	S				B	F	S	
Belgium (*Malus domestica*)	Braeburn_ P03a01	12	11	12		**European wild apple Belgium (*Malus sylvestris*)**	syl_be 148	10	10	10	
	Elstar_ P03a02	12	11	12			syl_be 4	11	11	12	
	Fuji_ P03a12	11	11	12			syl_be 54	11	10	11	
	Granny Smith_ P03a04	12	11	12			syl_be 60	11	10	11	
	Wellant_ V05a1	11	11	12			syl_be 76	12	11	12	
	Total Belgian trees	**58**	**55**	**60**	**173**		syl_be 93	11	11	12	
France (*Malus domestica*)	Api_Noir_	12	11	12			**Total Belgian wild apple trees**	**66**	**63**	**68**	**197**
	Clochard_A5	12	11	12		**European wild apple Spain (*Malus sylvestris*)**	syl_es B	11	11	11	
	Reale_d'Entraygues	11	11	11			syl_es D	10	9	10	
	Reinette_Franche	12	11	12			syl_es F	12	11	12	
	Reine Des Reinettes Tasse	12	11	12			**Total Spanish wild apple trees**	**33**	**31**	**33**	**97**
	Total French trees	**59**	**55**	**59**	**173**		**Total European wild apple trees**	**99**	**96**	**101**	**296**
Spain (*Malus domestica*)	Limón_Montés_M0236	12	11	12		**Tolerant control**	*Malus floribunda* _X6518	11	11	11	
	Perico_M0056	11	11	12			Florina_ X2775	11	10	11	
	Raxao_M0174	12	11	12			Priscilla X2851	12	11	12	
	Regona_M0239	11	11	12			**Total tolerant trees**	**34**	**32**	**34**	**100**
	Xuanina_M0084	12	11	12			**Total per site (for infestations: modality 1)**	244	220	246	710

	Total Spanish trees	**58**	**55**	**60**	**173**		**Total per site (control without infestations: modality 2)**	76	83	80	239
Susceptible control	Golden Delicious cv.	12	12	12	**36**		Total	**320**	**305**	**326**	**951**
						Aphid rearing and synchronization (February 2021)	Golden Delicious cv.	63	80	63	**206**
						TOTAL over sites	(infestation + rearing)				**1193 trees**

Table 2. Number of leaves infested with aphids planned in the Spring of 2021 at each common garden orchard in Belgium, France, and Spain, on each of the 28 apple genotypes (*Malus domestica* and *Malus sylvestris*, respectively). The apple genotypes included 15 *M. domestica* genotypes: five genotypes from Belgium (B1 to B5), five genotypes from France (F1 to F5), and five genotypes from Spain (S1 to S5); three tolerant apple genotypes from France (T1 to T3: two *M. domestica* apple genotypes, 'Priscila' cv. and 'Florina' cv., and one *Malus floribunda* Siebold ex Van Houtte); one susceptible genotype "Golden Delicious" (GD); Nine European wild apple genotypes *M. sylvestris* (W1 to W9, six from Belgium and three from Spain). For the aphid, three genotypes per locality, with BE_X = Belgian aphid genotype X; FR_X = French aphid genotype X; SP_X = Spanish aphid genotype X. Sympatric combinations are highlighted in grey and allopatric combinations are not highlighted.

Malus domestica																			**Controls**					***Malus sylvestris***							
Belgian tres						**French trees**							**Spanish trees**							**Resistant**				**Susceptible**	**Belgian trees**						**Spani**	
1	**B2**	**B3**	**B4**	**B5**	**SUM**	**Aphid**	**F1**	**F2**	**F3**	**F4**	**F5**	**SUM**	**Aphid**	**S1**	**S2**	**S3**	**S4**	**S5**	**SUM**	**Aphid**	**R1**	**R2**	**R3**	**GD1**	**W1**	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**
9	9	9	9	8	44	**BE_1**	9	9	9	9	9	45	**BE_1**	9	9	9	8	9	44	**BE_1**	8	8	9	9	7	9	8	8	9	9	9	9
9	9	9	9	8	44	**BE_2**	9	9	9	9	9	45	**BE_2**	9	9	9	8	9	44	**BE_2**	8	8	9	9	7	9	8	8	9	9	9	9
9	9	9	9	8	44	**BE_3**	9	9	9	9	9	45	**BE_3**	9	9	9	8	9	44	**BE_3**	8	8	9	9	7	9	8	8	9	9	9	9
9	9	9	9	8	44	**FR_1**	9	9	9	9	9	45	**FR_1**	9	9	9	8	9	44	**FR_1**	8	8	9	9	7	9	8	8	9	9	9	9
9	9	9	9	8	44	**FR_2**	9	9	9	9	9	45	**FR_2**	9	9	9	8	9	44	**FR_2**	8	8	9	9	7	9	8	8	9	9	9	9
9	9	9	9	8	44	**FR_3**	9	9	9	9	9	45	**FR_3**	9	9	9	8	9	44	**FR_3**	8	8	9	9	7	9	8	8	9	9	9	9
9	9	9	9	8	44	**SP_1**	9	9	9	9	9	45	**SP_1**	9	9	9	8	9	44	**SP_1**	8	8	9	9	7	9	8	8	9	9	9	9
9	9	9	9	8	44	**SP_2**	9	9	9	9	9	45	**SP_2**	9	9	9	8	9	44	**SP_2**	8	8	9	9	7	9	8	8	9	9	9	9
9	9	9	9	8	44	**SP_3**	9	9	9	9	9	45	**SP_3**	9	9	9	8	9	44	**SP_3**	8	8	9	9	7	9	8	8	9	9	9	9
SUM					**396**		**SUM**					**405**		**SUM**					**396**		**SUM**											
1	**B2**	**B3**	**B4**	**B5**	**SUM**	**Aphid**	**F1**	**F2**	**F3**	**F4**	**F5**	**SUM**	**Aphid**	**S1**	**S2**	**S3**	**S4**	**S5**	**SUM**	**Aphid**	**R1**	**R2**	**R3**	**GD1**	**W1**	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**
9	9	9	9	9	45	**BE_1**	9	9	9	9	9	45	**BE_1**	9	9	9	8	9	44	**BE_1**	8	8	9	9	7	9	8	9	9	9	9	9
9	9	9	9	9	45	**BE_2**	9	9	9	9	9	45	**BE_2**	9	9	9	8	9	44	**BE_2**	8	8	9	9	7	9	8	9	9	9	9	9
9	9	9	9	9	45	**BE_3**	9	9	9	9	9	45	**BE_3**	9	9	9	8	9	44	**BE_3**	8	8	9	9	7	9	8	9	9	9	9	9
9	9	9	9	9	45	**FR_1**	9	9	9	9	9	45	**FR_1**	9	9	9	8	9	44	**FR_1**	8	8	9	9	7	9	8	9	9	9	9	9
9	9	9	9	9	45	**FR_2**	9	9	9	9	9	45	**FR_2**	9	9	9	8	9	44	**FR_2**	8	8	9	9	7	9	8	9	9	9	9	9
9	9	9	9	9	45	**FR_3**	9	9	9	9	9	45	**FR_3**	9	9	9	8	9	44	**FR_3**	8	8	9	9	7	9	8	9	9	9	9	9
9	9	9	9	9	45	**SP_1**	9	9	9	9	9	45	**SP_1**	9	9	9	8	9	44	**SP_1**	8	8	9	9	7	9	8	9	9	9	9	9

9	9	9	9	9	45	**SP_2**	9	9	9	9	9	45	**SP_2**	9	9	9	8	9	44	**SP_2**	8	8	9	9	7	9	8	9	9	9	9	9	
9	9	9	9	9	45	**SP_3**	9	9	9	9	9	45	**SP_3**	9	9	9	8	9	44	**SP_3**	8	8	9	9	7	9	8	9	9	9	9	9	
SUM					**405**		**SUM**					**405**		**SUM**					**396**		**SUM**													
1	**B2**	**B3**	**B4**	**B5**	**SUM**	**Aphid**	**F1**	**F2**	**F3**	**F4**	**F5**	**SUM**	**Aphid**	**S1**	**S2**	**S3**	**S4**	**S5**	**SUM**	**Aphid**	**R1**	**R2**	**R3**	**GD1**	**W1**	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**	
8	8	8	8	8	**40**	**BE_1**	8	8	8	8	8	40	**BE_1**	8	8	8	8	8	40	**BE_1**	8	7	8	8	7	8	8	8	8	8	8	8	
8	8	8	8	8	40	**BE_2**	8	8	8	8	8	40	**BE_2**	8	8	8	8	8	40	**BE_2**	8	7	8	8	7	8	8	8	8	8	8	8	
8	8	8	8	8	40	**BE_3**	8	8	8	8	8	40	**BE_3**	8	8	8	8	8	40	**BE_3**	8	7	8	8	7	8	8	8	8	8	8	8	
8	8	8	8	8	40	**FR_1**	8	8	8	8	8	40	**FR_1**	8	8	8	8	8	40	**FR_1**	8	7	8	8	7	8	8	8	8	8	8	8	
8	8	8	8	8	40	**FR_2**	8	8	8	8	8	40	**FR_2**	8	8	8	8	8	40	**FR_2**	8	7	8	8	7	8	8	8	8	8	8	8	
8	8	8	8	8	40	**FR_3**	8	8	8	8	8	40	**FR_3**	8	8	8	8	8	40	**FR_3**	8	7	8	8	7	8	8	8	8	8	8	8	
8	8	8	8	8	40	**SP_1**	8	8	8	8	8	40	**SP_1**	8	8	8	8	8	40	**SP_1**	8	7	8	8	7	8	8	8	8	8	8	8	
8	8	8	8	8	40	**SP_2**	8	8	8	8	8	40	**SP_2**	8	8	8	8	8	40	**SP_2**	8	7	8	8	7	8	8	8	8	8	8	8	
8	8	8	8	8	40	**SP_3**	8	8	8	8	8	40	**SP_3**	8	8	8	8	8	40	**SP_3**	8	7	8	8	7	8	8	8	8	8	8	8	
SUM					**360**		**SUM**					**360**		**SUM**					**360**		**SUM**													

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Table 3. Description of the indexes, terms, and the effects included in our proposed statistical model General Linear Mixed Model (GLMM) to test for local adaptation of the rosy apple aphid (*Dysaphis plantaginea*).

Index		Term	Effect
α	h	$Aphid\_origin_h$	Aphid country of origin (Spain, France, Belgium), **fixed** effect
β	i	$Apple\_origin_i$	Apple country of origin (Spain, France, Belgium), **fixed** effect
γ	j	$Site_j$	Common garden site (Spain, France, Belgium), with a covariance-variance matrix of difference of temperature (or humidity) between each apple tree before (or after) the infestation, **fixed** effect
B	k	$Block_k$	Block (each block consists of 28 apple genotypes infested with 9 aphid genotypes), **random** effect
	l	Gh_l	Apple host genotype, random effect
μ	m	$Leaf\_level_m$	Leaf level (Position of the infested apple leaf on the main stem. Three levels: upper, middle, or lower), **random** effect
P	n	Gp_n	Aphid parasite genotype, random effect
ω	o	$Tree\_clone_o$	Apple clone of a given genotype, **random** effect
δ	t	$Time\ of\ infestation_t$	Day of infestation, **random** effect
ζ	t2	$Time\ of\ infestation_{t2}$	Hour of infestation, **random** effect
κ	x	$Tolerant\_status_x$	Tolerant or susceptible genotype status assessed from previous studies (Miñarro and Dapena, 2008), **fixed** effect
η	y	$Crop\_wild\_status_y$	Cultivated or wild apple host (*Malus domestica* and *Malus sylvestris*, respectively), **fixed** effect
	z		Effect of each observation
ε			Residual error

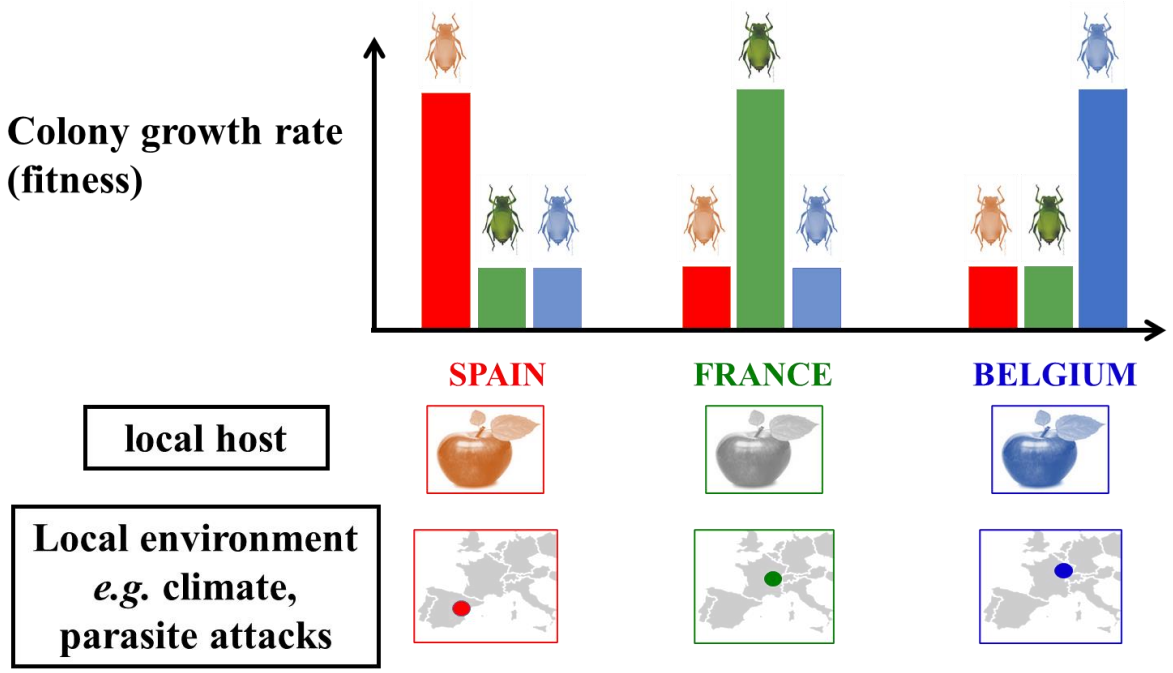

Figure 1. Expected patterns in the case of the rosy apple aphid (*Dysaphis plantaginea*) are locally adapted to its local environment and host. The rosy apple aphid populations that present the highest fitness in their local abiotic environment and host will reflect local adaptation.

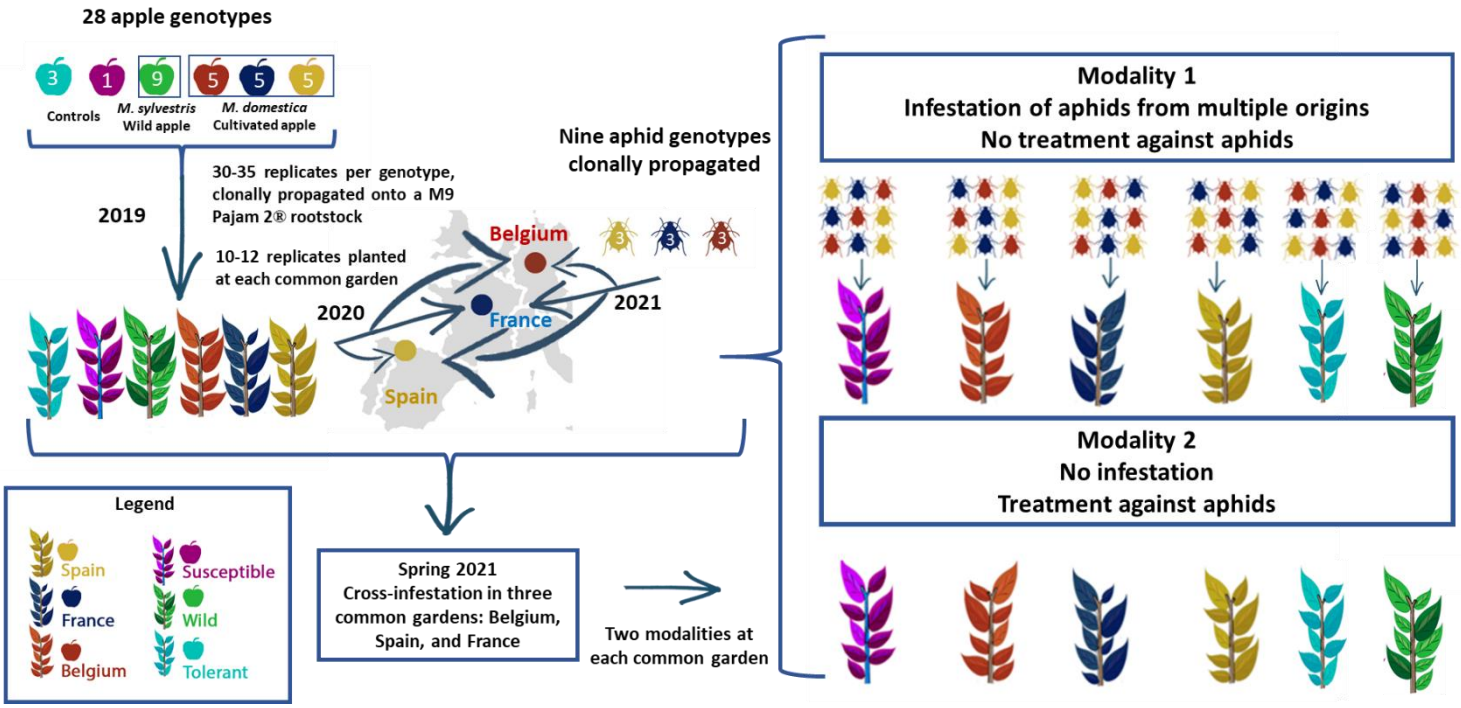

Figure 2. General scheme of the aphid cross-infestation experiment that will be performed in the Spring of 2021 at the three common garden orchards in Belgium, Spain, and France. At each common garden orchard, 28 clonally propagated apple genotypes are grown with 10 to 12 replicates per genotype, depending on the survival of the grafted trees at each common garden. The apple genotypes included 1) *Malus domestica* genotypes from Belgium (five genotypes, red color), France (five genotypes, dark blue color), and Spain (five genotypes, yellow color). Additionally, 2) nine wild apple genotypes (*Malus sylvestris*), including six from Belgium and three from Spain (light green color), 3) *M. domestica* genotypes (Priscilla and Florina cultivars), and *Malus floribunda* Siebold ex Van Houtte, used as "tolerant to aphid infestation" controls (light blue color), and 4) the Golden delicious *M. domestica* genotype that will be used for aphid rearing as well as the "susceptible to aphid infestation" control (purple). Meanwhile, nine rosy apple aphid genotypes (*Dysaphis plantaginea*) were clonally propagated: three from Belgium (red color), three from France (dark blue color), and three from Spain (yellow color). A total of 10-12 replicates of each of the 28 apple genotypes were transferred in February 2020 to each of the three common gardens. The aphid genotypes will be transferred for rearing locally in February 2021 at each site.

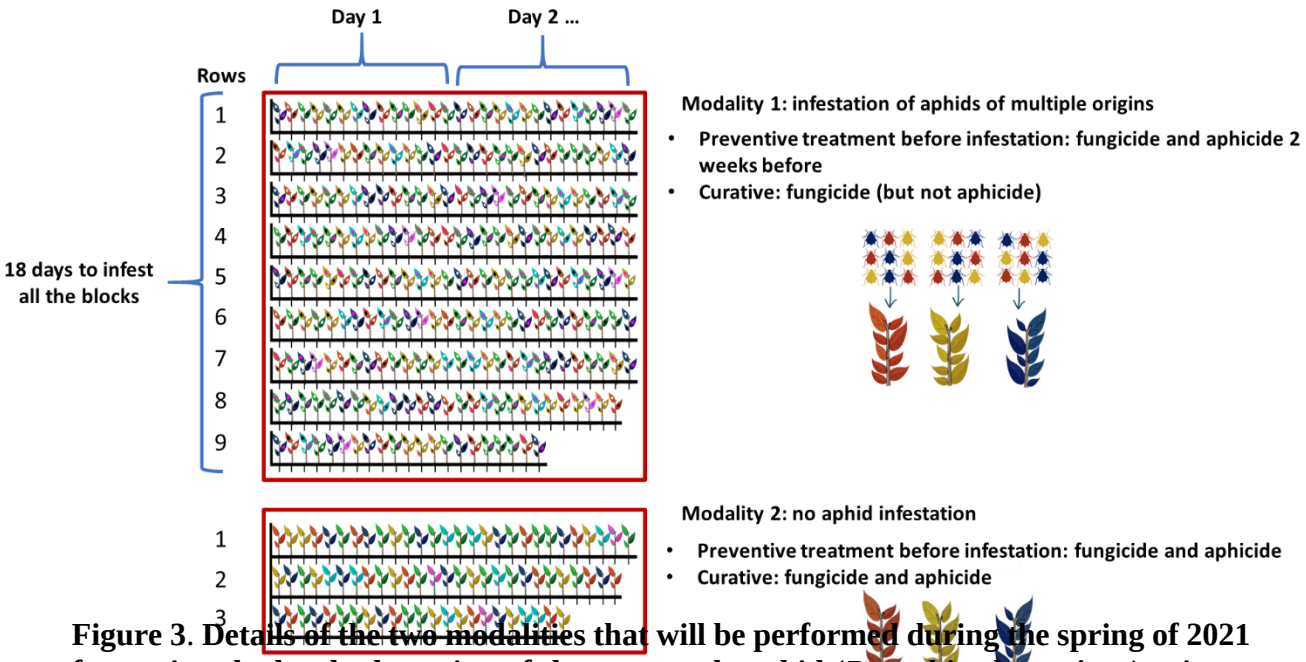

Figure 3. Details of the two modalities that will be performed during the spring of 2021 for testing the local adaptation of the rosy apple aphid (*Dysaphis plantaginea*) using a cross-infestation experiment. Here an example of the common garden in Belgium (Sint-Truiden). The experimental field of each common garden consists of rows, each including the 28 apple genotypes positioned at random in the row; the final rows lack a few genotypes due to the death of certain apple genotypes in 2019 and 2020. All trees will receive an aphicide and fungicide treatment two weeks before the infestation begins. Nine different aphid genotypes from each of the three locations (three from Belgium, three from France, and three from Spain) will then be infested on the 28 apple trees (five genotypes from Belgium, five from France, and five from Spain, six European wild apple *M. sylvestris* genotypes, three tolerant controls, and one susceptible cultivated apple control) in mid-April 2021. Modality 1 will consist of the infestation of as many apple trees as possible per day but we think we will need about 18 days to complete the infestation of all trees. We aimed to infest 14 apple trees as the minimal number of infested trees per day. For modality 2 (control), there will no infestation and we will apply treatments against aphids and fungi. Different colors of aphids and trees represent different genotypes. Apple trees and aphid genotypes will be spatially randomized in each block.

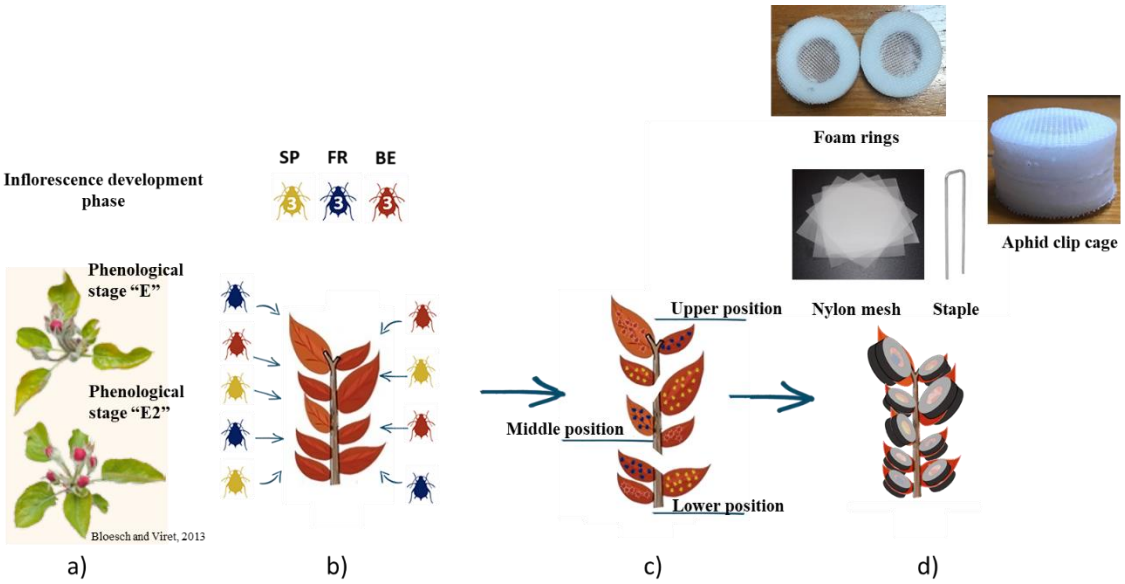

Figure 4. Representation of rosy apple aphid infestation on the different apple genotypes. a) The aphid infestation will be performed at the phenological stages "E" and "E2". During the E stage the sepals open slightly, the petals lengthen and become visible while in the E2 stage the flowers form a hollow balloon with their petals. During both stages, there are tender light green leaves. b) Nine aphid genotypes from different origins (three from Belgium, three from France, and three from Spain) will be infested on an apple tree. c) Synchronized micro-colonies of female aphids from the nine aphid genotypes will be infested on a leaf of a tree: three aphid genotypes from France, Belgium, and Spain will be randomly infested in the upper part of the tree, three in the medium and three in the lower part of the tree. d) Each infestation will be protected with a clip-cage. The clip cage is comprised of two circular plastazote foam rings (each ring 25 mm diameter and 1cm thickness) covered by a nylon screen and clip together with an angle-shaped staple. BE = Belgium, FR = France, SP = Spain.

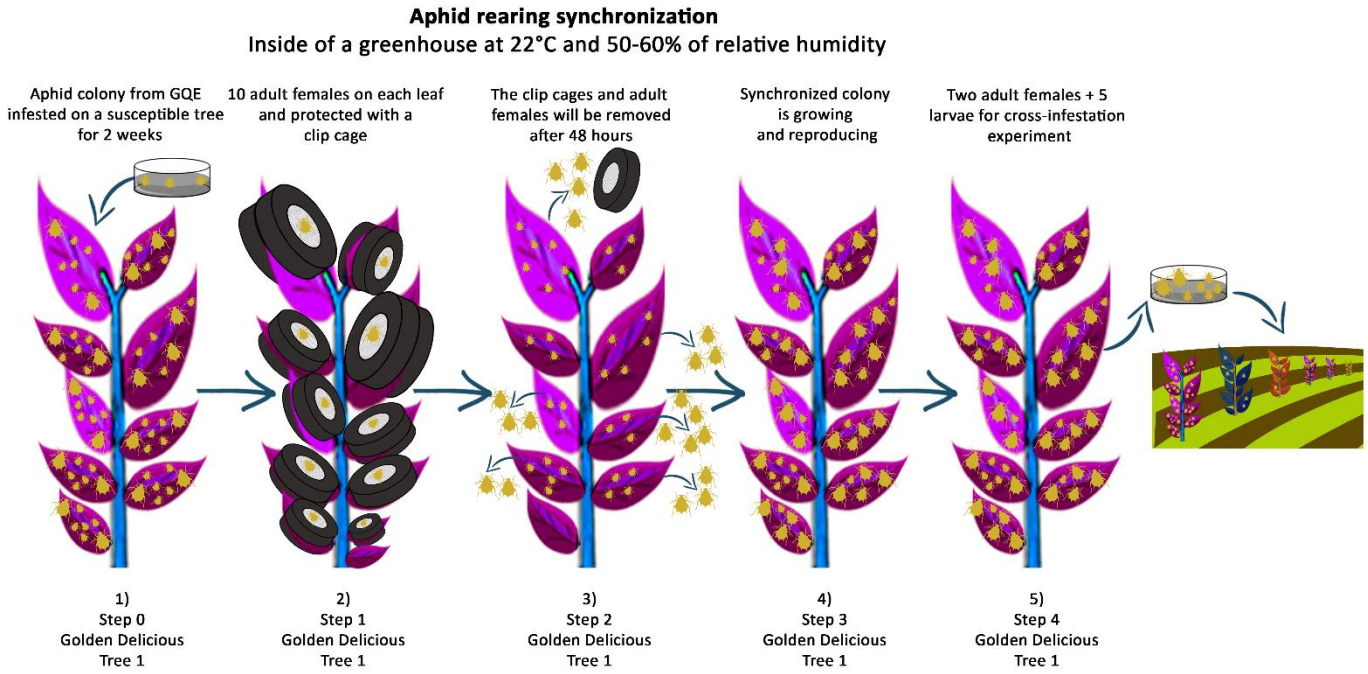

Figure 5. Aphid rearing synchronization steps explained for clone 1 from Spain. We will follow the same protocol for each aphid genotype. Step 0: a colony from the GQE-Le Moulon laboratory is received and placed onto an M9 grafted Golden Delicious susceptible apple genotype. The colony will grow for two weeks. Step 1: Ten adult females are put on a new M9 grafted Golden Delicious susceptible apple genotype, separately on different leaves, for 48 h and protected by a clip cage. Step 2: after 48 h, the clip cages and the adult females are removed and put back on the tree 1. The larvae are let grown for 10-12 days. Step 3: The larvae have grown and became adults and have started to produce larvae themselves. A synchronized colony of a single aphid genotype now grows on the tree. Step 4: Two adult females and five larvae will be selected to infest a leaf of each tree on the field. Steps 1 to 4 will be repeated every two or three days to synchronize aphid colonies for about 18 days to follow the infestation plan (see section "Aphid genotypes and preparation for the infestation").

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