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*) to its host (*Malus domestica*) and to its climate in Europe.

2.- Authors

Olvera-Vazquez S.G.[1], Alhmedi A.[2], Miñarro M.[3], Shykoff J. A.[4], Marchadier E.[1], Rousselet A. [1], Remoué C.[1], Gardet R.[5], Degrave A. [5], Robert P. [5], Chen X.[1], Porcher J. [5], Giraud T. [3], Vander-Mijnsbrugge K.[6], Raffoux X. [1], Falque M. [1], Alins, G.[7], Didelot F.[8], Beliën T.[2], Dapena E.[3], Lemarquand A. [8], Cornille A.[1]

1. GQE–Le Moulon, INRAE, Université Paris-Saclay, CNRS, AgroParisTech, Université Paris-Saclay, 91190, Gif-sur-Yvette, France.
2. Department of Zoology, pcfruit vzw, Sint-Truiden, Belgium.
3. Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Carretera AS-267, PK. 19, E-33300, Villaviciosa, Asturias, Spain.
4. Laboratoire d'Ecologie, Systématique et Evolution, Université Paris-Saclay, CNRS, AgroParisTech, 91400 Orsay cedex, France.
5. AgroCampus-Ouest, UMR1345 Institut de Recherche en Horticulture et Semences (IRHS), 49045 Angers, France
6. Department of Forest Ecology and Management, Research Institute for Nature and Forest, 9500 Geraardsbergen, Belgium
7. IRTA Fruitcentre, PCiTAL, Park of Gardeny, Fruitcentre Building, 25003 Lleida, Spain
8. INRAE, Unité Expérimentale Horticole N34 0449, Centre d'Angers-Nantes, 49071 Beaucouzé Cedex, France

Corresponding author: amandine.cornille@inrae.fr

Abstract

Understanding the extent of local adaptation in natural populations and the mechanisms enabling individuals to adapt to their native environment is a major avenue in ecology research. Host-parasite coevolution 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 of local adaptation processes in host-parasite interactions will also help to tackle pressing issues, such as the ways in which environmental change alters the emergence of pathogens leading to host extinction, how to promote sustainability of agroecosystems in the face of emerging crop diseases or in guiding for 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 carry out experimental tests for *D. plantaginea* fitness differences among three aphid populations from Belgium, France, and Spain infested in three common garden orchards located in Belgium France, and Spain, comprised each of a panel of cultivated apple varieties from Belgium, France, and Spain. This experiment that will start in the Spring of 2021 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, 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 climate and/or 2) its cultivated apple host (*Malus domestica* Borkh)? To that aim, we will ask several questions, outlined below.

Question 1 and hypotheses: Is there evidence of rosy apple aphid adaptation to the local climate?

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

Hypothesis 0: No, the rosy apple aphid is not locally adapted to its climate. There is a lack of variation in the rosy apple aphid fitness across the three different environments (i.e. common garden orchards located in Belgium, France, and Spain). For example, there is similar fitness of the Spanish rosy apple aphids infested among the three common garden orchards (Belgium, France, and Spain).

Hypothesis 1: Yes, the rosy apple aphid is locally adapted to its local climate. There is a higher fitness of rosy apple aphids from a particular origin in their local environment. For example, there is higher fitness of Spanish rosy apple aphids infested at the Spanish common garden orchard.

Hypothesis 2: the rosy apple aphid is maladapted to its local climate: rosy apple aphids show higher fitness in their foreign climate than in their local climate. For example, there is higher

fitness of the Spanish rosy apple aphids infested in the common garden orchards in Belgium and France than in its local environment, the common garden orchard in Spain.

Question 2 and hypotheses: Is there evidence of rosy apple aphid adaptation to the locally 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: no, the rosy apple aphid is not locally adapted to its cultivated apple host. There is a lack of variation in the rosy apple aphid fitness of different origins on different local cultivars. For example, there is no significant difference in the fitness of the rosy apple aphids from France infested on the cultivated apple genotypes from the three different origins (i.e., Belgium, France, and Spain).

Hypothesis 1: yes, the rosy apple aphid is locally adapted to its cultivated apple host. There is a higher rosy apple aphid fitness of particular origin on the apple cultivars locally cultivated from the same origin. For example, the fitness of the French rosy apple aphids is higher on the French cultivated apple genotypes.

Hypothesis 2: The rosy apple aphid is maladapted to its cultivated apple host: aphid populations show higher fitness on their foreign host than in their local host. For example, the fitness of the French rosy apple aphids is higher on Belgian and Spanish cultivated apple genotypes rather than on the French cultivated apple genotypes. This pattern of maladaptation to the host was already reported for the obligate parasite *Microbotryum violaceum* Demi and Oberw using cross-infection on several host populations (Kaltz et al., 1999). However, there is limited information in other model systems, including aphids.

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

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

Hypothesis 0: no, the rosy apple aphid is not locally adapted to its host and climate. There is no variation in the rosy apple aphid fitness across the different environments and origins of the cultivated apple. For example, there is no difference in the fitness of Belgian rosy apple aphids infested on the cultivated apple genotypes from the three origins (Belgium, France, and Spain) and across the three common garden orchards (Belgium, France, and Spain).

Hypothesis 1: yes, the rosy apple aphid is locally adapted to its local host and climate. There is a higher fitness of the rosy apple aphids from a particular origin infested on cultivated apple genotypes from the same origin only when these are growing in the environment of origin. For example, the Belgian rosy apple aphids infested on cultivated apple genotypes from Belgium that are growing in the Belgian common garden orchard present higher fitness than the Belgian rosy apple aphids infested on different cultivated apple genotypes from different origins and on different common garden orchards.

Hypothesis 2: the rosy apple aphid is maladapted to its local host and climate. The fitness of the rosy apple aphids from a particular origin is higher on the cultivated apple genotypes from different origins and grown in common gardens from a different origin. For example, the Belgian rosy apple aphids infested on cultivated apple genotypes from different origins and on common garden orchards from different origins than Belgium exhibit a higher fitness than the Belgian rosy apple aphids infested on cultivated apple genotypes locally cultivated in Belgium and on the common garden orchard located in Belgium.

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 is a lack of variation in the fitness of the rosy apple aphid infested either on wild and cultivated apple genotypes. For example, the fitness of the rosy apple aphids, whatever their origins, is similar in the infestations on the cultivated apple genotypes and the wild apple genotypes.

Hypothesis 1: there is a higher fitness of the rosy apple aphid on wild apple genotypes than on cultivated apple genotypes. Indeed, the European wild apple is the local wild apple in Europe and is present there for at least the past 120,000 years. In contrast, the cultivated apple is 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). Additionally, the rosy apple aphid can only be found in Europe and the Caucasus. Therefore, the rosy apple aphid has probably been associated with the European wild apple longer 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: no, there is a lack of variation in the fitness between the rosy apple aphid infested on apple genotypes known to be tolerant to the rosy apple aphid and on other ~~sensible~~ apple genotypes.

Hypothesis: Yes. Previous studies suggested that the apple genotypes tolerant to the rosy apple aphid infestations induce lower fitness of the rosy apple aphid (Pagliarani et al., 2016).

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 that had been collected in Belgium, France, and Spain, with three lines from each country. Below we describe the detailed material that will be used.

Apple trees

Each common garden includes a total of 28 apple genotypes (Figure 2, Table 1), comprising **five local cultivated apple varieties** (*M. domestica*) from each country, thus, 15 apple genotypes from three countries, five from Belgium, five from France, and five from Spain. We also considered **nine wild apple genotypes** (*M. sylvestris*), six from Belgium, and three from Spain. Finally, we also included four apple genotypes, **three tolerant apple genotypes** (two *M. domestica* apple genotypes, 'Priscila' and 'Florina' cultivars, and one genotype of the species *Malus floribunda* Siebold ex Van Houtte) and **one susceptible genotype**, the *M. domestica* Golden Delicious cultivar. The selection of the cultivated apple genotypes was based on several criteria. First, whenever possible the chosen cultivars needed to represent the apple cultivars cultivated locally in the chosen locations of the common gardens. For

Spain and France, the local cultivars included traditional cultivars, in Belgium, the cultivation of apple includes recent commercial cultivars. Second, we chose cultivated genotypes known to not be genetically closely related based on microsatellite genetic characterization by each local laboratory involved in the project (Cornille et al., 2012). Third, unpublished qualitative assessments of *D. plantaginea* attacks onto several cultivated apple varieties also allows choosing five apple varieties per locality that showed variability in their response to *D. plantaginea* (from susceptible to tolerant). Concerning the European wild apple (*Malus sylvestris*), previous studies showed that Spanish and Belgian wild apples formed distinct populations in Europe (Cornille et al. 2013, 2015), we therefore obtained scions from mother trees maintained in a conservation orchard in Belgium, and from sampling in a forest in Northern Spain. Note that the 28 genotypes used in this experiment have been genetically characterized using 13 microsatellite markers (Chen et al. in prep), and we have also just received their genome sequences, that will be processed during fall 2020.

Depending on the availability of the scions at the beginning of the project, we grafted 10 to 12 times each of the 28 apple genotypes (Figure 2, Table 1). Besides, for the rearing and synchronization steps that will be performed at each common garden orchard (see method below), we also grafted 206 clones of the Golden Delicious variety (Table 1), to get at least 60 trees per locality available for the rearing. Therefore, in total, early 2019, 1,157 apple trees (Table 1, 951 for the infestation experiment and 206 for the rearing step) were grafted on an M9 Pajam 2® apple rootstock and maintained for one year (February 2019-2020) at an outdoor nursery at La Retuzière, Les Hauts d'Anjou, Angers, France (47°28'57" N, 0°36'52" W). Early February 2020, the trees were transferred and planted in the three common garden orchards (Figure 2). Each tree was sprayed with 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). Then new treatments will be used (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 including 12 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, represented by one to several genotypes, are currently being reared and maintained 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 cultivar) provided by the CRA-W (Micropropagation laboratory, Biological Engineering Unit, Gembloux, Belgium) in preparation for the cross-infestation experiment.

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 for the infestation in March 2021. Indeed, while the aphid colonies were collected to avoid mixing several clonal lineages, this can happen. Therefore, once grown up enough, each "single-genotype" colony will be genetically characterized using newly developed microsatellite markers (Olvera-Vazquez in prep). This step will allow us to build a collection of at least three distinct lineages from each locality (i.e., Belgium, France, Spain) that will be available for the infestation experiment in March 2021. To be safe, we will also maintain more genotypes until March of 2021 in control conditions in case any colony dies. In the end, from our complete set of 36 rosy apple colonies, we will maintain at least nine "single-genotype" colonies from Belgium, France, and Spain. In February 2021, some descendants of each of the nine "single-genotype" rosy apple aphid colonies will be sent to each local department in Belgium, France, and Spain. Locally, each lab will rear and synchronize each of the nine colonies in a greenhouse onto Golden Delicious cultivar clones (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 cloned replicates of each of the 28 apple genotypes (Table 1). These are planted in 10 to 12 rows, each that includes each available genotype placed at random (Figure 3). We will record ecophysiological traits on each of the apple genotypes before (March 2021), during (April-Jun 2021), and after (July 2021) the infestations. In total, we will measure 951 apple trees, including 320 trees in Belgium, 305 trees in France, and 326 trees in Spain. The experiment will be divided into two modalities (Figure 3):

-modality 1: apple genotypes that will be 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.

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 acclimated 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 or leaf cluster on the same apple tree of each of the 28 apple genotypes in the three common garden orchards (Figures 2, 3, and 4 and Tables 1 and 2). The choice of which aphid genotype is placed on which leaf or leaf cluster will be chosen at random within each tree. Performing the infestation is delicate and time-consuming and will, therefore, require several days to complete (we estimate 18 days, see Figure 3). 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 different aphid genotypes 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 not that sample sizes may be reduced at the start of the experiment if any trees are lost 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*). Besides, each aphid genotype will experience the climatic condition from its native origin and two different climatic conditions. This will allow us to experimentally test for local adaptation of the rosy apple aphid to the cultivated apple host and climate, 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 the infestation

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 place each of the nine aphid genotypes on Golden Delicious grafted onto an M9 Pajam® and wait for colony growth. One Golden Delicious tree will host a given aphid genotype; each bud of the tree will be surrounded by a cellophane bag used to maintain different synchronized aphid colonies of a given genotype (Figure 5). After two weeks, we will expect to have enough females to start the aphid synchronization. The aim of the aphid synchronization is to ensure the same developmental stage of the females that will be infested on a plant. Aphid synchronization will start mid-March 2021. Details of the synchronization procedure are described in Box 1 and Figure 5. For each aphid genotype (Figure 4), we will launch the aphid synchronization gradually, at different times, on different leaves or leaf clusters of a Golden Delicious tree. We will need at least 40 synchronized females of each aphid genotype each day to perform the cross-infestation schedule (Figure 5).

Detailed of modalities 1 and 2

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

-**modality 1:** apple genotypes that will be 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 nine different leaves or leaf clusters, each isolated in a cellophane bag, of each of the 28 apple genotypes by nine different aphid genotypes. Note that preliminary tests in our lab show that these bags do not influence aphid's behavior. Each leaf or leaf cluster 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 of 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 or leaf cluster together with the cellophane bag and transfer this into a Falcon tube previously filled with isopropanol >90%. In the laboratory, we will count the number of adults and nymphs

with the software ImageJ (Schneider et al., 2012) and record the degradation of each infestation following the Rat-Morris scale (Figure 6).

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. In addition, we will take the apple tree diameter at 50 cm height and photosynthesis measurements with a Dualex© clip (flavonoid and chlorophyll contents, and nitrogen balance index) two times per month.

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 climates) against 1/3 sympatric comparisons (i.e. aphid genotypes infested on their local apple genotypes and climates) (Table 2).

We choose to perform all infestation treatments with all aphid genotypes on each individual apple tree. This minimizes the error variance associated with differences among trees due to their condition or microsite variation and therefore, maximizes our power to detect differences among aphid and apple genotypes and among 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. However, we do not replicate our common garden orchards within the different areas of origin, i.e., Belgium, France, and Spain. Therefore, though we can adequately test for local adaptation we cannot assess how generalizable are our results at the level of the three areas of origin.

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 are either cultivated (*M. domestica*) or wild (*M. sylvestris*). The former is of different cultivated apple genotypes. The cultivated apple genotypes were selected to represents local genotypes, genetically far from each other, and show variability in the response against rosy apple aphid attacks. Alternatively, for the wild apple genotypes, we chose them because of already-characterized population differentiation that has been observed in the European wild apple (Cornille et al 2015, Chen et al. in prep). However, we acknowledge that the current experiment will definitively give a first insight into the natural

response of the wild apple genotypes to the attacks of the rosy apple aphid and will not be the core question of the current study.

On the other hand, as previously described, 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. Indeed, we will use recently developed microsatellite markers for *D. plantaginea* to select the aphid genotypes with a significant different allelic variation.

Finally, the sites chosen for settling the common garden orchards represent a European latitudinal gradient to test the effect of local climate 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. We will estimate rosy apple aphid fitness as the growth rate of the colony (cumulative number of nymphs produced per surviving females produced on the infested plant; Warneys et al., 2018), and the insect life cycle (aphid stages (L1 to L5), apterous adults, nymphs, and winged forms (Angeli and Simoni, 2006)). We will have 6,408 infestation spots (single aphid genotype on a single apple genotype leaf or leaf cluster) 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).

Apple genotypes - Before the infestation and after the infestation, and then once a month on each tree, we will measure: chlorophyll (µg per cm2) and polyphenol contents (relative absorbance units) on at least 10 leaves per tree with the Dualex® optical leaf clip, presence of any disease or pest (e.g. *Venturia inaequalis* (Cooke) G., Winter or apple scab

and *Erwinia amylovora* (Burril) Winslow, fire blight or other aphid species), trunk diameter (beyond scion and at 50cm from the ground) and tree height (starting from the graft union). In the case there are branches on a tree, we will count and qualitatively measure them: we will categorize them in darts (between 1cm and 10 cm) or branches (equal or more than 10 cm). Finally, we will also record the bursting and flowering times using a reported phenological stages scale of the apple (Fleckinger, 1948).

After the infestation experiment, during the leaving-out of the infested leaves: for each tree, we will evaluate the leaf damage caused by aphids following the scale by Miñarro and Dapena (2007) (Figure 6) and the height of the leaf or leaf cluster used for the infestation.

Abiotic environment: we will record the temperature, humidity, and photoperiod along with the experiment. The data will be obtained using a data logger (Log32 THP TFA®) on each common garden.

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 utmost 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 coded the localization of each apple tree at each common garden orchard. Now 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 added in the statistical models (see above 15.1.3 section for details). 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 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 comprised of rows with randomized apple trees to prevent spatial autocorrelation of error variance 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 climate of the same origin: France or Belgium or Spain) against allopatric combinations (i.e. aphid genotypes infested on apple

genotypes and climate of a different origin: France, Belgium, and Spain). We will also consider that an aphid population is locally adapted to its host and climate if its fitness is the highest on its local host and climate (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. The other effects will be fixed (see below). Then, we will gradually remove interactions and effects depending on their significance. In addition, we will evaluate the differences in the effect on aphid fitness using a contrast analysis.

To test for local adaptation, we will partition the three-way interaction among sites (common garden orchards), apple origin, and aphid origin into a sympatric *versus* allopatric comparison. This sympatric versus allopatric contrast will also be tested both within each locality, i.e., separately for the three different common garden orchards in a similar way in order to determine whether local adaptation is expressed differently 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 and hypothesis 1- ($G_{parasite}$*climate): $aphid\_origin_h$*$site_j$
Question and hypothesis 2- ($G_{parasite}$ *G_{host}): $aphid\_origin_h$ * $apple\_origin_i$
Question and hypothesis 3 - ($G_{parasite}$ *G_{host}* climate): $aphid\_origin_h$*$apple\_origin_i$*$site_j$

Below the following factors will be used

Equation 1

$$W_{hijklmntt2z} = \mu_W + aphid\_origin_h + apple\_origin_i + site_j + site_j(block_k) + Gh_l(leaf_m(Gp_n)) + day\_of\_infestation_t + hour\_of\_infestation_{t2} + aphid\_origin_h*site_j + aphid\_origin_h*apple\_origin_i + aphid\_origin_h*apple\_origin_i*site_j + \varepsilon_{hijklmntt2z}.$$

Mathematic equation:

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

Index		Term	Effect
α	h	$Aphid\_origin_h$	Aphid country of origin (Spain, France, Belgium)
β	i	$Apple\_origin_i$	Apple country of origin (Spain, France, Belgium)
γ	j	$Site_j$	Common garden site (Spain, France, Belgium)
B	k	$Block_k$	Block (Each block is comprised of 28 apple genotypes infested with 9 aphid genotypes. Either the position of the tree in the field and the infested leaf are randomized)
	l	Gh_l	Apple host genotype
	m	$Leaf_m$	Leaf ID treatment (Position of the infested apple leaf on the main stem)
P	n	Gp_n	Aphid parasite genotype
δ	t	$Time\ of\ infestation_t$	Day of infestation
ζ	t2	$Time\ of\ infestation_{t2}$	Hour of infestation
κ	x	$Tolerant\_status_x$	Tolerant or susceptible genotype status assessed from previous studies (Miñarro and Dapena, 2008)
η	y	$Crop\_wild\_status_y$	Cultivated or wild apple host (*Malus domestica* and *Malus sylvestris*, respectively)
	z		Effect of each observation
ε			Residual error

where $W_{hijklmntt2z}$ is the absolute fitness value of an aphid genotype *Gp* (i.e. parasite genotype) from the country of origin *n* on the apple genotype *l* in block *k* on *leaf m* and in the common garden *j* infested at day t and hour t2, 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. genotype name) and $\varepsilon_{hijklmntt2z}$ is the residual term. *Block* is random and nested within site, and aphid $genotype_n$ is nested within *leaf* ID_m, and *leaf* ID_m is nested within apple genotype Gh_l, and they were added to the models as random-effect terms. The *site* term measures the quality or suitability of the common garden locations, *aphid_genotype*

and *apple* accounts for differences in fitness intrinsic to each local aphid genotype and apple genotype country of origin, and the $aphid\_origin_h*site_j$ accounts for differences in local adaptation to the climate among the three aphid origins, the $aphid\_origin_h*$ $apple\_origin_i$ account for differences in local adaptation to the host among the three aphid origins, the $aphid\_origin_h*apple\_origin_i*site_j$ accounts for differences in local adaptation to the host and climate 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*.

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

Equation 2

$$W_{hijklmntt2z} = \mu_W + aphid\_origin_h + crop\_wild\_status_y + site_j + site_j(block_k) + Gh_l(leaf_m(Gp_n)) + day\_of\_infestation_t + hour\_of\_infestation_{t2} + aphid\_origin_h*site_j + aphid\_origin_h* crop\_wild\_status_y + aphid\_origin_h* crop\_wild\_status_y *site_j + \varepsilon_{hijklmntt2z}$$

Mathematic equation:

$$y_{hyjklmntt2z} = \alpha_h + \eta_y + \gamma_j + B_{jk} + P_{lmn} + \delta_t + \zeta_{t2} + \alpha_h* \gamma_j + \alpha_h*\eta_y + \alpha_h*\eta_y* \gamma_j + \varepsilon_{hyjklmntt2z}.$$

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

Equation 3:

$$W_{hxjklmntt2z} = \mu_W + aphid\_origin_h + tolerant\_status_x + site_j + site_j(block_k) + Gh_l(leaf_m(Gp_n)) + day\_of\_infestation_t + hour\_of\_infestation_{t2} + tolerant\_status_x *site_j + aphid\_origin_h* tolerant\_status_x + aphid\_origin_h* tolerant\_status_x*site_j + \varepsilon_{hijklmntt2z.}$$

Mathematic equation:

$$y_{hxjklmntt2z} = \alpha_h + \kappa_x + \gamma_j + B_{jk} + P_{lmn} + \delta_t + \zeta_{t2} + \alpha_h* \gamma_j + \alpha_h*\kappa_x + \alpha_h*\kappa_x* \gamma_j + \varepsilon_{hxjklmntt2z}.$$

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 over-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 the 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, we will first check for the 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?

Not observing any aphids will be a key parameter, this will be counted as a true observation: the absence of growth (growth rate equal to 0). However, aphids are very sensitive to any change in environmental conditions and it might occur that some infestation fails for a technical reason. Thus, in case after one day of infestation the female has died, we will consider that the infestation has failed. In that case, we will infest again the next day and we will note this re-infestation and take it into account for statistical analyses (section 18).

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. **N**

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 cultivar has an identification including 1) the cultivar name and 2) the accession ID. cv : cultivar.

Origin of the genotypes	ID	Common garden orchards			TOTAL	Origin of the genotypes	ID	Common garden orchards			TOTAL
		B	**F**	**S**					**F**	**S**	
Belgium (*Malus domestica*)	Braeburn_ P03a01	12	11	12		**European wild apples 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	11	11	
	Granny Smith_ P03a04	12	11	12			syl_be 60	11	11	11	
	Wellant_ V05a1	11	11	12			syl_be 76	12	11	12	
	Total Belgian cultivars	**58**	**55**	**60**	**173**		syl_be 93	11	11	12	
France (*Malus domestica*)	Api_Noir_	12	11	12			**Total Belgian wild apple**	**66**	**65**	**68**	**199**
	Clochard_A5	12	11	12		**European wild apples 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**	**33**	**31**	**33**	**97**
	Total French cultivars	**59**	**55**	**59**	**173**		**Total European wild apple**	**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 cultivars**	**34**	**32**	**34**	**100**
	Xuanina_M0084	12	11	12			**Total per site (for infestations: modality 1)**	244	222	246	712
	Total Spanish cultivars	**58**	**55**	**60**	**173**		**Total per site (control without infestations: modality 2)**	76	83	80	239
Sensitive 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 aphid infestations 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*										
Common garden	Belgian trees						French trees							Spanish trees							Resistant				Susceptible	Belgian trees						Spanish trees				Overall
Belgium	Aphid	B1	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	W9	SUM	SUM
	BE_1	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	111	244
	BE_2	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	111	244
	BE_3	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	111	244
	FR_1	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	111	244
	FR_2	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	111	244
	FR_3	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	111	244
	SP_1	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	111	244
	SP_2	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	111	244
	SP_3	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	9	111	244
		SUM					396		SUM					405		SUM					396		SUM													999	2196
Spain	Aphid	B1	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	W9	SUM	SUM
	BE_1	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	112	246
	BE_2	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	112	246
	BE_3	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	112	246
	FR_1	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	112	246
	FR_2	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	112	246
	FR_3	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	112	246
	SP_1	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	112	246
	SP_2	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	112	246
	SP_3	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	9	112	246
		SUM					405		SUM					405		SUM					396		SUM													1008	2214
France	Aphid	B1	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	W9	SUM	SUM
	BE_1	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	102	222
	BE_2	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	102	222
	BE_3	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	102	222
	FR_1	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	102	222
	FR_2	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	102	222
	FR_3	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	102	222
	SP_1	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	102	222
	SP_2	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	102	222
	SP_3	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	8	102	222
		SUM					360		SUM					360		SUM					360		SUM													918	1998

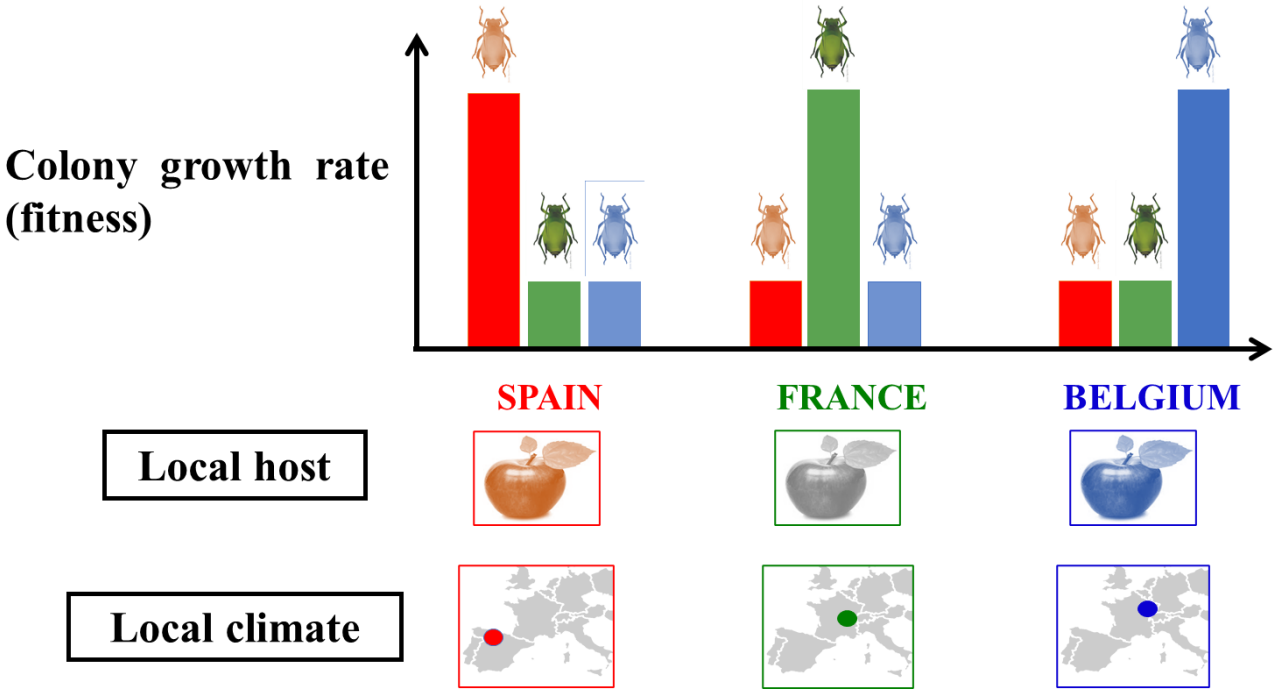

Figure 1. Expected pattern in the case of the rosy apple aphid is locally adapted to its climate and host. The rosy apple aphid populations that present the highest fitness in their local abiotic environment and host will reflect local adaptation.

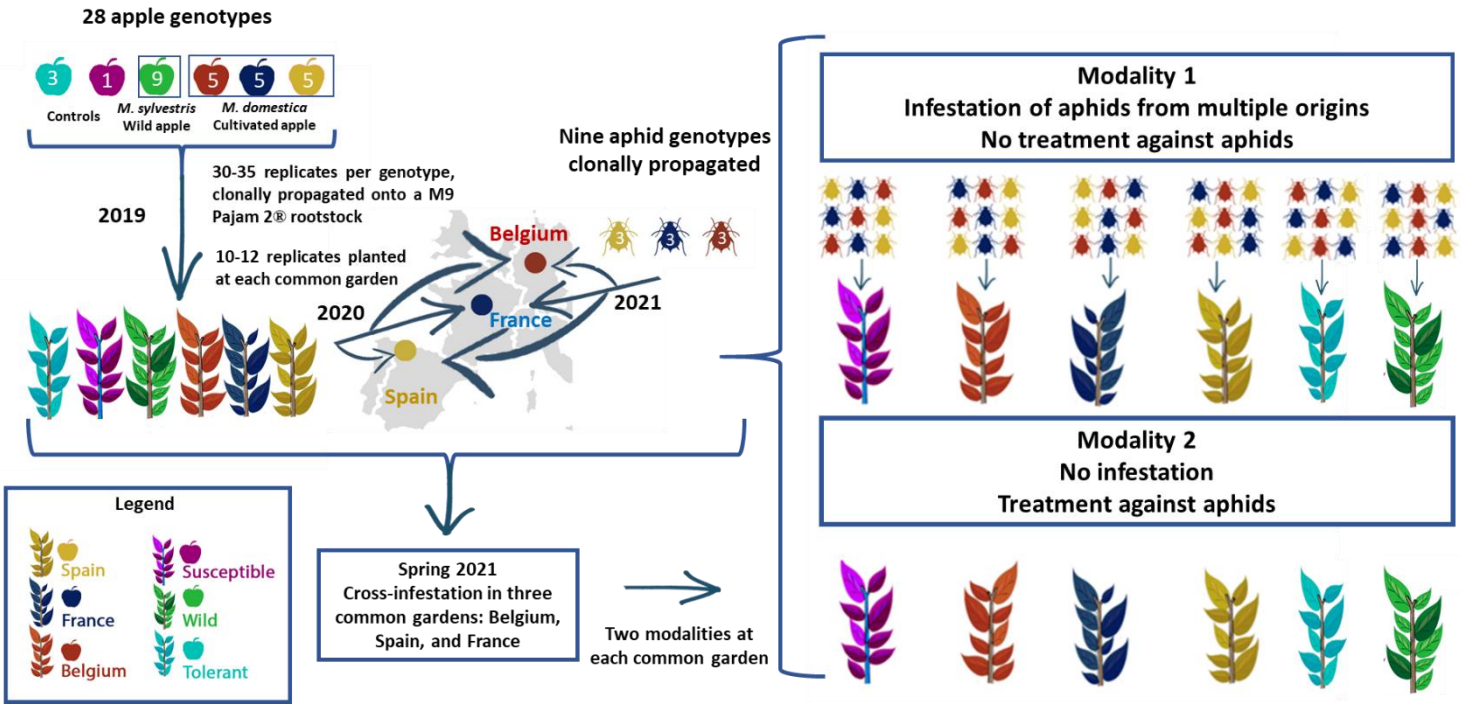

Figure 2. General scheme of the aphid cross-infestation experiment that will be performed in the Spring of 2021 at the three common garden orchards in Belgium, Spain, and France. At each common garden orchard, 28 clonally propagated apple genotypes are included with 10 to 12 replicates per genotype, depending on the availability 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 and *Malus floribunda* Siebold ex Van Houtte used as "tolerant against aphid infestation" controls (light blue color), and the Golden delicious *M. domestica* genotype that will be used for aphid rearing as well as "susceptible for 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.

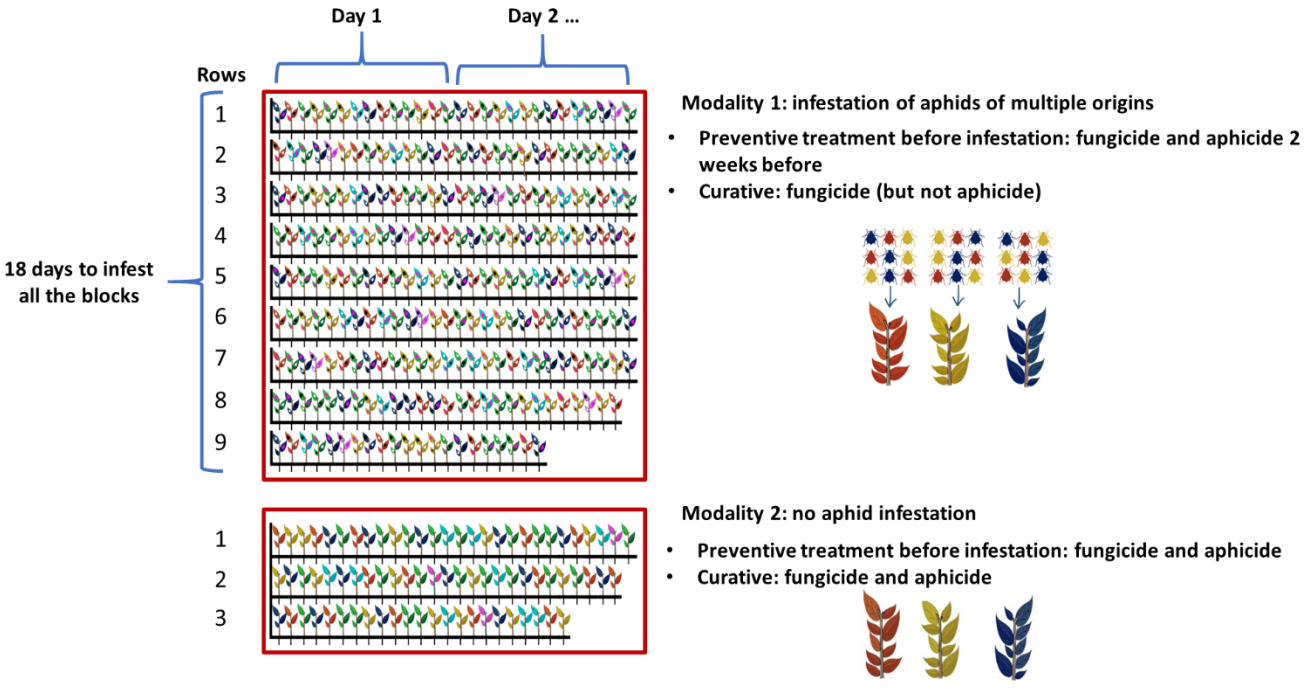

Figure 3. Details of the two modalities that will be performed in spring 2021 for testing the local adaptation of the rosy apple aphid (*Dysaphis plantaginea*) using a cross-infestation experiment. Here an example of the common garden in Belgium (Sint-Truiden). The experimental field of each common garden is comprised of rows, each with all available 28 apple genotypes at a random position in the row, with the final rows lacking few genotypes due to apple genotype availability. 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. The modality 1 will consist in the infestation as many apple trees as possible per day but 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 not be an 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 for each block.

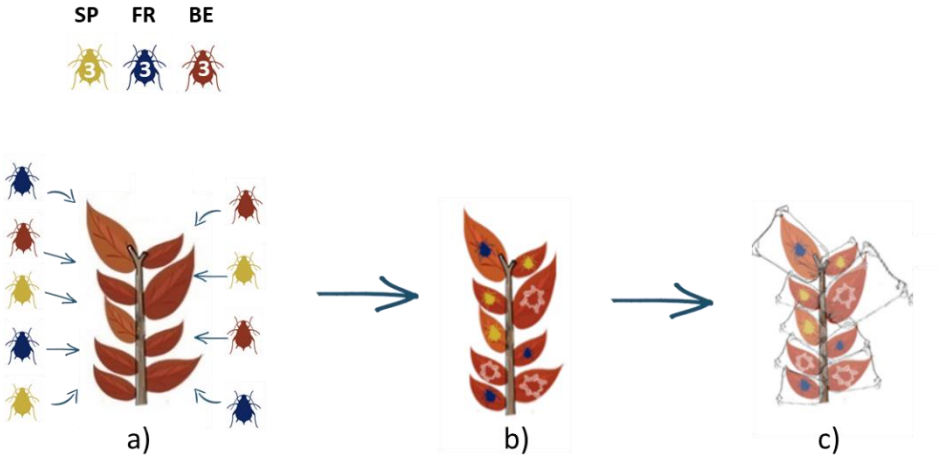

Figure 4. Representation of rosy apple aphid infestation on the different apple genotypes. a) Nine aphid genotypes from different origins (three from Belgium, three from France, and three from Spain) will be used to infest a cultivated apple tree. b) A single synchronized adult female aphid from each 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.

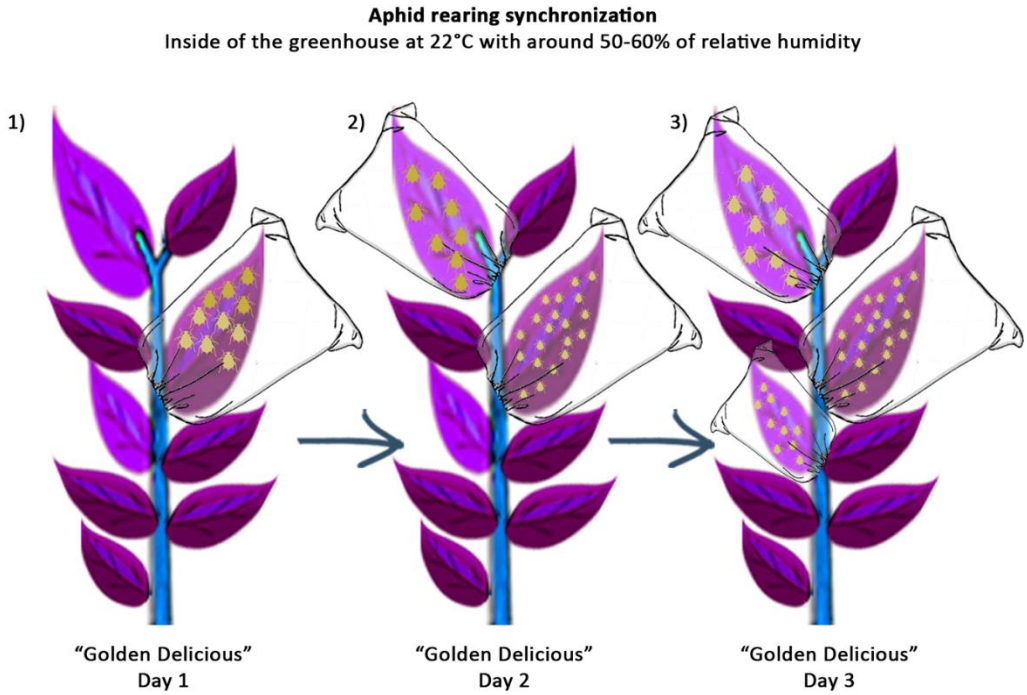

Figure 5. Aphid rearing synchronization, for example for clone 1 from Spain. 1) Day1: 10 adult parthenogenetic aphid females will be placed on an M9 grafted susceptible apple genotype Golden Delicious surrounded by a cellophane bag; 2) After 24 hours, each female will have laid about four to five larvae, then, the founder female aphids will be removed. Four to five larvae of each of the ten founder aphids will start to establish their colonies. 4) after 12 days each of the larvae will be adult female ready for the infestation. 5) We will repeat these steps every two days for each aphid genotype to have enough aphids for the cross-infestation experiment.

Box 1. Aphid synchronization (Figure 5)

For each aphid genotype, the synchronization procedure will be as explained:

1. At each site, the aphid genotype colonies will be placed onto a leaf and the rearing will be launched early March 2021.
2. As soon as we will have 10-to-20 parthenogenetic adult females for a given genotype, we will place them placed on a leaf of a grafted Golden Delicious genotype (one apple tree with one aphid genotype, each bud with one set of synchronized females), and then surrounded by a cellophane bag. The same Golden Delicious genotype will be used at the three common gardens.
3. After 24 hours, the 10 female founders will be removed. During these 24 hours, each founder female will produce around four to five nymphs.
4. Hence, the final number of synchronized nymphs from the 20 founders will be around 40 to 50 per tree (Figure 6).
5. We estimate that every day during the infestation we will need to produce about 40 adult females.

Steps 1, 2, and 3 will be repeated every two days, on the same Golden Delicious tree, but on different buds that will be tagged with a date to record the day of synchronization. This gradual synchronization along the 18 days of the infestation will allow us to get enough aphid females each day to infest at least 20 apple trees per day mid-April with each aphid genotype. In total, we have 80 Golden Delicious trees available for the synchronization steps.

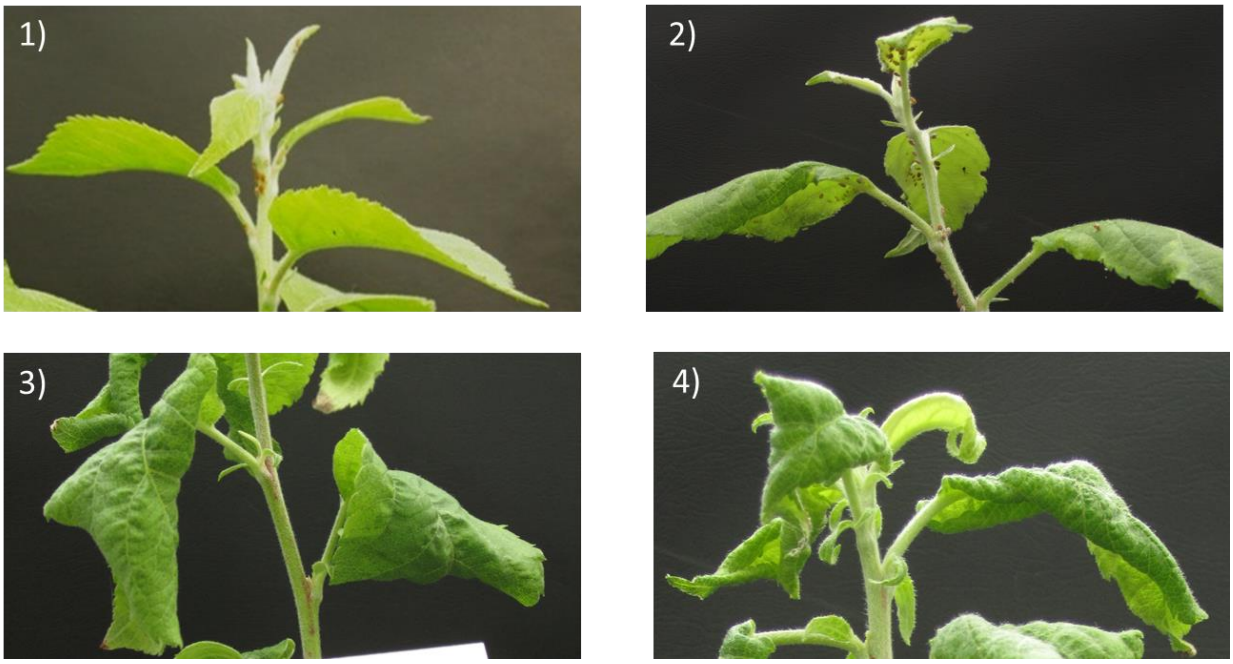

Figure 6. Shoot damage will be coded from 0 to 3 based on Rat-Morris scale (1993): 1) value of 0: no damage; 2) value of 1: leaf slightly curled at the edge; 3) value of 2: borders of the leaf curled longitudinally; 4) value of 3: typically, rosy apple aphid rolled leaves (Miñarro and Dapena, 2007).

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