Predation assessment on fake caterpillars and leaf sampling

Protocol for partner schools

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1 - Protocol summary and key steps

This protocol describes in detail the different tasks you and your pupils will carry out during the project. It only focuses on the scientific parts that need to be standardized among partners. Pedagogical choices are yours. If you want to involve several classes, then you will need to select several trees, one oak per class. For your personal archives, take many photos at each step!

Key steps

▶ Autumn 2018

- Before leaf fall, identify one or several pedunculate oaks of at least 6 cm in diameter as measured at 1.30m, with low branches and preferably acorns to facilitate the tree species identification.

▶ From April 2019

- Make **40 fake caterpillars** per tree, using material provided by the project leader.
- **6 weeks** after the first leaves have appeared (usually between May 1st and May 20th), install the caterpillars in the oak(s).
- 15 days after caterpillars were installed, **carefully remove the caterpillars** and assess the predator marks with help of the "field bite guide". **Carefully pack all caterpillars** in the small boxes that you have received from project leader.
- Install 20 new caterpillars.
- Another 15 days latter, **carefully remove the caterpillars** and assess the predator marks with help of a "field bite guide". **Carefully pack all caterpillars** in the other small box that you received.
- During the second assessment randomly pick 120 oak leaves. Then, randomly pick 60 leaves out of the 120. Them the leaves together with damaged caterpillars to Bastien Castagneyrol.

▶ Mid June 2019

- End of the “field work” part of the project

Key numbers

- 1 oak tree (*Quercus robur*)
- 4 branches
- 2 × 20 fake caterpillars (5 caterpillars × 4 branches × 2 dates)
- 30 leaves per branch

2 - Before you start…

Once you have confirmed your willingness to participate in the project, *we will send you the material* necessary to make and install caterpillars. The package that you will receive will consist in:

- Modelling clay Staedler (Noris Club 8421, green[5]) to model caterpillars
- Thin metallic wire, 0.5 mm diameter (3m)
- One already made caterpillar to serve as an example.

For this project, you will also need a few material that we will not include in the package that you will receive:

- Portable magnifier glass (for instance ×10)
- Measuring tape
- Camera or smartphone with powerful camera (> 10 Mpixels)

Before mid April 2019, make sure that you have gathered all the material and could download and print this protocol and the field scoring sheet (see at the end of this protocol). If you have any trouble, please contact the project coordinator (bastien.castagneyrol@inra.fr).

3 - Selecting your oak tree

Some important restrictions regarding the choice of the oak tree will be applied in this experiment:

- In this experiment, all scientists and teachers will focus on **pedunculate oaks (*Quercus robur*)**, also known as English oak. It is very important that all participants use the same species[1]. The map on **Figure 1** shows the geographic range of pedunculate oaks in Europe. If you are outside of this range, you will not find them. Do beware! Pedunculate oaks sometimes look similar to sessile oaks (*Quercus petraea*), Turkey oak (*Quercus cerris*), pubescent oak (*Quercus pubescens*) or even pyrenean oak (*Quercus pyrenaica*).
  - *Quercus robur* (the one we need), *Q. petraea* and *Q. cerris* are easier to recognize in autumn, when acorns are still hanging and leaves are still attached to branches. *Quercus robur* has acorns hanging on a long peduncle, while acorns of *Quercus petraea* are directly attached to the branch (botanists say they are *sessile*). This video will help you (see appendix 3). The Turkey oak cannot be confounded with another oak species: the cupula is covered by long and thick expansions making it “hairy”.
  - *Quercus pubescens* and *Quercus pyrenaica* are even easier to recognize: their leaves are fluffy on the lower side (*Q. pubescens*) or on both sides (*Q. pyrenaica*).
  - If you have any doubt, please take some photos and send them to the local scientific partner, or on Twitter (@BCastagneyrol and #chenillesparticipatives) and the community will help you with oak identification.

1 - If you absolutely want to participate in the project but do not find any pedunculate oak around you, please contact the local scientific coordinator of the project.

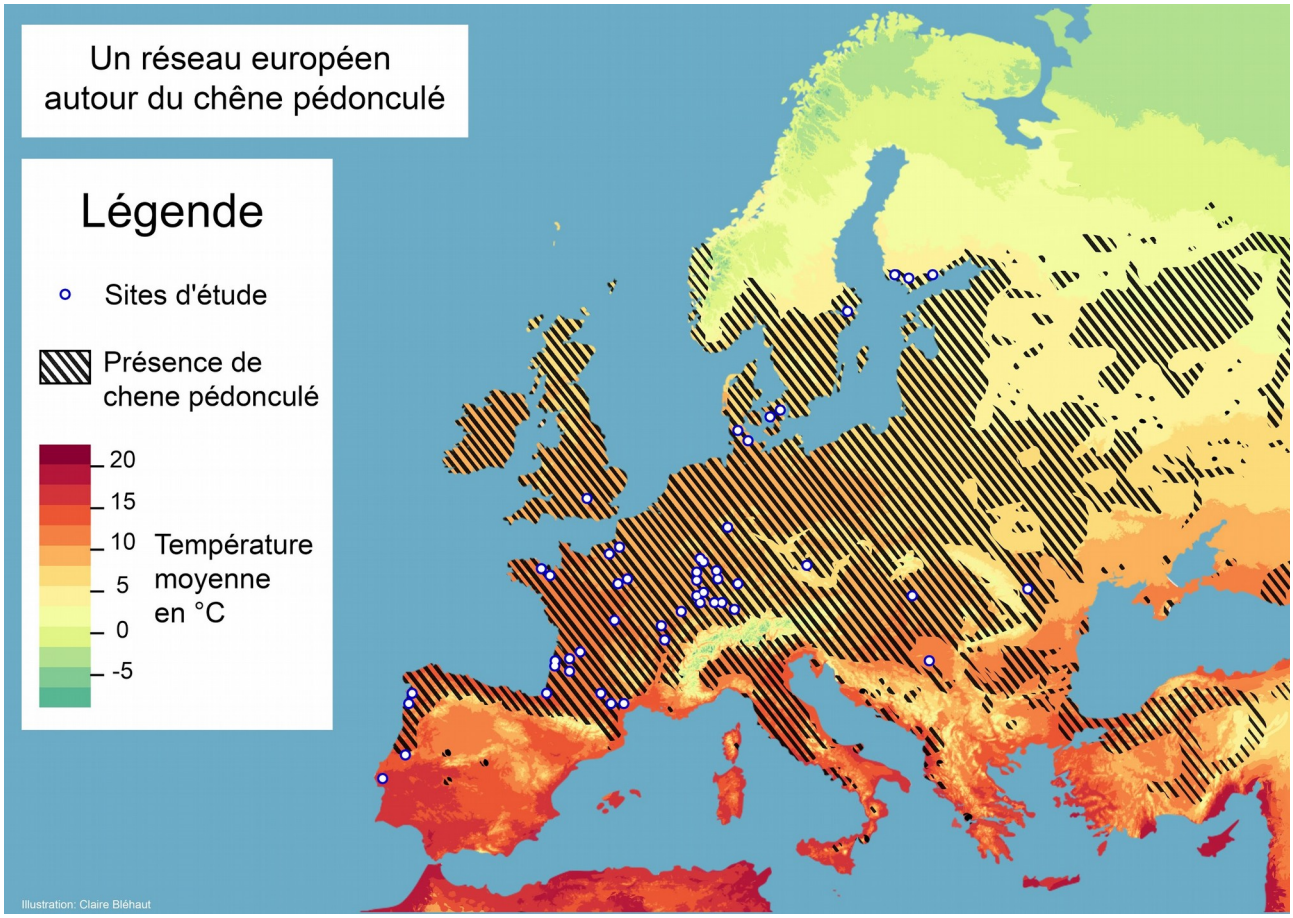

Figure 1: Geographical range of *Quercus robur*. Background colours represent mean annual temperatures (°C). The hashed area represent the geographic range of the pedunculate oak. Dots correspond to school and scientific partners who participated in the project in 2018.

- The chosen oak must be **mature**, i.e., must produce acorns. That's why it is important to select the tree in autumn[2]. Mature trees commonly have a diameter at breast height (i.e., 1.30 m from ground level) > 6 cm.
  - The chosen tree must have **low branches**, easy to reach from ground level.
  - If you are lucky enough to have a mature pedunculate oak in your schoolyard, you can use it. Otherwise, it is better to use the closest accessible oak, or the safest for your pupils. If your school is in the countryside, you can usethe closest isolated oak, or the closest hedgerow or the closest wood. If you have any doubt, do not hesitate to contact your local scientific coordinator.
  - You will need **one tree per group** of children. Therefore, if you want to do the experiment with more than one class, you will have to select as many trees as classes or groups of children.
- Each partner teacher will be given a **unique partner ID**, simply consisting of his/her "*surname.name*". For instance, *Bastien.Castagneyrol*. This notation only aims at facilitating project management and correspondence between teachers and scientific partners. Oak trees will also receive a single oak ID. It will simply consist in partner ID, plus oak number. For instance, *Bastien.Castagneyrol_1* and *Bastien.Castagneyrol_2* if this partner selects two trees because he involves two classes. If you include only one oak tree in the survey, then your oak ID will be ***surname.name_1***. Carefully store your partner and tree IDs.

2 - You have been late in selecting your oak tree? No worries, you will have a second chance in spring. Contact your local scientific coordinator to find out how to proceed.

4 - Measuring the tree

After you have selected your oak tree, some basic information is needed:

- Measure the **circumference at breast height (in cm)**, i.e., at 1.30 m from ground level with a measuring tape. This video shows you how to do it.
- Estimate the tree height by applying basic trigonometry rules method. There are plenty of videos on youtube explaining how to do.
- Record the GPS coordinates of the tree using Google Maps. Write down coordinates of the form **Latitude ; Longitude**. For instance 44.737145 ; -0.775294 for the oak tree we use at INRA station in Bordeaux **(Figure 2)**.

Carefully write down this information in your field scoring sheet (printed from the website). Later on, copy these data in the Excel field scoring sheet on your computer.

Figure 2: Screenshot showing how to extract coordinates from Google Maps. Simply click on the exact location of the tree to make coordinates appear at the bottom of the screen. In this example, coordinates (latitude, longitude) are: **44.737145, -0.775294**.

5 - Making and installing the caterpillars

You will prepare **20 fake caterpillars** and install them all at the same time on your tree.

5.1 - Choosing the right time

Climate varies a lot from Southern (Spain and Portugal) to Northern (Sweden and Finland) Europe. This has a strong impact on tree phenology, *i.e.*, the timing of budburst and leaf fall. It may also have a strong impact on insect herbivores feeding on oak leaves and on the enemies of herbivores such as predators and parasites. It is therefore important that each partner installs the caterpillars at the same phenological stage (*i.e.*, at the same time, from the tree point of view).

We will install caterpillars approximately **6 weeks after the first leaves have appeared on the chosen oak**. This should be between mid April and mid May, depending on your country.

The exact date of budburst also varies from year to year, therefore we cannot tell you when you will need to install the caterpillars. We are also aware of organisational constraints you may face, in particular spring and summer holidays. **We will accept some flexibility regarding the date of caterpillar installation**. If you see that the predation assessment will fall within school holidays, we ask you to anticipate or postpone the date of installation.

If you have any doubt about your trees phenological stage, please take several photos of your oak tree (from a distance, and details of the leaves) the week before you plan to install the caterpillars with your pupils and send them to your local scientific coordinator who will confirm that the timing is good.

5.2 - Making the caterpillars

There are only 3 steps needed to make a fake caterpillar **(Figure 3)**. **All these steps are summarised in this video (appendix 4).**

1. Prepare a ball of modelling clay of 1 cm diameter and 12 cm of thin wire **(Figure 3A)**
2. Gently press the clay between your fingers onto the middle of the wire **(Figure 3B-C)**
3. Roll the modelling clay around the wire so as to obtain a 3-cm long "caterpillar" **(Figure 3D)**

Prepare 20 caterpillars before going to your tree. Make sure that they are not damaged on the way to the tree. For example, you can pin them on a foam board (**Figure 4**) in order to transport them without damaging them.

5.3 - Installing the caterpillars

Prior to installing the caterpillars, select **4 low branches** easily reached from the ground, one facing North, one South, one East and one West. Attach a short (< 5 cm) coloured ribbon or string of wool **at the branch collar (i.e., at the base of each branch)** to make it easier to retrieve. It is important that the ribbon is attached at the bottom of the branch, near the trunk, otherwise, there may be a risk that it will frighten birds.

Attach 5 caterpillars per branch, leaving approximately 15 cm between caterpillars **(Figure 3E-H)**. This video shows you how to do (Appendix 5).

1. Find a thin branch (< 1 cm diameter) with a clear area of about 10-15 cm without leaves. You may remove one or two leaves if there is a risk that they will brush the caterpillar.
2. Align the caterpillar along the branch and wrap one of the free ends of the wire around the branch **(Figure 3E)**
3. Carefully grip the caterpillar between the thumb and index finger **(Figure 3F)** and wrap the other end of the wire around the branch **(Figure 3G)**
4. Tada! The caterpillar is ready **(Figure 3H). Make sure that caterpillar surface is absolutely smooth** (there should be no marks left by buds or fingernails).

Indicate the date at which you installed the caterpillars in your field scoring sheet printed from the website. You can enter these data in the Excel field scoring sheet on your computer later on, but the sooner the better. And take a lot of photos for your personal archives, including one photo of your tree as a whole and one photo of one branch with caterpillars.

We have never experienced vandalism on our trees so far. However, you can prepare a sign to inform people that this is an ongoing experiment that should not be disturbed.

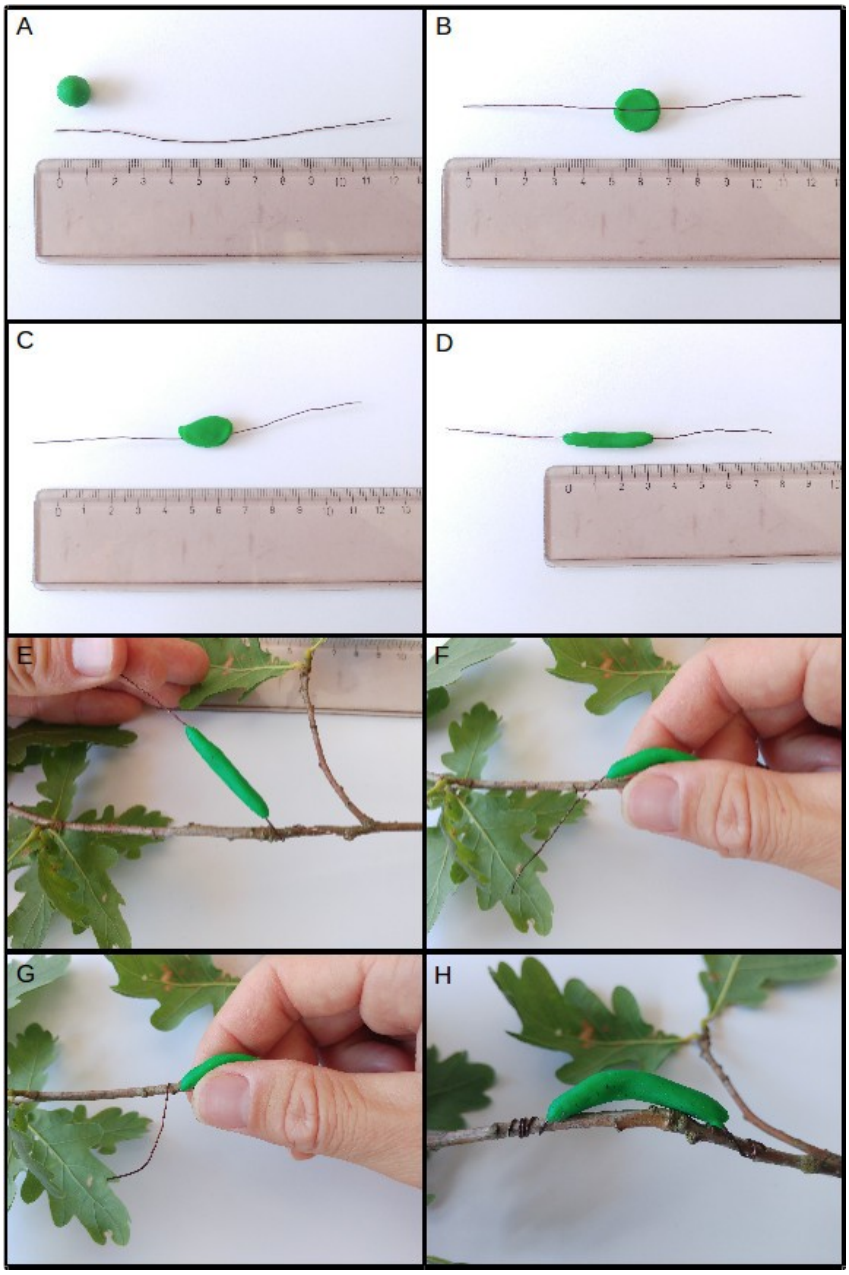

Figure 3: How to make and install fake caterpillars.

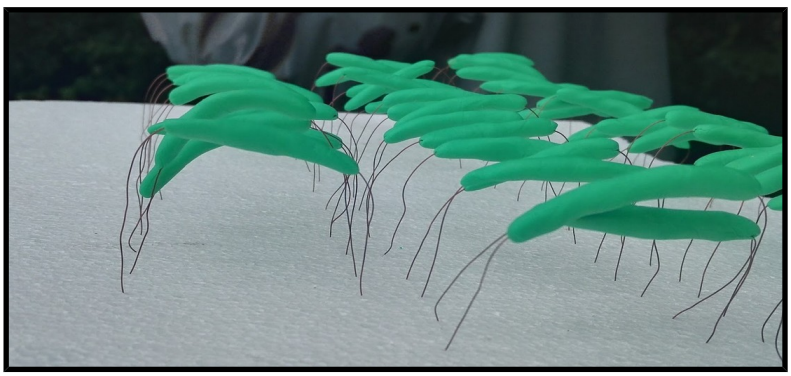

Figure 4 : Example of a foam board polystyrene plate with caterpillars.

6 - Assessing predation rate

Predation rate will be assessed by *reading* predation marks twice, first **15 days**, then **30 days** after caterpillar installation.

To *read* predation rate, you will need the "**guide to predation marks**" (see section 7, below). You will need to use a **magnifying glass** as some damage may be poorly impressed into the modelling clay. The most common marks are left by bird beaks and insect mandibles. Less often, you may see teeth marks left by mammals (e.g., shrews) or lizards.

You will have to write down your observations in the field scoring sheet (see appendices, below). Make sure your printed it before starting.

It is crucial that caterpillars are handled with care to avoid "false positive", *i.e.* marks that are not left by predators but by leafs, branches or finger nails.

For each caterpillar:

1. Gently remove each caterpillar from the branch. You can pin them again on a foam board (**Figure 4**) in order to transport them without damaging them.
2. **Once all caterpillars have been removed and saved** (should be 20, but some may have disappeared), replace the caterpillars with 20 new ones as indicated in the above section (**Figure 3**), making sure that their surface is absolutely smooth.
3. Once back in the classroom, inspect each caterpillar, first with bare eyes, then with the magnifying glass. If any mark is visible on the caterpillar surface, refer to the "field guide to predation marks" to identify what caused the damage. Fill field scoring sheet in (both paper in numeric versions):
    A. Indicate the date when caterpillars were installed.
    B. Indicate the date when the caterpillars were removed.
    C. Clearly write down the number of installed caterpillars, the number of undamaged caterpillars, the **total number of damaged caterpillars** (regardless of predator type) and the number of caterpillars with predation marks left by each predator type. For example, if predation marks left by both birds and insects are visible on the same caterpillar, you will count this caterpillar as damaged once in total, and once by birds and once by insects. Indicate 0 (i.e., zero) instead of leaving the cell empty if no damage was observed.

The caterpillars will be sent to the scientific coordinator of the project. In order to prepare the packages:

4. We will provide you with two mailing boxes in which you will introduce the caterpillars. Each box can fit 20 caterpillars.
5. In each box, introduce a thin foam board polystyrene plate. Pin the caterpillars on the board. Close the box.
6. On the box, indicate:
    A. The corresponding tree ID (e.g. Bastien.Castagneyrol_1)

B. The date when the caterpillar was removed from the tree.

C. The number of caterpillars (in principle, it should be N = 20 if all the caterpillars were retrieved, but it may be less than 20).

D. Optionally, you may want to know exactly which caterpillar was attacked by a predator (for instance, if each pupil was responsible of one caterpillar). In this case, you can give each caterpillar a number, with a paper tag, or directly written on the foam board or on the box.

At this stage, you should have:

- One box filled in with 20 caterpillars (or possibly less), with relevant information written on the box
- The paper field scoring sheet filled in
- The Excel field scoring sheet filled in on your computer
- 20 new caterpillars secured on the four branches.

Follow exactly the same procedure for the second assessment, with exception of step 2.

At this stage, you should have:

- **Two boxes filled in with 20 caterpillars each** (or possibly less), with relevant information written on the box
- The paper field scoring sheet filled in
- The Excel field scoring sheet filled in on your computer.

At the end of the project, you will send all the boxes together with sample leaves to Bastien Castagneyrol (see below).

7 - Field guide to predation marks

In the present procole, we only give few pictures showing the most typical predation marks. Additional pictures can be found (and downloaded and printed) here.

7.1 - Predation marks left by birds

Predation marks left by birds are the most frequent. They are characterized by a 'V' shape impressed in the modelling clay **(Figure 5, Figure 8I-M)**. These marks are often deep but may in some cases be superficial.

Figure 5: Examples of caterpillars showing predation marks left by birds. In these examples, the typical V-shaped marks are very pronounced, but they may also be more superficial.

7.2 - Predation marks left by insect mandibles

Insect mandibles leave more or less deep marks forming pairs of holes in the modelling clay. Pairs of holes are usually 1 to 3 mm apart **(Figure 6, Figure 8A-H)**. These marks can be very superficial. One of the two marks of a pair may be deeper than the other.

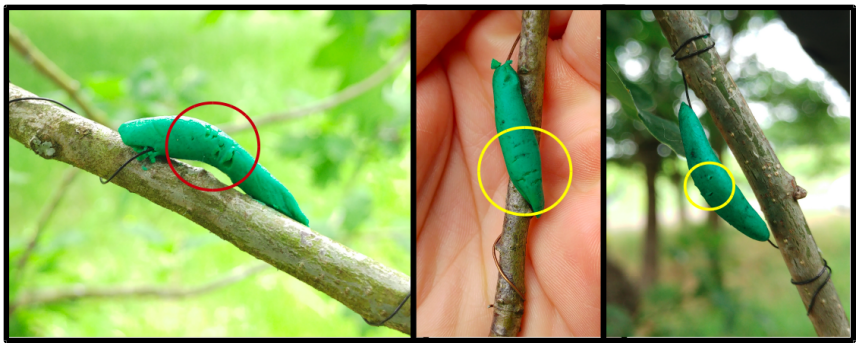

Figure 6: Examples of caterpillars showing predation marks left by insects. We recognise the pairs of holes left by the mandibles.

7.3 - Other predation and non-predation marks

You may find caterpillars with marks that do not look like those described above. Some may have been left by mammals or lizards (but this is rare), while others may be non-predation marks, what we will refer to has “false positive”. Additional photos of the different predation and non-predation marks can be found in the field bite guide.

Predation marks left by mammal and lizard teeth

Mammals leave traces of incisors. These are generally two parallel strips on the clay surface **(Figure 7, Figure 8N-P)**.

Figure 7: Examples of caterpillars with mammal tooth marks. The red circle locates parallel marks left by teeth.

Traces left by lizards are the least frequent. The U-shape of the jaws (unlike the V of the birds), with small holes at the location of the teeth (Figure 8Q-R) is typical.

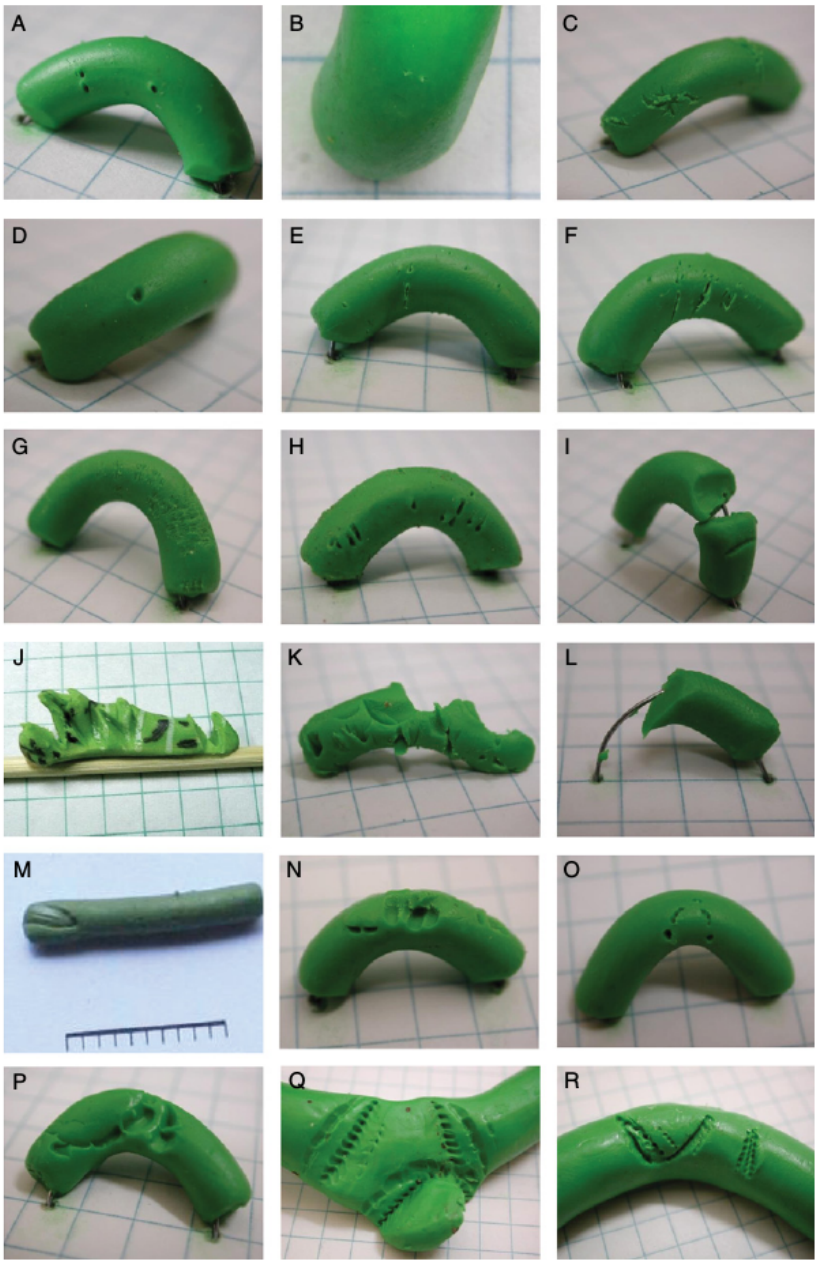

Figure 8: Examples of caterpillars with insect (A-H), bird (I-M), mammal (N-P) and reptile (Q-R) predation marks. Source: Low et al. (2014) *Entomologia Experimentalis and Applicata*, 152: 120-126.

False positive

The caterpillars may show traces that do not fall into the previous categories:

- Several thin, parallel, and superficial scratches can be made by the friction of adjacent leaves (Figure 9D).
- Marked grooves may appear at the junction between the modelling clay and the wire (both ends of the caterpillars from Figure 9A-B)
- Bud imprints can be impressed between the branch and the caterpillar (Figure 9A-C).

These are obviously not predation marks. They should not be taken into account.

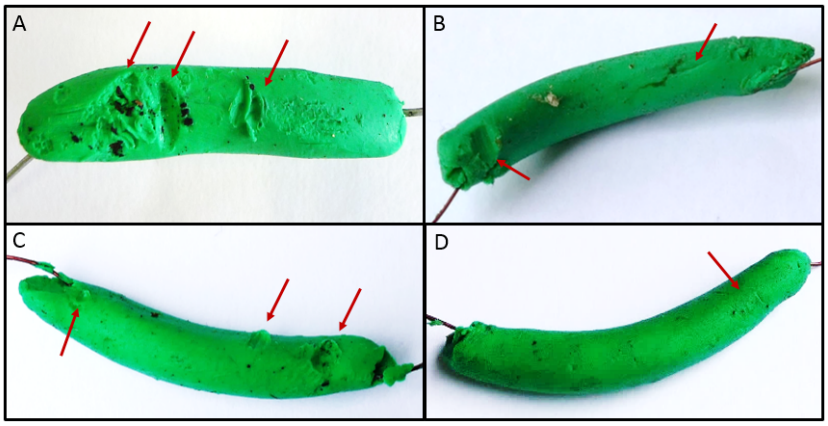

Figure 9: Example of caterpillars with bud (A-C) and leaves marks (D). Red arrows indicate non-predation marks.

Unidentifiable traces

If you have any doubt, it is better to indicate that you have not been able to identify the damage rather than venture to a questionable interpretation. In the field scoring sheet, then count these caterpillars as undamaged.

8 - Leaf collection

With this project, we ultimately aim at linking leaf insect damage with predator activity at the European scale. To do so, we therefore need to estimate leaf insect damage on oak leaves.

1. During the second assessment, **collect 30 leaves per branch** starting with the first at the tip of the branch and going down to the basis (which makes **30 × 4 = 120 leaves in total)**. Under no circumstances should the leaves be torn during sampling.
2. Even the most impartial observer cannot help but have their eyes drawn to the leaves with clearly visible damage. To reduce bias in the sampling, you will **randomly draw 60 leaves from the 120 initially collected**. This will be the final sample:
   A. Place all collected leaves into an opaque bag;
   B. Randomly draw 60 leaves **without damaging them**;
   C. Gather the 60 leaves into two small envelopes, inserting 30 leaves per envelope.Using small envelopes helps keeping the leaves as flat and tight as possible.
   D. If you want your pupils to estimate insect damage as well, keep the remaining 60 leaves and refer to the section 9. Otherwise, you can throw them away.
3. On each of the two envelopes, note:
   A. The name of your school
   B. Tree ID

C. Teacher's e-mail address

The leaves will be sent to the project coordinator together with the two boxes containing the caterpillars. You can use any type of package, provided that empty spaces are filled with paper or plastic chips to make sure boxes containing caterpillars will not move within the package. This package should contain:

- At least 2 boxes, with 20 caterpillars each. If you involved more than one group of children, and therefore surveyed more than one tree, then your package may contain more than two boxes. However, in this eventuality, make sure tree ID is properly indicated on each box.
- At least two envelopes with 30 oak leaves each. Again, if you surveyed and sampled more than one tree, then you package may contain more than two envelopes. However, in this eventuality, make sure tree ID is properly indicated on each envelope.
- The scoring field sheet to this package properly filled in with relevant information.

Send the package to:

Bastien Castagneyrol
INRA UMR BIOGECO
69 route d'Arcachon
33612 Cestas Cedex
France

Please send the package no more than one week after leaf collection. Meanwhile, the package with caterpillars must be stored in a room. Please do not forget it in you car during a hot sunny day!

9 - Quantifying insect damage (optional)

This part is not obligatory for participation in this project. But you can do it with your students to complete the whole process and compare the results of your class with other project partners and with insect damage estimated by scientists on your tree.

A fast and reliable method to assess insect damage is to mentally reconstruct the initial shape of the leaf and to visually estimate the percentage of area consumed or impacted by herbivores.

Insect damage may occur in different forms: herbivores may have chewed all leaf tissues (e.g., caterpillars and many beetles, **Figure 10**, Leaf-chewers), or only leaf mesophyll such that the two epiderms were detached by a leaf-miner larva (**Figure 10**, Leaf-miner), or only the epidermis has been scraped (**Figure 10**, Skeletonizers). You may also find some galls on leaves and buds.

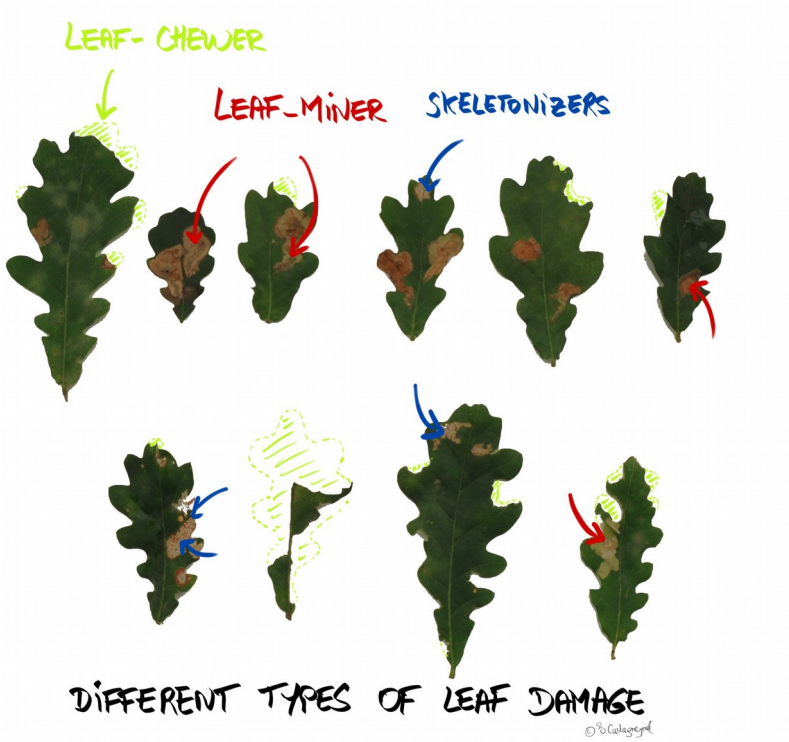

Figure 10: Different types of insect damage on oak leaves. You will consider the total damage, without referring to the different types of damage.

To help you estimate the damage and to reduce differences among observers, we have prepared a template (Figure 11). Using intact oak leaves that we have digitized and then colored, we mimicked several levels of defoliation (red parts on **Figure 11**) by removing a known number of pixels using an image manipulation software (GIMP) . The comparison of the number of pixels before and after the simulated defoliation allowed us to estimate the missing leaf area **(Figure 11)**.

For each leaf, you will assign a score corresponding to a defoliation class **(Table 1)**, regardless of whether the damage is due to chewers, skeletonizers or leaf-miners. You will rely on the template to estimate whether the defoliation observed on the leaf is greater or lesser than the threshold values shown in **Figure 11**, which will help you determine in which class each leaf falls. Additional photos can be found here. In addition, you will count the total number of galls and total number of mines.

Table 1: Correspondence between damage score, defoliation range and % defoliation. The mean defoliation at the tree scale is obtained by averaging the % defoliation of the 60 sampled leaves.

Score	Defoliation range	% défoliation
A	0	0
B	1-5	3
C	6-10	8
D	11-15	13
E	16-25	20,5
F	26-50	38
G	51-75	63
H	76-100	88

Use the Excel field scoring file to enter your data. Mean % defoliation will be calculated automatically. Indicate the total number of galls and total number of mines found on the leaves. This will be used to calculate the mean number of galls and mines by simply dividing the number of galls or mines by the number of sampled leaves.

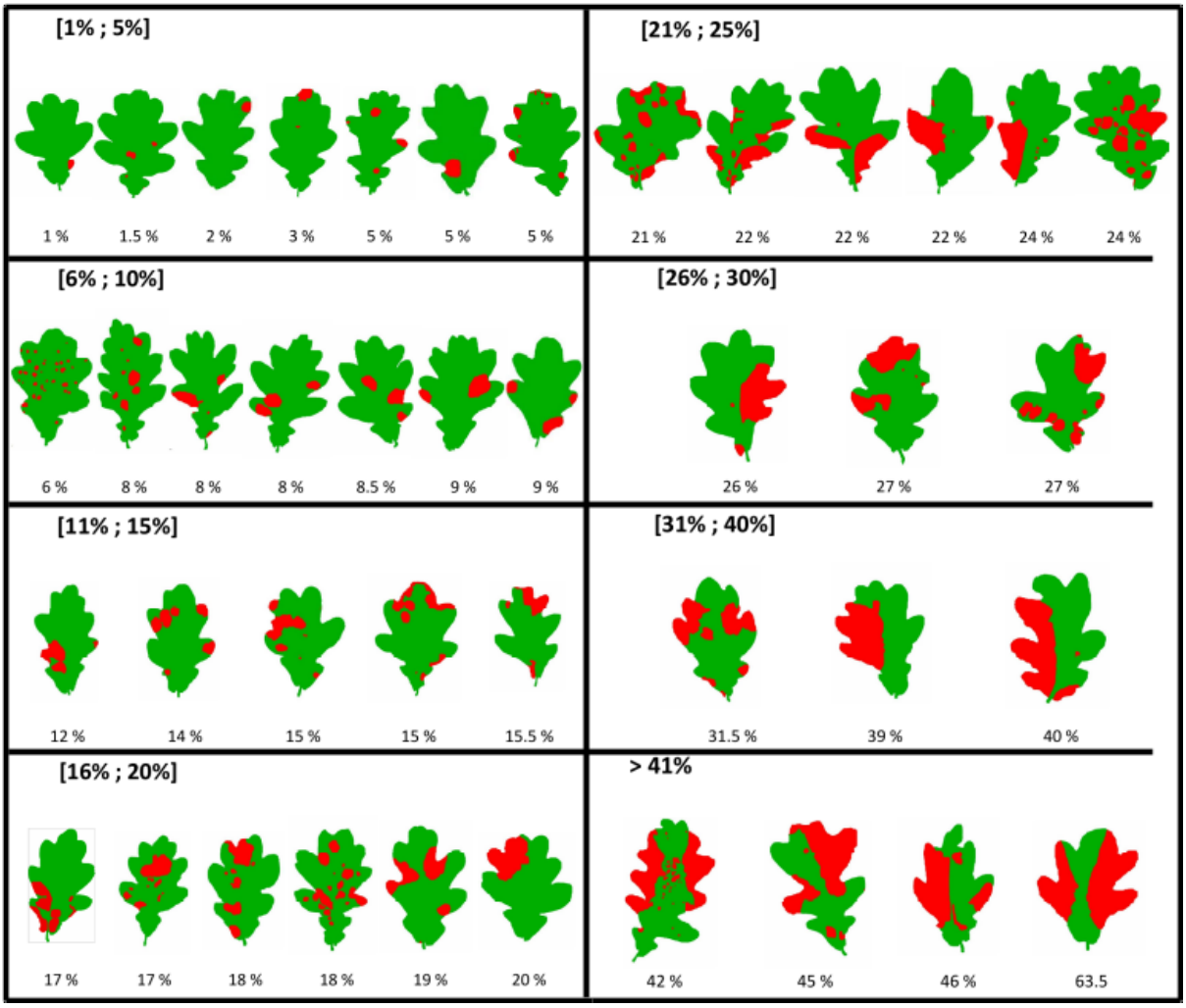

Figure 11: Template showing different types and levels of damage. Red parts are those removed or damaged by herbivores.

10 - FAQ (Frequently Asked Questions)

The present protocol has been updated based on the most frequently questions teacher partners had during the 2018 field campaign. However, if you have any question after having read this protocol, please contact the project coordinator.

11 - Appendices

Appendix 1 – Field scoring sheet (below, and online)
Appendix 2 – Additional scientific context (online)
Appendix 3 – How to identify a pedunculate oak (online video)
Appendix 4 – How to make a fake caterpillar (online video)
Appendix 5 – How to install a fake caterpillar (online video)

Field scoring sheet	
Print this sheet. Once filled in, make a copy. Send the original sheet along with oak leaves	
Your oak	
Partner number	
Latitude (read on Google maps, ex : 44.6784)	
Longitude (read on Google maps, ex : 0.7784)	
Circumference at 1.30 m from ground level (cm)	
Estimated tree height (m)	
You	
School name	
Teacher NAME and Surname (e.g., CASTAGNEYROL, Bastien)	
Teacher's e-mail	
Mean pupil age	
Your caterpillars	
Date of installation (Year-Month-Day, e.g. 2018-05-13)	
Date of first assessment	
Date of second assessment	
Date of leaf collection	
Your observations	
First assessment	
Number of installed caterpillars	
Number of caterpillar with **any type** of predation mark(s)	
Number of predation marks left by **birds**	
Number of predation marks left by **arthropods**	
Number of predation marks left by **mammals**	
Number of predation marks left by **lizards**	
Number of **unindentified** predation marks	
Number of **lost** caterpillars (i.e., not retrieved)	
Second assessment	
Number of installed caterpillars	
Number of caterpillar with **any type** of predation mark(s)	
Number of predation marks left by **birds**	
Number of predation marks left by **arthropods**	
Number of predation marks left by **mammals**	
Number of predation marks left by **lizards**	
Number of **unindentified** predation marks	
Number of **lost** caterpillars (i.e., not retrieved)	
Insect herbivory (optional)	
Number of observed leaves	
Total number of galls	
Total number of mines	
Number of leaves in class A (0 % leaf area loss)	
Number of leaves in class B (1-5 % leaf area loss)	
Number of leaves in class C (6-10 % leaf area loss)	
Number of leaves in class D (11-15 % leaf area loss)	
Number of leaves in class E (16-25 % leaf area loss)	
Number of leaves in class F (26-50 % leaf area loss)	
Number of leaves in class G (51-75 % leaf area loss)	
Number of leaves in class H (>75 % leaf area loss)	
Your comments	
Indicate below any additional observation you made	